MONASH University

Emerging Issues in Sexual Health: *Mycoplasma Genitalium* and 'Stealthing'

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Abstract

Sexual health is a dynamic area of medicine, with rapidly emerging issues dependent on societal practices, and changing prevalence of diseases. The studies in this thesis contribute to the literature on two emerging issues in sexual health; *Mycoplasma genitalium* (*M.genitalium*), a relatively recently discovered sexually transmitted infection (STI), and 'stealthing', a recently described non-consensual practice of removing a condom without consent. This thesis will be split into Section A, discussing *M.genitalium* and Section B discussing 'stealthing'.

Section A: Chapters 1 and 2 establish the background by reviewing the literature regarding *M.genitalium*: the bacterium, its pathogenic effects and treatment options, and highlights the need for further research.

Chapter 3 describes a meta-analysis which aimed to establish *M.genitalium* prevalence amongst gay, bisexual and other men who have sex with men (MSM) by anatomical site. Forty-six studies met inclusion criteria, with *M.genitalium* prevalence estimated to be 5.0% at the urethra, 6.2% at the rectum, and 1.0% at the pharynx. *M.genitalium* was more commonly detected in symptomatic men, and more common in HIV-positive men at the urethra.

Chapter 4 presents a cross-sectional study of consecutively collected rectal swabs from MSM, that tested positive for *Chlamydia trachomatis* (*C.trachomatis*) (N=212) or *Neisseria gonorrhoeae* (*N.gonorrhoeae*) (N=212), as well as consecutively collected pharyngeal samples (N=480) from MSM. One in seven men treated for rectal-*C.trachomatis* or rectal-*N.gonorrhoeae* had undiagnosed *M.genitalium* detected, that would have been potentially exposed to azithromycin during treatment of *C.trachomatis* or *N.gonorrhoeae*. Pharyngeal *M.genitalium* was uncommon, detected in 2% (95%CI: 1-3%) of samples.

In chapter 5, I determine the prevalence of *M.genitalium* and macrolide-resistance, and its association with common genital symptoms in women, to inform indications for testing and clinical practice. Between April 2017-April 2019, 1318 women were tested for *M.genitalium* and answered a questionnaire about genitourinary symptoms in the week prior to presentation. *M.genitalium* was not associated with genital symptoms, but was significantly associated with cervicitis. One in two women with *M.genitalium* had macrolide resistant *M.genitalium.*

Chapter 6 details a retrospective study of 92 women diagnosed with *M.genitalium* and pelvic inflammatory disease (PID) at Melbourne Sexual Health Centre between 2006-2017. The clinical features of *M.genitalium*-associated PID (*M.genitalium*-PID) were compared to *C.trachomatis*-associated PID (*C.trachomatis*-PID), and treatment outcomes of women with *M.genitalium*-PID

were examined. Symptoms and signs in women diagnosed with *M.genitalium*-PID did not significantly differ to those with *C.trachomatis*-PID. Ninety-five percent of women treated with moxifloxacin were microbiologically cured.

Section B: Chapters 7 and 8 introduce the concept of 'stealthing' and highlight the importance of condoms for contraception and prevention of STIs, consent, and the gaps in the literature around non-consensual condom removal. The subsequent study in chapter 9 presents a cross-sectional survey of 1189 women and 1063 MSM, of whom 32% of the women and 19% of the men reported having ever experienced stealthing.

Overall, this thesis provides further evidence around the prevalence of *M.genitalium* in both women and MSM, data on the clinical features of *M.genitalium* in women, and estimates as to the prevalence of 'stealthing' in our society.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: 

Print Name: Rosie L. Latimer

Date: 16/03/2021

Publications during PhD enrolment

Peer-reviewed Scientific Publications that Contributed to the Thesis

Latimer RL, Shilling HS, Vodstrcil LA, *et al.* 'Prevalence of Mycoplasma genitalium by anatomical site in men who have sex with men: a systematic review and meta-analysis.' *Sexually Transmitted Infections* 2020;96:563-570. (Chapter Three)

Latimer RL, Vodstrcil L, De Petra, *et al.* 'Extragenital Mycoplasma genitalium infections among men who have sex with men.' *Sexually Transmitted Infections*. 2019 Jun 19. pii: sextrans-2019-054058. doi: 10.1136/sextrans-2019-054058. (Chapter Four)

Latimer RL, Vodstrcil LA, Plummer E, *et al.* 'Informing clinical indications for testing women for Mycoplasma genitalium.' Accepted May 2021 in *Sexually Transmitted Infections*. (Chapter Five)

Latimer, RL, Read, TRH, Vodstrcil, LA, *et al.* 'Clinical Features and Therapeutic Response in Women Meeting Criteria for Presumptive Treatment for Pelvic Inflammatory Disease Associated With *Mycoplasma Genitalium*.' *Sexually Transmitted Diseases*. 2019; 46(2):73-79 (Chapter Six)

Latimer RL, Vodstrcil LA, Fairley CK, *et al*. (2018) Non-consensual condom removal, reported by patients at a sexual health clinic in Melbourne, Australia. *PLoS ONE* 13(12): e0209779. https://doi.org/10.1371/journal.pone.0209779 (Chapter Nine)

Peer-reviewed Scientific Publications that did not Contribute to the Thesis

Plummer EL, Vodstrcil LA, Bodiyabadu K, Murray GL, Doyle M, Latimer RL, Fairley CK, Payne M, Chow EPF, Garland SM, Bradshaw CS. 'Are Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum associated with specific genital symptoms and clinical signs in non-pregnant women?' *Clinical Infectious Diseases*. 2021 Jan 27:ciab061. doi: 10.1093/cid/ciab061. Epub ahead of print. PMID: 33502501.

Cornelisse VJ, Chow EPF, Latimer RL, et al. 'Getting to the bottom of it: Sexual positioning and stage of syphilis at diagnosis, and implications for syphilis screening.' *Clinical Infectious Diseases*. 2019 Aug 17. pii: ciz802. doi: 10.1093/cid/ciz802.

Oral Presentations given during PhD Enrolment

Latimer, R. (2020). Informing clinical indications for testing women for Mycoplasma genitalium. Oral Presentation O182. Joint Australasian HIV&AIDS and Sexual Health Conferences: Virtual, November 16th-20th 2020.

Latimer, R. (2019). Extragenital Mycoplasma genitalium infections among men who have sex with men. Oral presentation O02.6. STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019.
Published: Latimer R, Vodstrcil L, Read T, et al. O02.6 Extragenital mycoplasma genitalium infections amongst men who have sex with men. *Sexually Transmitted Infections* 2019; 95:A41.

Latimer, R. (2019). Oh MG! The symptoms of Mycoplasma genitalium in women. Oral presentation. Central Clinical School Graduate Research Symposium, Melbourne, October 7th 2019.

Latimer, R. (2018). Clinical Features of Mycoplasma Genitalium Associated Pelvic Inflammatory Disease and Response to Moxifloxacin: A Case Series. Oral presentation #196. IUSTI Asia Pacific Sexual Health Congress, Auckland, New Zealand, November 1st-3rd 2018.

Latimer, R. (2018). Clinical Features of Mycoplasma Genitalium Associated Pelvic Inflammatory Disease and Response to Moxifloxacin: A Case Series. Oral presentation. Sexual Health Society of Victoria's annual World AIDS Day & Post Conference event, Melbourne, December 4th 2018.

Latimer, R. (2018). Clinical Features of Mycoplasma Genitalium Associated Pelvic Inflammatory Disease and Response to Moxifloxacin: A Case Series. Oral presentation. CCS Postgraduate Symposium, Melbourne, November 12th 2018.

Poster Presentations during PhD Enrolment

Latimer, R. (2019). Prevalence of Mycoplasma genitalium by anatomical site in men who have sex with men: A systematic review and meta-analysis. Poster P525. STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019.
Published: Latimer R, Shilling H, Vodstrcil L, et al. P525. Prevalence of Mycoplasma genitalium by anatomical site in men who have sex with men: a systematic review and meta-analysis. *Sexually Transmitted Infections* 2019; 95:A239-A240.

Latimer, R. (2019). Oh MG! The symptoms of Mycoplasma genitalium in Women. Poster P606. STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019.

Published: Latimer R, Vodstrcil L, Read T, et al. P606 Oh MG! the symptoms of mycoplasma genitalium in women. *Sexually Transmitted Infections* 2019; 95:A268.

Latimer, R. (2018). Non-Consensual Condom Removal in a Sexually Transmitted Infection Clinic Population. Poster presentation #18. IUSTI Asia Pacific Sexual Health Congress, Auckland, New Zealand, November 1st-3rd 2018.

Media appearances/interviews related to the PhD thesis

Hunt, Richelle and Epstein, Raf. 'Afternoons' ABC Radio Melbourne. Discussing non-consensual condom removal, live-to-air. June 4th 2019. Retrieved from: https://www.abc.net.au/radio/melbourne/programs/afternoons/afternoons/11158548

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Tencic, Nat. 'The Hook Up' triple ABC radio. Discussing negotiating condom use. November 4th 2018. Retrieved from: https://www.abc.net.au/radio/programs/the-hook-up-podcast/condom-negotiation/10941048

Honours and Recognitions

First place at the Australian Medical Student Association Three Minute Thesis Competition 2020.

Second place oral presentation at the Central Clinical School Graduate Research Symposium, Monash University 2019.

Second place at the Central Clinical School Three Minute Thesis Competition 2018.

Recipient of an Australian Government Research Training Program (RTP) Scholarship.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes four original papers published in peer reviewed journals and one original paper that has been submitted for publication in a peer reviewed journal. The core theme of the thesis is sexual health. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Melbourne Sexual Health Centre, Alfred Health and the Central Clinical School, Monash University, under the supervision of Professor Catriona Bradshaw.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 3, 4, 5, 6 and 9, my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision)*	**Nature and % of student contribution**	**No author, Co-author name, % and nature of Co-author's contribution**	**Co-author, Monash student Y/N**
Chapter 3	Prevalence of *Mycoplasma genitalium* by anatomical site in men who have sex with men: a systematic review and meta-analysis.	Published	65%, first author: Wrote the study protocol and submitted to PROSPERO, created the literature searching strategy, performed the literature search for articles, acted as reviewer 1 for inclusion/exclusion of studies, extracted data from included studies, contacted authors for missing data, performed analyses, wrote the first draft of paper and	2) Hannah Shilling (10%): acted as reviewer 2 for inclusion/exclusion of studies, checked extracted data from included studies for accuracy, provided input into the manuscript. 3) Lenka Vodstrcil (10%): assisted with performance of the analysis, provided input into manuscript and reviewers comments. 4) Dorothy Machalek (2%): provided input into the study protocol, assistance with the analysis, and input into the manuscript. 5) Christopher Fairley (1%): provided input into the protocol and input into the manuscript.	N N N N

			incorporated co-authors and reviewers' comments.	6) Eric Chow (1%): provided statistical support and input into the manuscript. 7) Tim Read (1%): provided input into study protocol, assisted with decisions around data extraction, and provided input into the manuscript. 8) Catriona Bradshaw (10%): formulated study concept, provided input into the study protocol, input into analysis, and input into manuscript and reviewers comments.	N N N
Chapter 4	Extra genital *Mycoplasma genitalium* infections amongst men who have sex with men.	Published	65%, first author: Wrote the ethics application, identified samples to be included in the study, extracted necessary data from patient files, performed the data analysis, wrote the first draft of the paper, and incorporated co-authors and reviewer's comments into the manuscript.	2) Lenka Vodstrcil (10%): assisted with the data analysis and provided input into manuscript and reviewers comments. 3) Vesna De Petra (1%): provided input into the study protocol, coordinated testing of study samples, and provided input into the manuscript 4) Christopher Fairley (1%): provided input into the protocol and manuscript	N N N N

				5) Tim Read (10%): co-wrote the protocol and assisted with the ethics application, assisted with the data analysis, and provided input into manuscript 6) Deborah Williamson (1%): provided input into the protocol and manuscript 7) Michelle Doyle (1%): provided input into the manuscript 8) Eric Chow (1%): provided input into manuscript 9) Catriona Bradshaw (10%): formulated the study concept, assisted with data analysis, provided input into the manuscript and reviewers comments.	N N N N
Chapter 5	The clinical indications for testing women for *Mycoplasma genitalium*.	Submitted	65%, first author: Wrote the ethics application and study protocol, created questionnaire, study packs, and set up study database. Data entry and cleaning, performed the data analysis, wrote the first draft of the paper, and incorporated co-	2) Lenka Vodstrcil (5%): assisted with the data analysis and provided input into manuscript and reviewers comments. 3) Erica Plummer (3%): assisted with data analysis and provided input into the manuscript, and assistance with reviewer's comments. 4) Michelle Doyle (5%): put together study packs, assisted with data entry and maintained patient records,	N Y N

			authors and reviewers' comments into the manuscript.	notified patients of test results, provided input into the manuscript. 5) Gerald Murray (1%): provided input into the study protocol, coordinated testing of study samples, and provided input into the manuscript. 6) Christopher Fairley (1%): provided input into the study protocol and manuscript 7) Kaveesha Bodiyabadu (2%): performed testing of study samples, and provided input into the manuscript. 8) Tim Read (4%): assisted with the ethics application and study protocol, as well as design of the questionnaire and analysis. 9) Marti Kaiser (1%): assisted with recruiting and provided input into the manuscript. 10) Elisa Mokany (1%): provided input into the study protocol and manuscript 11) Rebecca Guy (1%): provided input into the study protocol and manuscript	N N N N N N N

				12) Eric Chow (1%): provided input into the study protocol, data analysis and manuscript. 13) Catriona Bradshaw (10%): formulated the study concept, assisted with study protocol and data analysis, provided input into the manuscript and reviewers comments.	N
Chapter 6	Clinical features and therapeutic response in women meeting criteria for presumptive treatment for pelvic inflammatory disease associated with *Mycoplasma genitalium*	Published	65%, first author: Conducted the data extraction from patient files, performed the analysis, wrote the first draft of the manuscript, and incorporated co-author and reviewer comments.	2) Tim Read (10%): Wrote the ethics application, assisted with the analysis, provided input into manuscript and reviewers comments. 3) LenkaVodstrcil (5%): provided input into manuscript and reviewers comments. 4) Jane Goller (2%): provided data and input into the manuscript. 5) Jason Ong (1%): provided statistical support and input into the manuscript. 6) Christopher Fairley (1%): provided input into the analyses and the manuscript. 7) Jane Hocking (1%): provided input into the manuscript.	N N N N N N

				8) Catriona Bradshaw (15%): formulated the concept, assisted with the analysis, provided input into manuscript and reviewers comments.	N
Chapter 9	Non-consensual condom removal, reported by patients at a sexual health clinic in Melbourne, Australia.	Published	85%: Devised concept and protocol, wrote questionnaire, analysed data, wrote the first draft of the manuscript and incorporated co-authors and reviewer's comments.	1) Lenka Vodstrcil (2%): provided input into the protocol, analysis and manuscript 2) Christopher Fairley (1%): provided input into the protocol and manuscript 3) Vincent Cornelisse (1%): assisted with questionnaire development and provided input for the manuscript 4) Eric Chow (1%): provided input into the manuscript 5) Tim Read* (5%): provided input into protocol, questionnaire, and ethics, assistance with data analysis, and input into the manuscript. 6) Catriona Bradshaw* (5%): input into protocol and questionnaire, assistance with data analysis, and input into the manuscript. *T.R.H.R and C.S.B are Joint Senior Authors and contributed equally to this work.	N N Y N N N

I have included PDFs of the published papers in my appendices, however have included the papers as written chapters to allow for inclusion of supplementary material and to generate a consistent presentation within the thesis. The content of the published work has not been altered in any way aside from necessary edits for formatting consistency.

Student signature:  **Date:** 16/03/2021

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 16/03/2021

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My list of acknowledgments is long and numerous, which is a testament to the sheer amount of support I received throughout my PhD journey.

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Cat was a ridiculously dedicated supervisor, and I am so grateful for the sheer amount of time and effort she put into teaching me the ins and outs of research. I felt that Cat tried to encourage me to reach my potential and push myself out of my comfort zone. Every opportunity that came my way, whether it was a conference, or the tiniest speaking competition, or talking on ABC radio, Cat encouraged me to take it, and made the time to help me practice and prepare. Cat is exceptionally caring as a person, and genuinely cares about her students as a whole person. It was inspiring to be Cat's student, and I consider her to be a role model professionally, as a female leader and clinician, but also a role model for general life and how to treat those under you. It was a privilege to be part of the 'Genital Microbiota and Mycoplasma Group', jokingly known as 'The Cat Empire'.

My co-supervisors Tim and Lenka were not only my STATA guides/life-savers, as well as being fantastic company. Tim taught me all I know about ethics applications, and also provided an endless stream of 'dad jokes', education on the Australian political system, and how to read between the lines of the newspaper. I look forward to pointing at the images of his political career for years to come and saying 'That's my supervisor!'. Having Lenka as a supervisor was fantastic; with predominately clinicians as supervisors, it was great to have Lenka to balance that and provide statistical support and education, which was particularly necessary when it came to the meta-analysis. Lenka always made herself available, no matter how busy, and tirelessly edited all my work for which I am so grateful. This was particularly necessary with the tables, and my never ending back to front < > signs (which at this point is something I will probably never get right).

Thank you to Kit, for supporting me as a supervisor and as director of Melbourne Sexual Health Centre. Kit is one of the cleverest people I think I have ever met, and witnessing how quickly Kit can process and develop ideas is truly awe-inspiring. I feel privileged to have worked with Kit these last few years, and to be a part of the Melbourne Sexual Health Centre team. Thank you for supporting not only my ideas, but also for the financial support for attending conferences etcetera.

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You cannot do several years of research without requiring some technical assistance, and so I must thank Mark Chung for all his assistance with formatting posters and logos etc. over the years, and the IT department, Jun Kit Sze, Afrizal, and Rashidur Rahman, for not only launching my various questionnaires, but also assisting me the 2000 times I forgot my computer passwords.

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I would like to thank all my friends, particularly Laura, Shagun, Macey, Maddy, Bird, and Tim. My friends have become my Melbourne family, and have supported me so much over the past few years. So many of you shouted me a fancy dinner or a coffee or two, and told me I was amazing when I was having a hard day and felt like the opposite. You all encouraged me so much, and I'm very lucky to have friends like you all- although I may have completed this thesis sooner if we didn't have quite so many fun adventures together.

My actual family, Mum, Dad, Rachel, Phoebe, Jess and Daisy. While you're not (for the most part) in Melbourne, thank you for supporting and encouraging me loudly from afar (because our family does nothing quietly). Thank you particularly to my parents for the example that you set, and for the continual push to try my hardest, no matter what. Submitting this thesis will conclude 25 years of education, and I think you're both as relieved as I am! Thank you as well to all my grandparents for reading my papers and for always being proud of me, even though my topic slightly confuses you!

I would also like to acknowledge this research was supported by an Australian Government Research Training Program (RTP) Scholarship (RTP Stipend and RTP Fees Offset).

I would finally like to thank all the participants in my research, the thousands of people who answered questionnaires or who agreed to samples being tested. Thank you, this research could not have happened without any of you.

Executive Summary

Sexual health is a dynamic area of medicine, with rapidly emerging issues, dependent on changing prevalence's of various infections, and changes in societal sexual behaviours and practices. *M.genitalium* is a recently discovered sexually transmitted infection, and determining its prevalence in populations within the community and examining its associations with clinical syndromes formed the core part of my PhD. The scope of the PhD was expanded to include a research project on non-consensual condom removal (known colloquially as 'stealthing'), in response to public reports of this behaviour occurring, and absence of academic evidence. This thesis will be split into Section A, discussing *M.genitalium* and Section B discussing 'stealthing'. The sections highlight recent literature on both *M.genitalium* and 'stealthing', the projects generated by literature gaps in these areas, and the subsequent research papers and outputs of these projects. The projects conducted as part of this PhD were:

Study 1 / Chapter 3: The prevalence of *M.genitalium* in men who have sex with men.
Study 2 / Chapter 4: *M.genitalium* pharyngeal infection and rectal co-infection in men who have sex with men.
Study 3/ Chapter 5: The clinical indications for testing women for *M.genitalium.*
Study 4 / Chapter 6: The clinical features and response to moxifloxacin of *M.genitalium* - associated Pelvic Inflammatory Disease.
Study 5 / Chapter 9: How often do patients report non-consensual condom removal when presenting to a sexual health clinic.

All compulsory ethics modules and site training has been completed. All Monash coursework requirements have been completed. Compulsory unit 'Translational research (TRM6002)', and elective unit 'Introductory biostatistics (MPH6041)' were completed in Semester One 2017.

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List of Abbreviations

AOR	Adjusted Odds Ratio
CDC	Centers for Disease Control and Prevention
CI	Confidence intervals
C.trachomatis	*Chlamydia trachomatis*
C.trachomatis-PID	*Chlamydia trachomatis* associated pelvic inflammatory disease
DNA	Deoxyribonucleic acid
et al.	et alia; and others
HIV	Human immunodeficiency virus
hpf	High Power Field
mg	Milligrams
M.genitalium	*Mycoplasma genitalium*
MgPars	MgPa repeats
M.genitalium-PID	*Mycoplasma genitalium* associated pelvic inflammatory disease
MSM	Gay, bisexual and other men who have sex with men
NAAT	Nucleic Acid Amplification Test
N.gonorrhoeae	Neisseria gonorrhoea
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PEACH	Pelvic Inflammatory Disease Evaluation and Clinical Health
PID	Pelvic Inflammatory Disease
PMNL	Polymorphonuclear Leucocyte
PrEP	Pre-Exposure Prophylaxsis
RCT	Randomised Control Trial
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
STI	Sexually Transmitted Infections
TMA	Transcription Mediated Amplification

T.vaginalis	*Trichomonas vaginalis*
WHO	World Health Organisation

SECTION A

MYCOPLASMA GENITALIUM

1. Introduction to '*Mycoplasma genitalium*'

Mycoplasma genitalium (*M.genitalium*) is a bacterium: the smallest prokaryote capable of self-replication (Fraser *et al.* 1995). It is a sexually transmitted infection (STI), with a recent meta-analysis estimating the prevalence in the general community to range from 1.3% to 3.9% (Baumann *et al.* 2018). Testing for *M.genitalium* is recommended in the clinical syndromes of urethritis and proctitis in men (BASHH *et al.* 2018; CDC 2015), and cervicitis and pelvic inflammatory disease (PID) in women by the majority of international guidelines (BASHH *et al.* 2018; Slifirski *et al.* 2017; ASHA 2018). However, the issue of screening for *M.genitalium* is more complex, largely due to the lack of clarity around it's natural history, and increasing challenges with treatment due to rising antimicrobial resistance (Read *et al.* 2019b). Due to these issues, medical guidelines do not currently recommend screening for *M.genitalium* in either men or women (CDC 2015).

While a meta-analysis has been conducted to determine community estimates for the prevalence of *M.genitalium* (Baumann *et al.* 2018), data on *M.genitalium* prevalence in gay, bisexual and other men who have sex with men (MSM) have been limited. The meta-analysis of community-based studies included only 8 studies of MSM and estimated the overall prevalence of *M.genitalium* in MSM to be 3.2% (95% confidence interval (CI): 2.1-5.1%) (Baumann *et al.* 2018). However, this review excluded studies conducted in clinical settings, and estimates were predominantly derived from urine samples (Baumann *et al.* 2018), failing to capture the rectum and pharynx as other possible sites of infection. Studies in clinical settings, such as urban STI clinics in Melbourne and Sydney, have estimated a higher overall proportion of *M.genitalium* infections in MSM of between 9.5 and 13.4%, respectively (Couldwell *et al.* 2018; Read *et al.* 2019a). These studies have also shown *M.genitalium* to be more commonly detected at the rectum, compared with the urethra (7.0-8.9% at the rectum, and 2.7-4.9% at the urethra)(Couldwell *et al.* 2018; Read *et al.* 2019a), meaning deriving estimates that have only come from studies that tested the urethral site may underestimate the true prevalence of *M.genitalium* in MSM. Uncertainty around the prevalence of *M.genitalium* in MSM led me to conduct a meta-analysis examining the prevalence of *M.genitalium* in MSM by anatomical site (pharynx, rectum and urethra) (Chapter 3).

Screening at the pharynx for the two most common bacterial STIs, *Chlamydia trachomatis* (*C.trachomatis*) and *Neisseria gonorrhoeae* (*N.gonorrhoeae*), has been widely recommended in MSM in the British, American and Australian STI screening guidelines (ASHA 2020; Clutterbuck *et al.* 2016; CDC 2015). However, there are limited data on the prevalence of *M.genitalium* in the pharynx of MSM which is needed to inform screening and testing practices. The limited published studies on the prevalence of pharyngeal *M.genitalium* have been conflicting, with two Australian

studies failing to detect *M.genitalium* at the pharynx in MSM (Couldwell *et al.* 2018; Bradshaw *et al.* 2009), while one Chinese study found a high prevalence of pharyngeal *M.genitalium* (13.5%) among 388 MSM recruited from gay bars in five cities across China (Jiang *et al.* 2015). Overall, more data is needed to provide accurate estimates for *M.genitalium* prevalence in MSM at the pharynx, to inform testing and clinical practice. This knowledge gap led me to undertake a study to determine the proportion of MSM who are infected with pharyngeal *M.genitalium* (Chapter 4).

M.genitalium has a marked propensity to develop antimicrobial resistance, which has greatly complicated its management (Bissessor *et al.* 2015). First line treatment of *M.genitalium* has included azithromycin, a macrolide antibiotic which is used widely in the STI field to treat common STIs such as *C.trachomatis* and *N.gonorrhoeae* (CDC 2015; ASHA 2018). Resistance to azithromycin has now detected in at least 50.8-58.0% of *M.genitalium* infections in many countries (Gesink *et al.* 2016; Dumke *et al.* 2016; Read *et al.* 2017a), and treatment of *M.genitalium* with 1g azithromycin has been shown to select macrolide resistance in at least 11.1-11.8% of *M.genitalium* infections (Read *et al.* 2017a; Bissessor *et al.* 2015). The high prevalence of macrolide resistance seen in *M.genitalium* is also likely to have been contributed to by inadvertent exposure of *M.genitalium* to azithromycin during the treatment of *C.trachomatis* or *N.gonorrhoeae* infections (Read *et al.* 2017a). It is important to understand how commonly *M.genitalium* is co-infected with *C.trachomatis* and *N.gonorrhoeae* in clinic populations, particularly among MSM who are at particular risk of STI acquisition. Furthermore, this would inform testing practices and to determine how commonly *M.genitalium* is being unintentionally exposed to antibiotics administered for the treatment of other STIs. As screening for *M.genitalium* is not recommended or practiced, data on how common *M.genitalium* co-infection is with *C.trachomatis* or *N.gonorrhoeae* has been limited. A Sydney study reported rectal *C.trachomatis* to be independently associated with anorectal rectal-*M.genitalium* (Odds Ratio (OR)=5.0, 95%CI: 2.1 to 11.8, p<0.001) (Couldwell *et al.* 2018), however a study at our centre did not support this finding (Read *et al.* 2019a). I undertook a study to determine the proportion of rectal *C.trachomatis* and rectal *N.gonorrhoeae* infections in MSM who are co-infected with rectal *M.genitalium* to inform clinical practice (Chapter 4).

While the pathogenic role of *M.genitalium* in male urethritis has been well established for many years, its role in infection and sequelae in women has long been debated. A meta-analysis in 2015 found *M.genitalium* to be significantly associated with an increased risk of cervicitis, PID, preterm birth, and spontaneous abortion (Lis *et al.* 2015). The majority of studies investigating *M.genitalium* in women have focused on the association between *M.genitalium* and clinical syndromes, with less published data on the association of *M.genitalium* with a range of genitourinary symptoms and signs. The available evidence is conflicting, with some studies finding no association between

M.genitalium and symptoms in women (Anagrius *et al.* 2005; Mobley *et al.* 2012), and others finding significant associations between *M.genitalium* and dysuria (Mobley *et al.* 2012), abnormal vaginal discharge (Vandepitte *et al.* 2012; Walker *et al.* 2011), and post-coital bleeding (Bjartling *et al.* 2012). As women are disproportionately affected by the adverse consequences of sexually transmitted infections (STIs) (Eng *et al.* 1997; Anderson 1995), it is important to have robust evidence that underpins recommendations for STI testing in women. This knowledge gap led me to undertake a study to determine the contribution of *M.genitalium* to specific genital symptoms and signs in women, to inform indications for testing (Chapter 5).

Although *M.genitalium* has been proven to cause a number of urogenital syndromes in women (Lis *et al.* 2015), there is little research conducted on the differences in clinical presentations between *M.genitalium* and other STI such as *C.trachomatis*. This is particularly important for syndromes such as PID, where serious sequelae can be averted by prompt treatment of the STI (Ness *et al.* 2002). Further research is needed to understand if there are clinically important differences between PID caused by *M.genitalium* (*M.genitalium*-PID), and PID caused by *C.trachomatis* (*C.trachomatis*-PID), the most common STI detected in women presenting with PID in most settings. This information can assist clinicians' decision making in terms of first line testing and presumptive management, particularly as currently recommended regimens for PID do not generally include antimicrobials to which *M.genitalium* is susceptible (Ross *et al.* 2011; CDC 2015; Haggerty *et al.* 2008). To inform testing and clinical management of PID, I conducted a study examining the clinical characteristics of *M.genitalium*-PID and its response to moxifloxacin (Chapter 6).

In summary, with a number of commercial diagnostic assays for *M.genitalium* recently emerging and increasing testing occurring in the community, it is important to understand the prevalence of *M.genitalium* in specific populations and at individual anatomical sites and to define its contribution to genital symptoms and syndromes in men and women. This is particularly important in MSM, a population at high risk of STI, and in women, as the population most affected by the consequences of STI. There are many gaps in the literature regarding *M.genitalium* and as outlined above, this PhD will seek to address some of these.

2. Literature Review A- *Mycoplasma genitalium*

2.1 *Mycoplasma genitalium*- The Organism

M.genitalium was discovered in 1981, when it was isolated from the urethral specimens of two men in London (Tully *et al.* 1981). It belongs to a class of bacteria called mollicutes (Taylor-Robinson *et al.* 2011), which are distinguishable by the absence of a bacterial cell wall. *M.genitalium* belongs to a sub-class of mollicutes, mycoplasmas, which include *Mycoplasma hominis* and *Mycoplasma pneumonia* (Tully *et al.* 1981). Key features of mycoplasmas include the absence of a cell wall, filterability (ability to travel through filters sized 0.45μm), lack of reversion to bacteria with cell walls when grown in an antibiotic free medium, penicillin resistance, and production of typical colonies on agar (Tully *et al.* 1981). In place of the rigid peptidoglycan cell wall bacteria usually possess, *M.genitalium* has a triple-layered self-limiting membrane. *M.genitalium* is an aerobic organism (Tully *et al.* 1981).

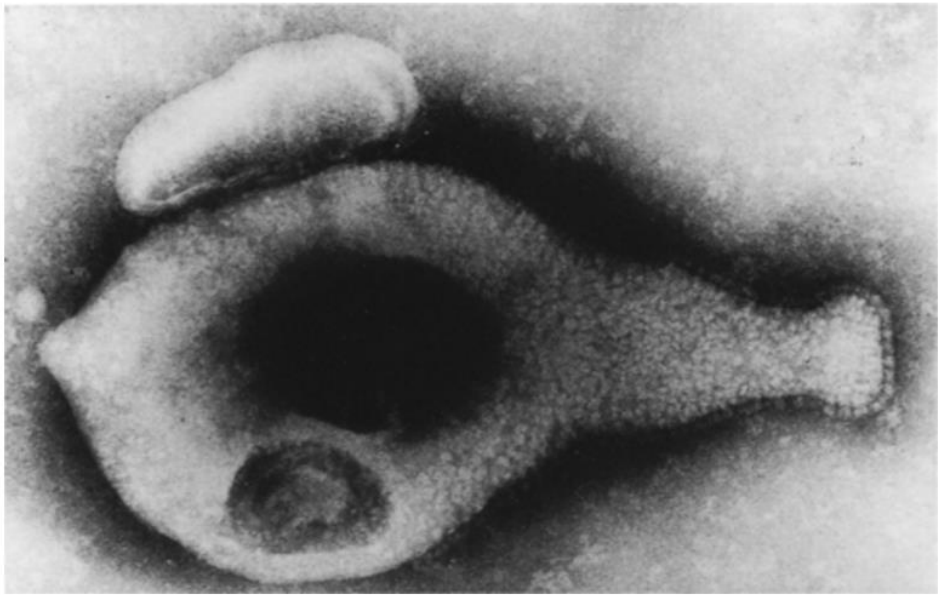

Figure 1. Transmission Electron Micrograph of *Mycoplasma genitalium*.
M.genitalium is negatively stained with ammonium molybdate. The characteristic flask shape and the terminal truncated portion with extracellular small projections are shown. The organism size is presented in the text (original magnification, ×120,000). *Image and text from Tully et al. Mycoplasma genitalium, a New Species from the Human Urogenital Tract, 1983 (Tully et al. 1983)*

M.genitalium is the smallest prokaryote capable of self-replication, with a genome size of around 580 kilo base pairs, 517 genes and 482 predicted protein coding regions (Figure 1) (Fraser *et al.* 1995). It remains the smallest known organism that can be grown in pure culture (Fraser *et al.* 1995) and thus is often used in minimal-genome research. *M.genitalium* contains an important operon named MgPa, a functioning unit of deoxyribonucleic acid (DNA), comprised of a cluster of genes, including mg190 also known as mgpA (encoding a predicted 29-kDa protein), mg191/mgpB (encoding the P140 protein), and mg192/mgpC (encoding the P110 protein)(Musatovova *et al.* 2003). Sequences of repetitive DNA elements from the MgPa operon form MgPa repeats (MgPars), which are distributed along the *M.genitalium* genome, although these do not function as protein-coding sequences (Fraser *et al.* 1995). MgPars represent 4.7% of the total genomic sequence, and are 78–90% identical to the corresponding sequences of MgPa (Fraser *et al.* 1995). *M.genitalium* adheres to host cells through its complex tip structure, known as the attachment or terminal organelle, which are comprised of *M.genitalium*'s major cell adhesins, MgpB (P140) and MgpC (P110) (Mernaugh *et al.* 1993), which reciprocally stabilise each other (Burgos *et al.* 2006).

Most mycoplasmas acquire change to their genomes through horizontal gene transfer, however *M.genitalium* remains unique amongst the mycoplasmas with regards to mutation (Blanchard *et al.* 2011). Recombination of the MgPa operon and MgPa islands contribute to the antigenic variations of MgPa proteins (Iverson-Cabral *et al.* 2006; Peterson *et al.* 1995). In the MgPa operon, class I mutants have large deletions of the mg192 gene, and class II mutants have deletions of both the mg191 and mg192 genes(Burgos *et al.* 2006). There has been some evidence to suggest that the immune system can detect proteins P140 (MgpB) and P110 (MgpC) (Baseman *et al.* 2004; Svenstrup *et al.* 2006), the proteins which form the terminal organelle, and thus *M.genitalium* may mutate to lose the terminal organelle, resulting in persistent infection (Burgos *et al.* 2006; Iverson-Cabral *et al.* 2006). A study which investigated the *M.genitalium* strain(s) in cervical specimens from a woman infected for 21 months, identified 17 mgpB variants within the single infecting strain, and confirmed mgpB heterogeneity occurs over the course of a natural infection (Iverson-Cabral *et al.* 2006). This observation suggests that recombination between the mgpB gene and MgPars sequences result in antigenically distinct MgPa variants which contribute to immune evasion and persistence of infection. Mutations have also been detected at the region V of the 23S ribosomal ribonucleic acid (rRNA) gene (Jensen *et al.* 2008). Non-mutated *M.genitalium* is referred to a wild-type sequence (Touati *et al.* 2014; Drud *et al.* 2020).

2.2 Diagnosis of *Mycoplasma genitalium*

M.genitalium cannot easily be cultured, and takes up to six months in a cell culture to grow due to its extremely fastidious nature (Tully *et al.* 1981; CDC 2015). As a consequence, there are only a few laboratories in the world currently consistently providing culture for *M.genitalium*. Enzyme-linked immunosorbent assays are unable to provide the sensitivity required to confidently detect *M.genitalium* in cervical or vaginal samples (Baseman *et al.* 2004). Studies were often performed using serology prior to the introduction of Nucleic Acid Amplification Testing (NAAT), as it was the only diagnostic measure available. Serology is however neither sensitive or specific for the detection of *M.genitalium* which is a mucosal infection (Waites *et al.* 2012).

M.genitalium infection is best detected through NAAT (Taylor-Robinson *et al.* 2012; CDC 2015). These assays were initially only available within laboratories that had adopted and validated research assays. However, several commercial *M.genitalium* assays were released in 2016 and 2017 (ASHA 2016), improving access to *M.genitalium* testing at non-research facilities, such as hospitals, obstetric and gynaecology clinics, and in primary care. Improved access to testing for *M.genitalium* in the community may lead to an increase in availability of data from a range of settings to inform clinical practice.

NAAT assays are usually polymerase chain reaction (PCR) assays that target either the 16S rRNA gene (Yoshida *et al.* 2002) or a major surface protein gene, MgPa (Jensen *et al.* 2003). The assays have comparable performance, however there are more variations in the MgPa gene than the 16S rRNA gene (Jensen *et al.* 2003; Eastick *et al.* 2003). Specimens for NAAT are collected through first pass urine or vaginal, cervical or anal swabs (Jensen *et al.* 2004). One study of heterosexual couples (n=1627) reported that men have higher mean bacterial loads than women, $p<0.001$, and women have higher mean bacterial loads in endocervical compared with urine samples ($p=0.001$) (Thurman *et al.* 2010).

Guidelines do not recommend screening for *M.genitalium* in any individuals (CDC 2015) due to its low prevalence in many populations (Andersen *et al.* 2007; Salado-Rasmussen *et al.* 2014) . Testing is recommended for patients presenting with symptoms indicative of a genital tract infection or those who are sexual contacts of *M.genitalium* patients (Oakeshott *et al.* 2010a; Walker *et al.* 2013). The literature suggests that *M.genitalium* has a similar clinical presentation to *C.trachomatis* (Anagrius *et al.* 2005), which is a predominately asymptomatic STI. The clinical syndromes and diseases associated with *M.genitalium* that prompt testing will be discussed in ‘Chapter 2.6 Health Outcomes in Men’ and ‘Chapter 2.7 Health Outcomes in Women’.

2.3 Epidemiology of *Mycoplasma genitalium*

2.3.1 Prevalence in the overall population

There are limited data on the prevalence and incidence of *M.genitalium* in the population. Commercial assays testing for *M.genitalium* were not available prior to 2017, and so testing had generally been restricted to health services with research affiliations, who had the capacity to develop and use in-house PCR assays. *M.genitalium* has not been screened for like other STIs such as *C.trachomatis*, and this has impacted on knowledge regarding its prevalence in populations, specific groups and geographical regions.

In early population studies of *M.genitalium, M.genitalium* was considered far less prevalent than *C.trachomatis*, with a prevalence of 1% in the US in 2001-02, compared with 4.2% for *C.trachomatis* (Manhart *et al.* 2007; Miller *et al.* 2004). However, the prevalence of *M.genitalium* may be increasing. The 2010-2012 NATSAL-3 study in Great Britain found *M.genitalium* to be as prevalent as *C.trachomatis*, with *M.genitalium* detected in 1.2% of men compared with *C.trachomatis* detected in 1.1%, and *M.genitalium* detected in 1.3% of women and *C.trachomatis* in 1.5% (Sonnenberg *et al.* 2015; Sonnenberg *et al.* 2013). This trend has also been noted elsewhere in studies of high risk populations. Early studies in high risk populations (those attending STI clinics) found a *M.genitalium* prevalence of 7.0% compared with 11.4% for *C.trachomatis* in the US (Manhart *et al.* 2003), and 5.6% compared with 9.7% for *M.genitalium* and *C.trachomatis* respectively in Sweden (Falk *et al.* 2005). Recent studies of high risk populations in the US have found the prevalence of *M.genitalium* in those attending STI clinics to exceed the prevalence of *C.trachomatis*, at 16.3% versus 9.3% respectively in women (OR 1.75; p=0.004), but remain equivalent in men at 17.2% and 17.8% (OR 0.961; p=0.822), respectively (Getman *et al.* 2016).

There has been one meta-analysis on the prevalence of *M.genitalium* in various populations conducted by Baumann *et.al.* (2018). Baumann estimated the overall prevalence of *M.genitalium* in countries with a high human development index at 1.3% (95%CI: 1.0-1.8, I^2 41.5%, n=9091) (Baumann *et al.* 2018), however this study had a number of limitations as it included few studies and estimates had high heterogeneity (Baumann *et al.* 2018). The analysis did not provide an estimate for the prevalence of *M.genitalium* in countries with a low human development index due to heterogeneity among estimates, but stated the estimated range was 3.9% (95%CI: 2.2-6.7, I^2 89.2%) in three studies that used probability sampling and 5.2% (95%CI: 2.4-11.5, I^2 86.8%) in two studies that used other methods to enrol participants. Their analysis found *M.genitalium* prevalence to be statistically higher in countries with a lower human development index (difference 3.1%, 95%CI: −0.1-6.3, P=0.057) (Baumann *et al.* 2018).

Several prevalence studies have noted ethnic differences in the distribution of *M.genitalium* prevalence, with studies conducted in the US showing a higher prevalence of *M.genitalium* amongst people identifying as either Afro-Caribbean (Horner *et al.* 1993), African Americans, or Hispanic (Manhart *et al.* 2007), compared with those who identify as Caucasian. Studies in the UK have found similar ethnic differences, with the highest prevalence of *M.genitalium* amongst those reporting black ethnicity (Sonnenberg *et al.* 2015). Authors concluded prevalence differences occur amongst different ethnicities due to differing social and behavioural factors, as well as ethnic differences in innate immunity (Lazarus *et al.* 2002) or in the vaginal microbiome (Ravel *et al.* 2011), which influence the risk of acquisition or persistence of infection.

2.3.2 Prevalence by gender

Two large epidemiological studies have investigated the prevalence of *M.genitalium* in the United States and the United Kingdom (Sonnenberg *et al.* 2015; Manhart *et al.* 2007). These found the prevalence of *M.genitalium* in women to be between 0.8-1.3%, and the prevalence of *M.genitalium* in men to be between 1.1-1.2%. in large epidemiological studies conducted in United States and the United Kingdom (Sonnenberg *et al.* 2015; Manhart *et al.* 2007). In Australia, a study of 1116 women attending Australian primary health care services found the prevalence of *M.genitalium* to be 2.4% (95%CI: 1-3), however this study did not examine men (Hocking *et al.* 2018). Prevalence in pregnant women before 14 weeks' gestation has been estimated at 0.9% (95%CI: 0.6-1.4, I^2 0%), however this summary estimate only included 4 higher HDI countries (Baumann *et al.* 2018). Overall Baumann's meta-analysis found no statistical difference in the prevalence of *M.genitalium* between men and women (p=0.47), although this analysis did not adjust for or stratify by sexuality (Baumann *et al.* 2018).

2.3.3 Prevalence amongst populations at higher risk of infection

Populations at greater risk of contracting STI include young people, MSM, sex workers, travellers, culturally and linguistic diverse people, people in custodial settings, and indigenous populations such as Aboriginal and Torres Strait Islander people in Australia (do Nascimento *et al.* 2014). Baumann's meta-analysis examined prevalence amongst MSM in the community from Australia, El Salvador, Guatemala and Honduras, and Nicaragua (n=3012). The summary estimated prevalence was 3.2% (95%CI: 2.1-5.1, I^2 78.3%) (Baumann *et al.* 2018). The analysis also examined *M.genitalium* prevalence amongst MSM presenting to STI clinics, with an estimate 3.7% (95%CI: 2.4-5.6, I^2 78.5%) from five clinics in Germany, the Netherlands, Norway and USA. Baumann's analysis included few studies, with estimates derived from urethral prevalence only (Baumann *et al.* 2018).

The meta-analysis by Baumann *et. al.* estimated prevalence of *M.genitalium* amongst female sex workers at 15.9%, from four studies based in China, Germany, Honduras and Uganda (Baumann *et al.* 2018).

2.4 Transmission and Risk Factors associated with *Mycoplasma genitalium* acquisition

M.genitalium has been shown to be a STI in a considerable number of epidemiological studies (Anagrius *et al.* 2005; Hjorth *et al.* 2006; Manhart *et al.* 2010; Slifirski *et al.* 2017), with acquisition of *M.genitalium* associated with typical risk factors for STI transmission. Sequence-based typing of *M.genitalium* in couples has confirmed transmission of the same *M.genitalium* strains between sexual partners (Hjorth *et al.* 2006).

M.genitalium acquisition has been associated with the classic risk factors common to STIs. The following demographics and epidemiological factors have been associated with increased risk of *M.genitalium* infection: younger age (Andersen *et al.* 2007; Manhart *et al.* 2007; Oakeshott *et al.* 2010a; Sonnenberg *et al.* 2015; Manhart *et al.* 2003), , lack of condom use (Walker *et al.* 2011; Sonnenberg *et al.* 2015; Manhart *et al.* 2007), lower levels of education (Manhart *et al.* 2003), younger age of sexual debut (Manhart *et al.* 2003), smoking (Oakeshott *et al.* 2010a), and increased number of sexual partners (Manhart *et al.* 2003; Manhart *et al.* 2007; Andersen *et al.* 2007; Svenstrup *et al.* 2014; Oakeshott *et al.* 2010a; Walker *et al.* 2011; Sonnenberg *et al.* 2015). Research from Denmark found the converse to be true, that long term relationships were associated with a decreased risk of contracting *M.genitalium* (Andersen *et al.* 2007). Ethnicity has also been found to play a role in risk of *M.genitalium* infection, with those identifying as black having significantly higher rates of *M.genitalium* infection, in both the UK and the USA (Manhart *et al.* 2007; Svenstrup *et al.* 2014; Oakeshott *et al.* 2010a), and those people identifying as Aboriginal and Torres Strait Islander at greater risk of *M.genitalium* infection in Australia (Walker *et al.* 2011). One French study performed at a sexual health centre, found no association between *M.genitalium* and demographic or epidemiological factors (Casin *et al.* 2002); however this study may have been subject to selection bias, as recruitment occurred at a sexual health clinic which represents a population at high risk of STI. The factors associated with increased risk of *M.genitalium* infection are associated with most STIs and are therefore unsurprising.

Studies have shown a high proportion of persons reporting sexual contact with a *M.genitalium* infected partner were infected. Amongst MSM, *M.genitalium* has been detected in 8.3% of contacts at the urethra, and 40.7% of contacts at the rectum (Slifirski *et al.* 2017). Amongst heterosexual couples, *M.genitalium* has been detected in 27.5-38.5% of male contacts of infection and 44.9-48.2% of female contacts of infection (Thurman *et al.* 2010; Slifirski *et al.* 2017; Anagrius *et al.* 2005). As such, female contacts of infection are significantly more likely to have *M.genitalium* detected than male contacts of female partners($p=0.004$) (Slifirski *et al.* 2017). Women may be more susceptible to *M.genitalium* infection due to immunological, hormonal, or physiological

factors that relate to the menstrual cycle (Manhart *et al.* 2003). Data suggests that women are more likely to be infected with *M.genitalium* during the proliferative phase of their menstrual cycle (Casin *et al.* 2002; Manhart *et al.* 2003).

Conflicting evidence exists over the natural history of *M.genitalium* and *M.genitalium* clearance rate, with very few studies investigating this issue. One study in Uganda found the median time taken to clear *M.genitalium* was two months, with an overall clearance rate was 26/100 person-years (Vandepitte *et al.* 2013). However a Kenyan study of female sex workers (n=299) found 47.7% of infections lasting ≥3 months, although there was a high risk of re-infection in this cohort (Cohen *et al.* 2006). Seven women in this study were persistently infected for more than 10 months (median, 14.4 months; minimum, 10.5 months; maximum, 21.1 months), with molecular strain typing analysis on cervical specimens confirming the same strain type throughout the study period, consistent with persistent infection with a single organism (Cohen *et al.* 2006). As discussed in Chapter 2.1, infections persisting for 21 months have been found to contain several mutated variants, with mutations assisting in avoidance of host detection and clearance (Iverson-Cabral *et al.* 2006).

2.5 Co-infection with other sexually transmitted infections

2.5.1 Co-infection with other bacterial sexually transmitted infections and genital infections

There is limited published literature reporting on coinfection rates of *M.genitalium* with other STIs. Studies of co-infection have been inconsistent, in part due to baseline variations of common STIs in populations globally. There has been one study examining whether participant demographics predicted *M.genitalium* co-infection, which did not find any associations (Harrison *et al.* 2019). Data on more than one co-infection with *M.genitalium* is exceptionally limited. Existing literature finds triple infections to be exceptionally uncommon, accounting for less than 1% of STI infections (Getman *et al.* 2016; Upton *et al.* 2017).

It is important to determine coinfection rates as acquisition of a STI may predispose to acquisition of a second STI, due to the similar risk factors, and endothelial damage from the initial infection (Zhang *et al.* 2000). Co-infection rates have further implications for *M.genitalium* infection as *M.genitalium* is rapidly developing antimicrobial resistance (see chapter 2.8), and it is likely *M.genitalium* is being inadvertently exposed to antimicrobials during the treatment of other STIs contributing to its antimicrobial resistance (Harrison *et al.* 2019).

2.5.1.1 Bacterial co-infection among heterosexual men

Co-infection data in men has been hampered by many studies failing to differentiate between heterosexual men and MSM, which are distinctly different populations. The reported frequency of *M.genitalium-C.trachomatis* co-infection ranged greatly in studies reporting on co-infection from 0.1-21.2% (Gesink *et al.* 2016; Mezzini *et al.* 2013; Yokoi *et al.* 2007; Gaydos *et al.* 2009a; Pépin *et al.* 2001; Fernández-Huerta *et al.* 2019).

Although a common infection among MSM, *N.gonorrhoeae* is an uncommon infection in heterosexuals outside of the USA. The British Nastal-3 study stated that men who were *M.genitalium* positive were more likely to have had *N.gonorrhoeae* or urethritis in the past five years, however when tested only one man had a *M.genitalium/N.gonorrhoeae* coinfection and one man had a *M.genitalium/C.trachomatis* coinfection, amongst 4828 samples (from all genders/sexual orientations) (Sonnenberg *et al.* 2015). The reported frequency of *M.genitalium/N.gonorrhoeae* co-infection ranged greatly in studies reporting on co-infection from 3.1%-37.9% (Mezzini *et al.* 2013; Uno *et al.* 1996; Yokoi *et al.* 2007; Pépin *et al.* 2001; Fernández-Huerta *et al.* 2019).

2.5.1.2 Bacterial co-infection among men who have sex with men

Data conflicts on the association between *M.genitalium* and *C.trachomatis* in MSM. At the urethra one large study found the two to be associated at the urethra among asymptomatic men (7.4% vs. 1.5%, p=0.03), but not among men with non-gonococcal urethritis (p = 0.001) (Read *et al.* 2019a). This study also found *M.genitalium* and *C.trachomatis* were not associated at the rectum (9.2% vs. 8.4%, p = 0.82) (Read *et al.* 2019a). However two other studies found *M.genitalium* and *C.trachomatis* to be strongly associated at the rectum [OR 3.5, 95%CI: 1.4-8.7 (Francis *et al.* 2008) and OR 5.0, 95%CI: 2.1-11.8, p<0.001 (Couldwell *et al.* 2018)]. Further research is needed to determine the true association between *M.genitalium* and *C.trachomatis* co-infection at the rectum amongst MSM.

There are less data on co-infection rates of *M.genitalium* and *N.gonorrhoeae* amongst MSM, compared to *M.genitalium* and *C.trachomatis*, somewhat paradoxically as MSM as a population suffer the highest burden of *N.gonorrhoeae* infections. A large Australian study examining prevalence amongst MSM found that in asymptomatic men *M.genitalium* was associated with *N.gonorrhoeae* detection at the urethra (7.4% vs. 0.5%, p = 0.002), but not at the rectum (6.1% vs. 6.2%; p = 0.98) (Read *et al.* 2019a). Other research by the same group a decade earlier had found no co-infection with *N.gonorrhoeae* was detected amongst MSM attending a male only sauna (Bradshaw *et al.* 2009).

2.5.1.3 Bacterial co-infection among women

There are a number of studies exploring *M.genitalium* co-infection with *C.trachomatis* in women. The reported frequency of *M.genitalium*/*C.trachomatis* co-infection ranged greatly in studies reporting on co-infection from 4.8%-42.9% (Casin *et al.* 2002; Svenstrup *et al.* 2014; Ljubin-Sternak *et al.* 2017; Chernesky *et al.* 2017; Mobley *et al.* 2012; Gaydos *et al.* 2009a; Harrison *et al.* 2019).

As stated above, *N.gonorrhoeae* is an uncommon infection in heterosexuals outside of the USA. In studies including both men and women, a NZ study detected 3/411 infections to be *M.genitalium*/*N.gonorrhoeae* co-infections (Upton *et al.* 2017), and a Greenland study stated *M.genitalium*/*N.gonorrhoeae* infections account for <1% of STI infections(Gesink *et al.* 2012). In the USA however, *M.genitalium*/*N.gonorrhoeae* appears to be a common co-infection in women. A study by Mobley *et al.* found that *M.genitalium* coinfections were detected in 30.4% of women with *N.gonorrhoeae* (Mobley *et al.* 2012), while a study by Manhart *et al.* detected *M.genitalium* in 6/50 women with *M.genitalium*-associated cervicitis (Manhart *et al.* 2003).

Literature to date has been conflicting on the relationship between *M.genitalium* and the common vaginal dysbiosis, bacterial vaginosis. While some cross-sectional studies have failed to detect a relationship between the two (Cox *et al.* 2016; Keane *et al.* 2000), or detected a significantly negative association between the two (Manhart *et al.* 2003), other research has found a significant positive association between the two [OR = 2.0 (95% CI: 1.1–3.4)] (Nye *et al.* 2020). A prospective study of female sex workers in Kenya found that bacterial vaginosis may enhance susceptibility to *M.genitalium*, with prior bacterial vaginosis significantly associated with *M.genitalium* acquisition when adjusted for age, human immunodeficiency virus (HIV) status and time [AOR (adjusted odds ratio)= 3.5 (95%CI: 1.9-6.6) (Lokken *et al.* 2017). Given bacterial vaginosis is associated with a significantly elevated risk for acquisition of *C.trachomatis* and *N.gonorrhoeae* infections (Brotman *et al.* 2010), it remains biologically plausible that bacterial vaginosis may enhance susceptibility to *M.genitalium*.

2.5.2 Co-infection with human immunodeficiency virus

There has been one systematic review and meta-analysis examining the association between HIV and *M.genitalium* (Mavedzenge *et al.* 2009). This study identified nineteen studies from sub-Saharan Africa (n=10), the United States (n=6), Europe (n=2), and South America (n=1), which analysed this relationship, with twelve of them finding a significant positive association. The meta-analysis concluded there was a significant positive relationship between these two infections, with a two-fold increased odds of detecting *M.genitalium* among people living with HIV [OR=2.0 (95%CI: 1.4-2.8)]. Importantly this review did not determine the temporal relationship between these infections, i.e. whether *M.genitalium* predisposes someone to HIV infection or if HIV infection predisposes someone to *M.genitalium* infection.

There has only been one longitudinal study examining whether or not HIV positivity impacted on *M.genitalium* acquisition in a group of women at high risk of infection. This study found that HIV was a significant risk factor for *M.genitalium* acquisition (hazard ratio [HR]=2.2, 95%CI: 1.3-3.7) (Cohen *et al.* 2006). Other research has been suggested that *M.genitalium* may predispose to HIV acquisition. *M.genitalium* has been shown to increase local inflammation and cause damage to genitourinary epithelial cells (Zhang *et al.* 2000; Tully *et al.* 1986; Das *et al.* 2014). *In-vitro* studies have found this both increases susceptibility to, and transmissibility of HIV infection (Zhang *et al.* 2000; Tully *et al.* 1986; Taylor-Robinson *et al.* 1985). *In-vitro* studies have also shown that *M.genitalium* infection promotes replication of the HIV virus once infected (Das *et al.* 2014). *In-vivo* research has disputed whether the relationship between *M.genitalium* and HIV is due to cervical inflammation, however it has shown that HIV DNA was three times more likely to be shed in women with *M.genitalium* [AOR=2.9 (95%CI: 1.1-7.6)] (Manhart *et al.* 2008). Clearance rates of

M.genitalium infection are associated with HIV status, with those who are HIV-positive with a CD4 count below 350 cells/mm having a significantly slower rate of clearance than those who are HIV-negative (Vandepitte *et al.* 2013).

Further research is needed to examine whether *M.genitalium* induced local inflammatory processes play a role in the acquisition and shedding of HIV, and to determine the temporal relationship between these two STIs. Whether this relationship represents behavioural and/or biological factors is not possible to determine from existing cross-sectional data.

2.6 Health Outcomes of *Mycoplasma genitalium* in Men

This chapter will explore the specific syndromes and clinical presentations that are associated with *M.genitalium* infection in men.

2.6.1 Urethritis

Urethritis is inflammation of the urethra, usually caused by a bacterial infection. Symptoms include dysuria, urethral discomfort and urethral discharge (Takahashi *et al.* 2006). Urethritis can be classified as gonococcal caused by *N.gonorrhoeae*, non-gonococcal generally caused by *C.trachomatis* or *M.genitalium*, or non-specific urethritis, where a pathogen is not detected. These classifications exist to guide treatment. *M.genitalium* is well-established as a cause of non-gonococcal urethritis in men, and is the second most common cause of non-gonococcal urethritis following *C.trachomatis* (Falk *et al.* 2004; Gaydos *et al.* 2009b; Moi *et al.* 2015).

The earliest review of *M.genitalium* and urethritis by Deguchi and Maeda concluded that in men with non-*C.trachomatis* non-gonococcal urethritis, *M.genitalium* prevalence ranged from 18-46% in men (Deguchi *et al.* 2002a). In 2004 a review by Jensen *et al.* found that *M.genitalium* was significantly associated with non-gonococcal urethritis [OR 3.8 (95%CI: 3.0-4.9; p<0.0001)], and accounted for 21% of non-gonococcal urethritis (Figure 2) (Jensen 2004). Jensen's review also found that *M.genitalium* accounted for 22% of non-*C.trachomatis* non-gonococcal urethritis, and that they were significantly associated [OR 5.2 (95%CI: 3.6-7.4; p<0.0001)](Figure 3) (Jensen 2004). A systematic review by Manhart *et al.* (2011) of thirty studies worldwide found *M.genitalium* to account for 13% of all non-gonococcal urethritis cases, and 25% of non-*C.trachomatis* non-gonococcal urethritis cases (Manhart *et al.* 2011). While numerous reviews have examined the relationship between *M.genitalium* and urethritis, only Manhart *et al.*'s was systematic review. The most recent review by Horner *et al.* in 2017 estimated that approximately 5.2% of *M.genitalium* infected men in England will develop non-gonococcal urethritis (Horner *et al.* 2017b). Development of non-gonococcal urethritis may be dependent on bacterial load, with studies reporting greater *M.genitalium* DNA loads in men with non-gonococcal urethritis, compared to *M.genitalium* positive men without non-gonococcal urethritis (Jensen *et al.* 2004; Jensen 2006).

M.genitalium has been associated with both asymptomatic and symptomatic urethritis (Falk *et al.* 2004; Anagrius *et al.* 2005). The definition of asymptomatic/microscopic urethritis varies between studies, but is generally defined as the presence of four or polymorphonuclear leukocyte (PMNLs) per high-powered field (hpf) in a urethral smear, in the absence of symptoms (Falk *et al.* 2004; Anagrius *et al.* 2005). Microscopic signs of urethritis (PMNLs) have been detected in up to ninety percent of men with *M.genitalium* (Falk *et al.* 2004). Those with symptomatic urethritis with

urethral discharge as either a symptom or sign are more likely to be *M.genitalium* positive compared to men with asymptomatic urethritis (OR 35.2, 95%CI 3.9-319.6, p=0.002) (Horner *et al.* 2002). Those with *M.genitalium*-associated urethritis have significantly more symptoms than those with *C.trachomatis*-associated urethritis [73% vs 40%, relative risk (RR) 1.8 (95%CI: 1.2-2.7)](Falk *et al.* 2004).

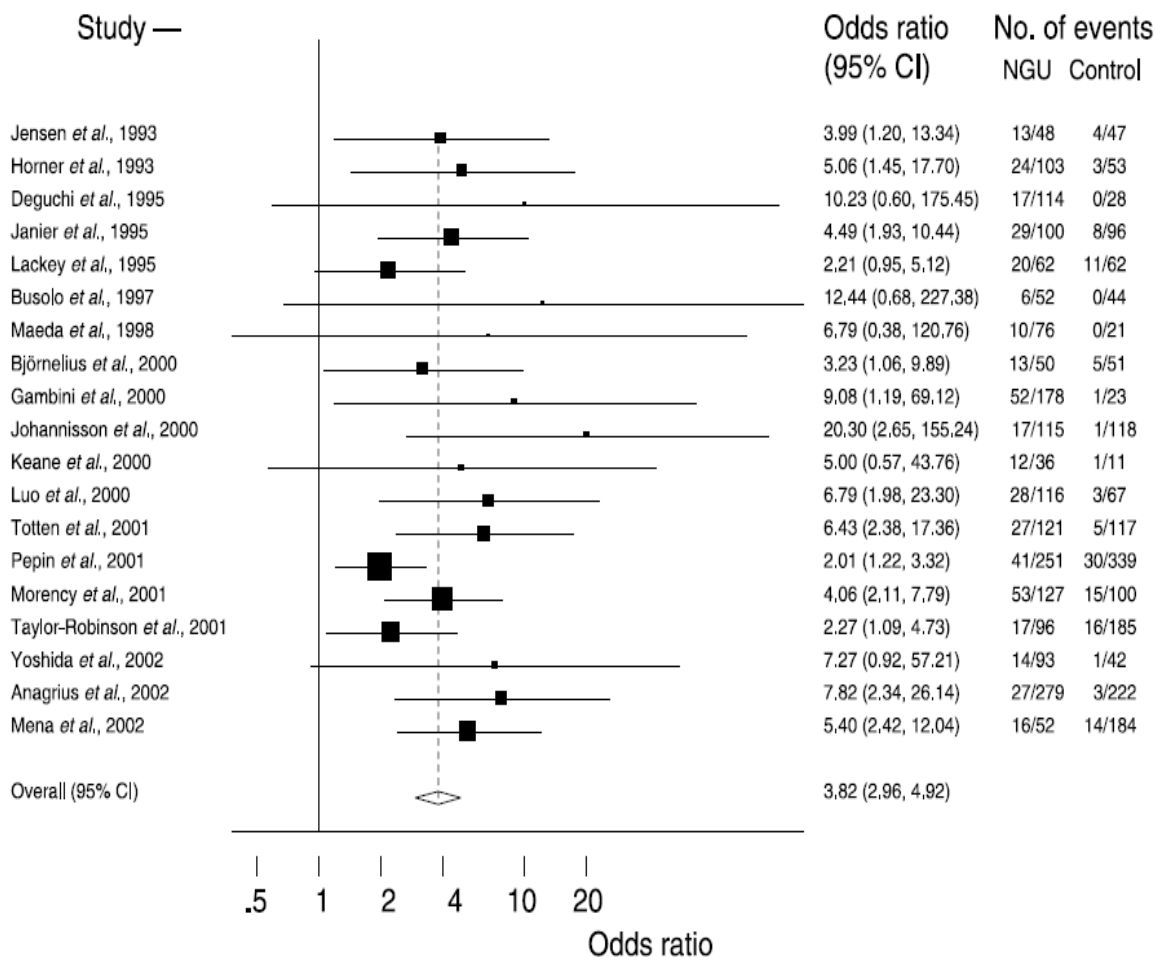

Figure 2. Forest plot of the association between Mycoplasma genitalium and non-gonococcal urethritis.

Forest plot and text from Jensen JS et al. Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases, 2004 (Jensen 2004)

Persistent or chronic non-gonococcal urethritis following acute infection with *M.genitalium* has been reported in many studies (Wikström *et al.* 2006; Deguchi *et al.* 2002b; Horner *et al.* 2001; Manhart *et al.* 2011), with *M.genitalium* accounting for 19-41% of men with the condition (Manhart *et al.* 2011). Horner *et al.* showed *M.genitalium* was associated with recurrent urethritis in those receiving treatment (OR 7.9, 95%CI: 1.0–65.9) (Horner *et al.* 2001). Men with recurrent urethritis

were less likely to be engaging in sexual activity than those without urethritis, indicating that ongoing infection rather than reinfection was responsible for their symptoms. Persistent *M.genitalium*-associated non-gonococcal urethritis is likely due to inadequate treatment due to either intrinsic or acquired antimicrobial resistance (Wikström *et al.* 2006; Taylor-Robinson *et al.* 2011) (please see Chapter 2.8).

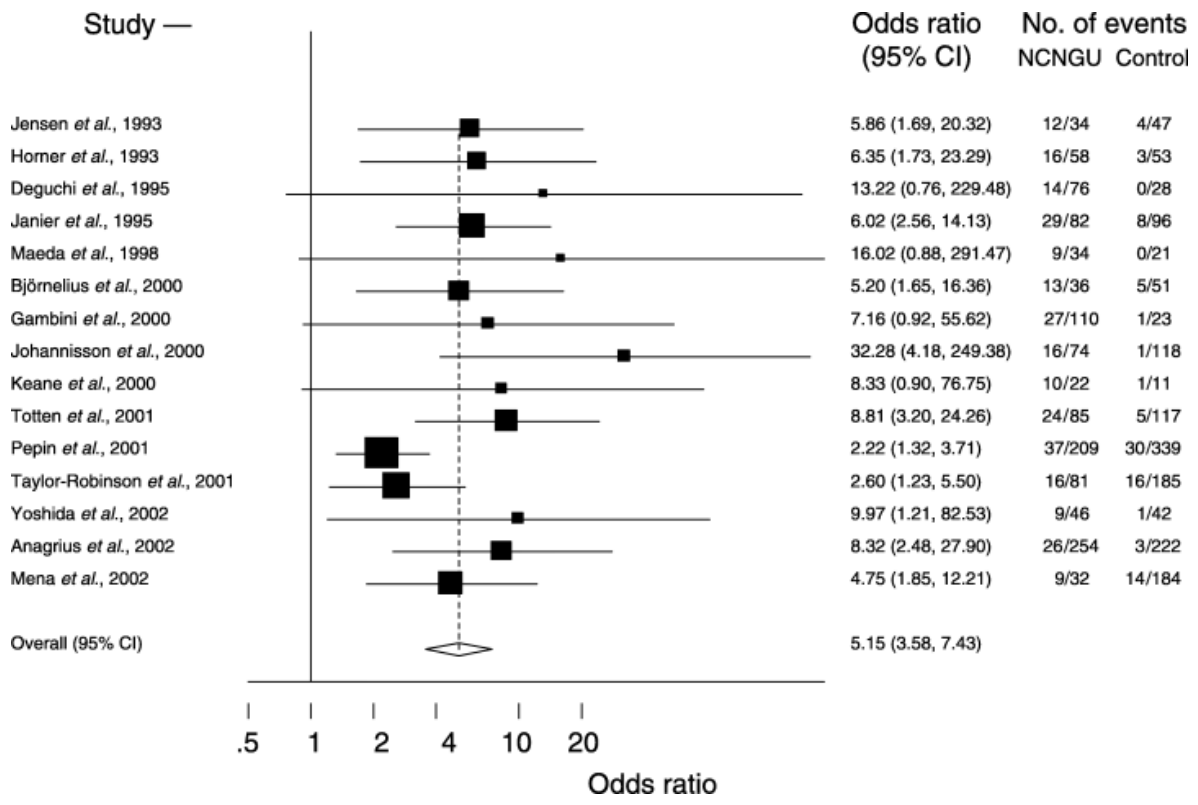

Figure 2. Forest plot of the association between Mycoplasma genitalium and non-*Chlamydia trachomatis* non-gonococcal urethritis.

Forest plot and text from Jensen JS et al. Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases, 2004 (Jensen 2004)

2.6.2 Proctitis

Proctitis is the result of inflammation of the anus and rectum, and symptoms include anal pain, tenesmus, anal discharge and bleeding, and pain when defecating. The role of *M.genitalium* in the syndrome of proctitis remains unclear.

Bissessor *et al.* were the first to detect *M.genitalium* amongst MSM with proctitis, with *M.genitalium* accounting for 11.7% (95%CI: 6.9-17.1) of proctitis within the study (Bissessor *et al.* 2016). However this study did not include a control group and as such could not provide a statistical

association between *M.genitalium* and proctitis (Bissessor *et al.* 2016). There have been three case control studies examining the association between *M.genitalium* and proctitis, of which all have failed to detect an association between *M.genitalium* and proctitis (Francis *et al.* 2008; Soni *et al.* 2010; Read *et al.* 2019a).

Studies examining rectal symptoms specifically have been conflicting, with the studies in the UK and Australia indicating no association between *M.genitalium* and rectal symptoms (Soni *et al.* 2010; Read *et al.* 2019a), and the study in San Francisco, USA finding *M.genitalium* was associated with rectal symptoms. Further research is needed to determine the contribution of *M.genitalium* to rectal symptoms in MSM and its contribution to the clinical syndrome of proctitis.

2.6.3 Balanitis, posthitis, prostatitis and epididymitis

Balanitis is inflammation of the glans (head) of the penis, and posthitis is inflammation of the prepuce (foreskin) of the penis. They may occur separately or concurrently and are usually caused by infection, irritants or trauma. There has been one study conducted by Horner and Taylor-Robinson in 2013, which examined the existence of balanitis and/or posthitis amongst 114 men with non-gonococcal urethritis. They found *M.genitalium* was significantly associated with balanitis and/or posthitis (OR 4.1, 95%CI: 1.3-13.4, p=0.01), with 37.0% of those positive for *M.genitalium* having balanitis or posthitis (Horner *et al.* 2011). Some patient treatment guidelines have recognised *M.genitalium* as a cause of balanoposthitis, however these are based on the single article by Horner and Taylor-Robinson (Edwards *et al.* 2014; Pandya *et al.* 2014). Further research is needed to determine the contribution of *M.genitalium* to this condition.

Prostatitis is inflammation of the prostate, which can be acute or chronic. There has been one study of 387 men which found *M.genitalium* to be statistically associated with prostatitis, in which men with prostatitis were compared with asymptomatic men presenting to a STI clinic in Shanghai, China. Prevalence of *M.genitalium* was significantly higher amongst men in the prostatitis group compared with the asymptomatic control group (10.2% vs 2.6%, p=0.005) (Mo *et al.* 2016). Other research in this field has looked into the detection of *M.genitalium* in men with prostatitis, with *M.genitalium* detected in biopsies of patients with chronic idiopathic prostatitis (Krieger *et al.* 1996), and in the semen of men with chronic prostatitis (Mändar *et al.* 2005). This study reported an association between prostatitis and mycoplasmas more broadly (p=0.023) (Mändar *et al.* 2005). Further research is needed to determine the contribution of *M.genitalium* to prostatitis.

Epididymitis is inflammation of the epididymis, usually caused by a bacterial infection. While it is biologically plausible that *M.genitalium* would be a cause of epididymitis (Horner *et al.* 2017b), the evidence has been particularly scant, with no studies yet demonstrating a statistical association

between the two. The first report of *M.genitalium*-associated epididymitis was a case report by Hamasuna, in which *M.genitalium* was the only pathogen identified in a man with epididymitis (Hamasuna 2008). A more recent study by Ito reported that *M.genitalium* accounted for 8.9% of epididymitis amongst 56 men (Ito *et al.* 2012). Aside from these six documented cases of *M.genitalium*-associated epididymitis the evidence is minimal, and much further research is needed to establish a causal relationship.

2.6.4 Male Infertility

A couple are defined as infertile if they are unable to conceive after twelve months of unprotected sexual intercourse during the fertile phases of the menstrual cycle (Evers 2002). There has been limited evidence that demonstrates *M.genitalium* may affect male factor infertility. One study of semen samples from 120 infertile men in Tunisia found that 5.0% of samples contained *M.genitalium*, with *M.genitalium* significantly associated with decreased spermatozoa concentration when compared with infertile male partners without *M.genitalium* ($p = 0.05$) (Gdoura *et al.* 2007). Svenstrup demonstrated in 2003 the ability of *M.genitalium* to bind to spermatozoa (Figure 4.) (Svenstrup *et al.* 2003). When numerous *M.genitalium* cells bound to the sperm, spermatozoon were rendered immotile, which could affect fertility. When fewer cells bound, the spermatozoon remained motile, demonstrating the potential for transmission. Factors relating to female factor infertility are described in Chapter 2.7.5.

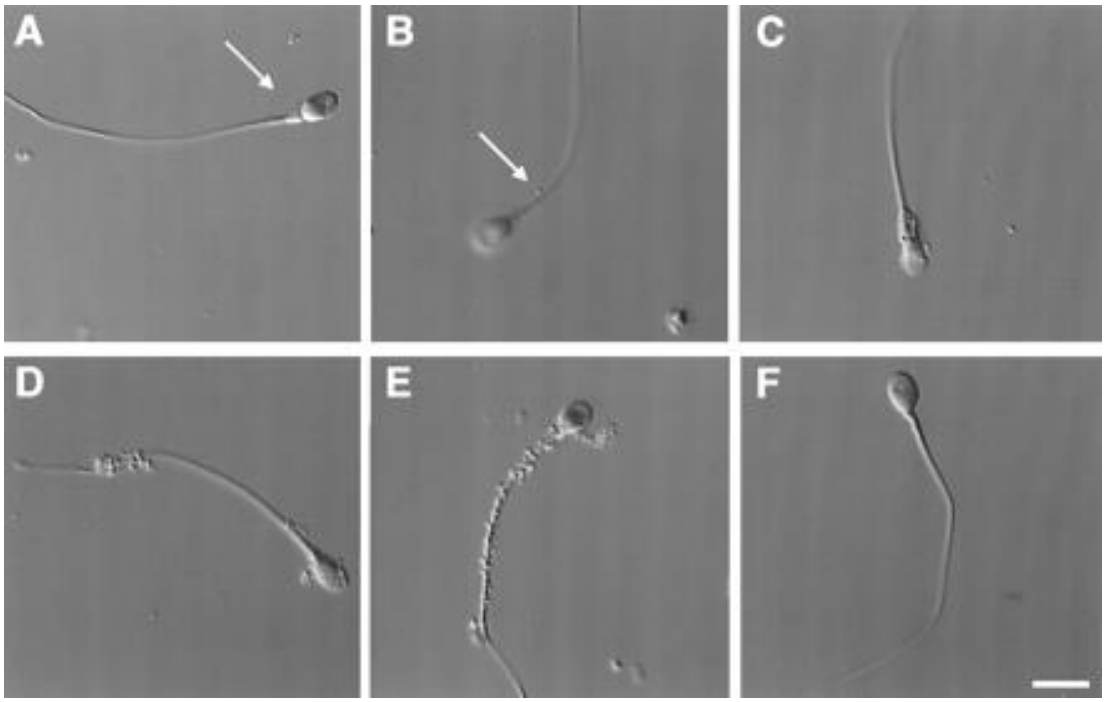

Figure 3. Nomarski microscopy (×100 objective) of sperm incubated in vitro with *M.genitalium*. (Svenstrup *et al.* 2003)

A single cell of *M.genitalium* is attached to (A) the midpiece region (arrow) and (B) the tail (arrow) of spermatozoon. (C) Several cells of *M.genitalium* attached to the head and midpiece. Note the swollen midpiece, which was not a general observation when spermatozoa with adhering mycoplasmas were studied. (D) Microcolonies of *M.genitalium* attached to the distal tail, mid-tail, midpiece and head of the spermatozoon. (E) A massive colonisation of the spermatozoon. Note that *M.genitalium* is also attached to the invisible head-vesicle of the spermatozoon. (F) A negative control of sperm incubated without mycoplasmas. Bar = 5 µm.

Photo from: Svenstrup et al. Hum Reprod, Volume 18, Issue 10, October 2003, Pages 2103–2109, https://doi.org/10.1093/humrep/deg392

2.7 Health Outcomes of *Mycoplasma genitalium* in Women

This chapter will explore the specific syndromes and sequelae that are associated with *M.genitalium* infection in women. These conditions may prompt testing for *M.genitalium* infection, or may be diagnosed subsequently following *M.genitalium* detection.

2.7.1 Cervicitis

Cervicitis is inflammation of the cervix, usually caused by an STI (Solnik 2018). Symptoms can include purulent or mucopurulent vaginal discharge, dysuria, intermenstrual bleeding, or post-coital bleeding (Solnik 2018; CDC 2015). Many women present asymptomatically (Solnik 2018; CDC 2015). Signs of cervicitis detected by a clinician on examination are higher vaginal and cervical PMNL count; increased cervical mucus or pus; cervical motion tenderness; abnormal vaginal discharge and increased cervical bleeding inducibility (Solnik 2018; CDC 2015). Cervicitis increases a woman's risk of developing PID (Lusk *et al.* 2008). It also increases the risk of contracting HIV from an infected partner (Lusk *et al.* 2008).

No definitive criteria exist to ensure a consistent diagnosis of cervicitis internationally (McGowin *et al.* 2011). This has led to difficulties establishing *M.genitalium* as a cause of cervicitis, as results between many studies are incomparable due to the use of varied criteria. The United States Centers for Disease Control and Prevention (CDC) states two major diagnostic signs are used to characterise cervicitis: 1) mucopurulent endocervical exudate noted on examination, and/or 2) inducible endocervical bleeding, whilst swabbing the cervical os (CDC 2015). Many studies of asymptomatic cervicitis rely on the presences of PMNLs only, yet the criteria of increased PMNLs has not been standardised and it therefore not reliable (CDC 2015).

Cervicitis has been detected in up to 50% of women with *M.genitalium* when examined in STI clinic populations (Falk *et al.* 2005). A meta-analysis by Lis *et al.* demonstrated a significant association between *M.genitalium* and cervicitis, with a pooled OR of 1.7 (95%CI: 1.4-2.0) (Figure 5) (Lis *et al.* 2015). This analysis included both studies that defined cervicitis clinically and studies that defined cervicitis by PMNL count (including asymptomatic cervicitis). The review found no difference in the pooled estimate when data was stratified by definition of cervicitis (Lis *et al.* 2015), however the data was not shown.

A review of *M.genitalium* and cervicitis by McGowin *et al*. determined that a high PMNL count (>30 PMNL/hpf) was not a specific sign of *M.genitalium* associated-cervicitis, and diagnosis based on PMNL count may fail to detect less severe inflammation (McGowin *et al.* 2011), with only three out of seven included studies showing a significant correlation between PMNL and

M.genitalium infection (Falk *et al.* 2005; Manhart *et al.* 2003; Moi *et al.* 2009a). This review found that discharge was the most common sign associated with *M.genitalium* and cervicitis (McGowin *et al.* 2011), with half the studies included in the review finding this association (Gaydos *et al.* 2009a; Arráiz *et al.* 2008; Manhart *et al.* 2008; Spence *et al.* 2007).

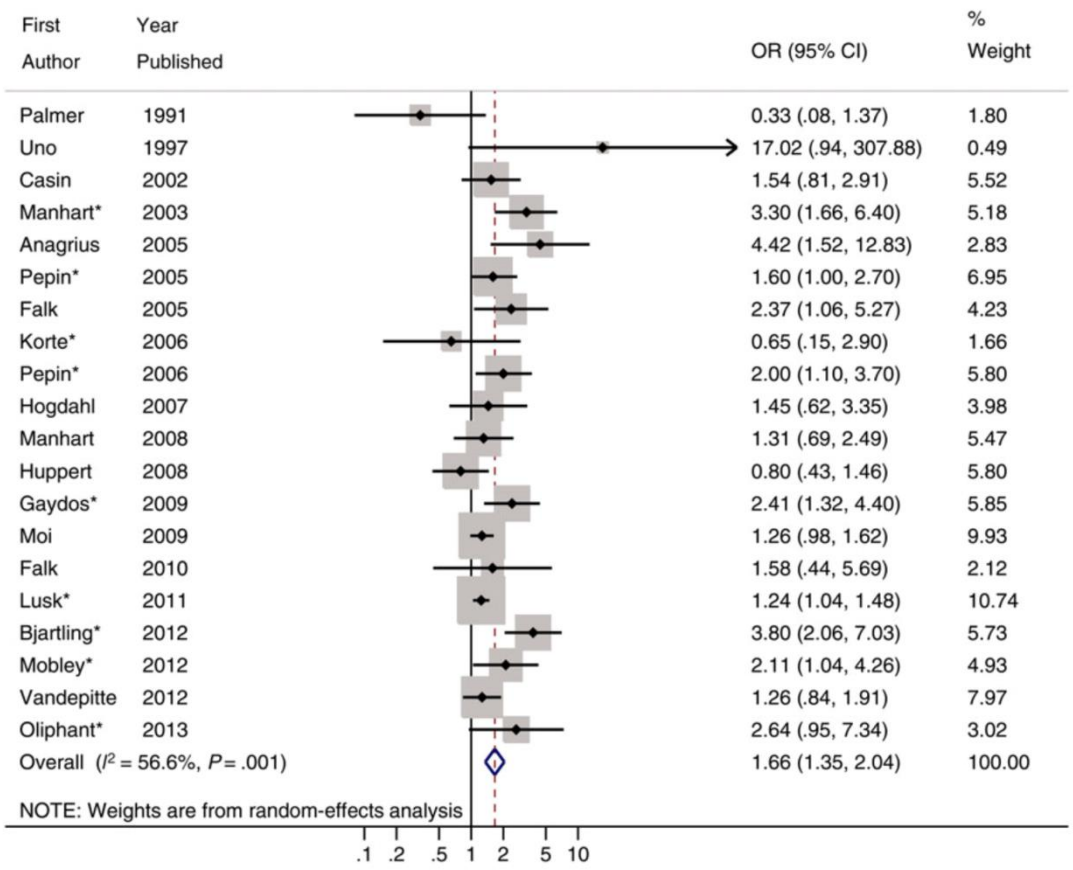

Figure 4. Forest plot of the association between *Mycoplasma genitalium* and cervicitis.
*Adjusted effect estimate (crude effect estimate in all other cases). *Forest plot and text from Lis et al. M.genitalium infection and female reproductive tract disease: a meta-analysis 2015.(Lis et al. 2015)*

2.7.2 Urethritis

As stated above, urethritis is inflammation of the urethra, usually caused by bacterial infection. Symptoms include dysuria, increased urgency, and hesitancy starting urination (Falk *et al.* 2005).

While the association between *M.genitalium* and non-gonococcal urethritis in men has been clearly determined, there are far less studies exploring this association in women. Many of the studies that do examine urethritis in women combine it as an outcome with cervicitis, making it difficult to

interpret the exact proportion of either in the study population being reported on (Anagrius *et al.* 2005; Falk *et al.* 2005). A review conducted in 2011 reported *M.genitalium* was detected in 4-9% of women with urethritis (Manhart *et al.* 2011), however this review only included three studies. Only two out of three studies reported a significant association between *M.genitalium* and urethritis in women (Anagrius *et al.* 2005; Moi *et al.* 2009a), with ORs between 2.1-2.5 (Manhart *et al.* 2011). One of these studies reported that *M.genitalium* was associated with microscopic signs of urethritis (PMNLs) but not symptoms such as dysuria (Anagrius *et al.* 2005). Further research is needed in this area, as there are too limited studies to determine the true association between *M.genitalium* and urethritis in women.

2.7.3 Pelvic Inflammatory Disease

2.7.3.1 Definition

PID encompasses all inflammatory disorders of the female upper genital tract. This includes any combination of endometritis, salpingitis, tubo-ovarian abscess, and pelvic peritonitis (Soper 2010). The primary cause is STIs, most commonly *N.gonorrhoeae* or *C.trachomatis* (Ness *et al.* 2002; Burnett *et al.* 2012).

2.7.3.2 Pathophysiology of Pelvic Inflammatory Disease

PID occurs after the protective cervical barrier is breached by pathogens, which then ascend to the upper genital tract (Weström *et al.* 1993). The cervical barrier is most commonly breached by bacteria following chronic cervical or vaginal infection (Weström *et al.* 1993). They may also ascend through medical interventions, surgical interventions, and the use of instrumentation such as in dilatation and curettage, termination of pregnancy, or insertion of an intrauterine device (Scholes *et al.* 1996). Epithelial damage to the cervix can occur due to *N.gonorrhoeae* or *C.trachomatis* infection, which allows for opportunistic bacteria to ascend (Scholes *et al.* 1996). Once bacteria have breached the cervical barrier they enter the endometrial cavity infecting the uterus, fallopian tubes, and/or ovaries (Dayan 2006).

Inflammation occurs after the upper genital tract is infected, with the degree of inflammation moderated by both microbial and host factors (Weström *et al.* 1993). Scarring may occur, with the amount of scarring dependent upon the degree to which inflammation occurred (Patton *et al.* 1987).

PID symptoms and sequelae are dependent on which structures are affected by inflammation and subsequent scarring (Weström *et al.* 1993; Patton *et al.* 1987). For example, if bacteria only infect the fallopian tubes, scarring may occur at this site and the expected subsequent sequelae will be tubal pathologies.

2.7.3.3 Symptoms and Signs of Pelvic Inflammatory Disease

PID can present with a wide variation of symptoms, which patients may experience to varying degrees. Some women present asymptomatically (with PID only detected upon examination), whilst others require hospital admission due to the severity of their symptoms. Symptoms of PID include lower abdominal pain, abnormal cervical or vaginal discharge (Ross *et al.* 2011; CDC 2015), abnormal bleeding (post-coital bleeding, inter-menstrual bleeding, or menorrhagia) (Jacobson *et al.* 1969), dysuria, chills or high fever, dyspareunia, nausea, and vomiting (CDC 2015; Ross *et al.* 2011).

On examination signs include uterine tenderness, cervical motion tenderness (CDC 2015), and adnexal tenderness (Peipert *et al.* 2001; CDC 2015).

2.7.3.4 Diagnosis of Pelvic Inflammatory Disease

Diagnosis of acute PID is difficult due to the large variation of signs and symptoms in each individual. Laparoscopy is the gold standard for diagnosis, as PID can be asymptomatic (Maleckiene *et al.* 2009; Gump *et al.* 1983). However, laparoscopy is not readily available in many settings, as it requires hospitalisation and is both costly and invasive. Therefore, PID is commonly diagnosed clinically using more subjective clinical criteria, with the sensitivity of clinical diagnosis ranging greatly between 65-90% (Jacobson *et al.* 1969; Peipert *et al.* 1997; Livengood *et al.* 1992).

The following criteria are used to diagnose PID according to the US CDC (CDC 2015):

- Sexually active young women, or other women who are at risk for STI
- Experiencing lower abdominal or pelvic pain
- No other cause for the illness other than PID is identifiable
- One or more of the following is found on examination:
    - Cervical motion tenderness
    - Uterine tenderness
    - Adnexal tenderness

The above criteria are considered diagnostic for PID however the following additional criteria increases the specificity of the diagnosis:

- Oral temperature >38.3° C
- Abnormal cervical mucopurulent discharge
- Cervical contact bleeding
- Presence of abundant numbers of white blood cells on saline microscopy of vaginal fluid

- Elevated erythrocyte sedimentation rate
- Elevated C-reactive protein
- Laboratory documentation of cervical infection with *N.gonorrhoeae* or *C.trachomatis*, through first pass urine or vaginal/cervical swab.

The Melbourne Sexual Health Centre adopted the CDC guidelines to diagnose PID presumptively in women attending their service with pelvic pain (CDC 2015; MSHC 2014).

2.7.3.5 Mycoplasma genitalium associated Pelvic Inflammatory Disease

Moller *et al.* first discovered *M.genitalium* to be associated with PID in 1985, when grivet monkeys and marmosets inoculated with *M.genitalium* developed moderate to severe endosalpingitis (Moller *et al.* 1985). It took much longer for the association between *M.genitalium* and PID to be confirmed in humans. In 2005, a Kenyan study established that *M.genitalium* had the ability to ascend into the fallopian tubes (Cohen *et al.* 2005). This study did not confirm its role in causing tubal pathology, it merely confirmed that *M.genitalium* could invade the upper genital tract in women. In 2010 McGowin *et al.* demonstrated that *M.genitalium* has the ability to ascend not only into the fallopian tubes, but to all of the upper genital tract in mouse models (McGowin *et al.* 2010). It has been suggested that *M.genitalium* can attach to the spermatozoa and can be directly introduced to the upper genital tract through intercourse (Svenstrup *et al.* 2003), in addition to introduction through injury or insult to the cervix as is usually seen in the pathogenesis of PID.

Clinical studies have confirmed the presence of *M.genitalium* in women with suspected PID (Haggerty *et al.* 2006; Simms *et al.* 2003). The Pelvic Inflammatory Disease Evaluation and Clinical Health (PEACH) study, an American multicentre randomised control trial (RCT) which used a stringent definition of PID, reaffirmed this data; 8.0% of women with clinically suspected PID tested positive for *M.genitalium* in endometrial specimens (Haggerty *et al.* 2008). These women had histologically confirmed endometritis at three times the rate of women without *M.genitalium* (Haggerty *et al.* 2008). The PEACH study also indicated that the relationship between *M.genitalium* and PID is independent and causal (Cohen *et al.* 2002).

In further analysis of the PEACH study, *M.genitalium* was found to account for 14% of non-*N.gonorrhoeae* non-*C.trachomatis* PID (Haggerty *et al.* 2006). *C.trachomatis* accounted for 13.5% of all cases of PID, and *N.gonorrhoeae* accounted for 14.7% of all cases (Ness *et al.* 2002). This association was further confirmed by a study of non-*C.trachomatis* adnexitis in which 4.1% of women with the condition tested positive for *M.genitalium*, compared to zero percent of the control group (Uno *et al.* 1997).

From research starting in 1985, *M.genitalium* was finally accepted as a cause of PID following a 2015 meta-analysis by Lis *et al.* as mentioned above (Simms *et al.* 2003; Lis *et al.* 2015). This analysis showed that *M.genitalium* was associated with a two-fold increased risk of developing PID [pooled OR of 2.1 (95%CI: 1.3-3.5)](Figure 6) (Lis *et al.* 2015). A study by Horner *et al.* collated data from multiple studies and used mathematical modelling to estimate that 4.9% (95%CI: 0.4–14.1) of *M.genitalium* infections progress to PID, compared with 14.4% (95%CI: 5.9–24.6) of *C.trachomatis* infections (Lewis *et al.* 2020). They estimated that in 2529 women included in the Prevention of Pelvic Infection (POPI) trial, a RCT designed to assess the incidence of clinical PID over 12 months (Oakeshott *et al.* 2010b), 9.4% (95%CI: 0.8–28.8) of PID was attributable to *M.genitalium* infection, compared to 37.4% (95%CI: 14.9–63.9) attributable to *C.trachomatis* (Lewis *et al.* 2020). The POPI study found that in sexually active female students in London (n=2529), the incidence of PID amongst women with *M.genitalium* was 3.9%, compared to 1.7% in those without the infection (Oakeshott *et al.* 2010a).

Importantly, a Danish study found untreated *M.genitalium* was associated with a six-fold increased risk of PID following the termination of pregnancy (OR 6.3; 95%CI: 1.6–25.2) (Bjartling *et al.* 2010). This is an important consideration in antibiotic treatment prior to termination of pregnancy, given 8.7% of women presenting for this procedure in New Zealand were found to have *M.genitalium* (Lawton *et al.* 2008).

2.7.3.6 Mycoplasma genitalium Associated Pelvic Inflammatory Disease Compared with Pelvic Inflammatory Disease of Other Causes

There are limited studies examining the clinical symptoms and signs of *M.genitalium*-PID specifically. *M.genitalium* has been shown to cause asymptomatic infection (Williams *et al.* 2007), however whether that can lead to asymptomatic PID as seen in *C.trachomatis* infections remains to be seen (Lan *et al.* 1995; Gump *et al.* 1983). In one study of *M.genitalium* and acute endometritis, common symptoms included mild abdominal pain and easily induced cervical bleeding (Cohen *et al.* 2002). There is little else published on symptoms and signs specifically regarding *M.genitalium*-PID.

There is also little published comparing *M.genitalium*-PID to PID of other causes. There is one American study by Short *et al.*, which utilised the PEACH study data, directly comparing *M.genitalium*-PID to other causes of PID. This study assessed oral temperature, WBC count, erythrocyte sedimentation rate, C-reactive protein level, bilateral adnexal tenderness, mucopurulent cervicitis, and bacterial vaginosis (Short *et al.* 2009). Signs and symptoms of nausea, abnormal bleeding, abnormal discharge, increased frequency of urination, and pelvic pain score were also

collected. *N.gonorrhoeae*-PID was associated with elevated markers of inflammation, mucopurulent cervicitis, elevated vaginal pH, and a high pelvic pain score. *M.genitalium*-PID and *C.trachomatis*-PID were suggested to have similar symptoms in this study, with no significant differences between the two noted (Short *et al.* 2009).

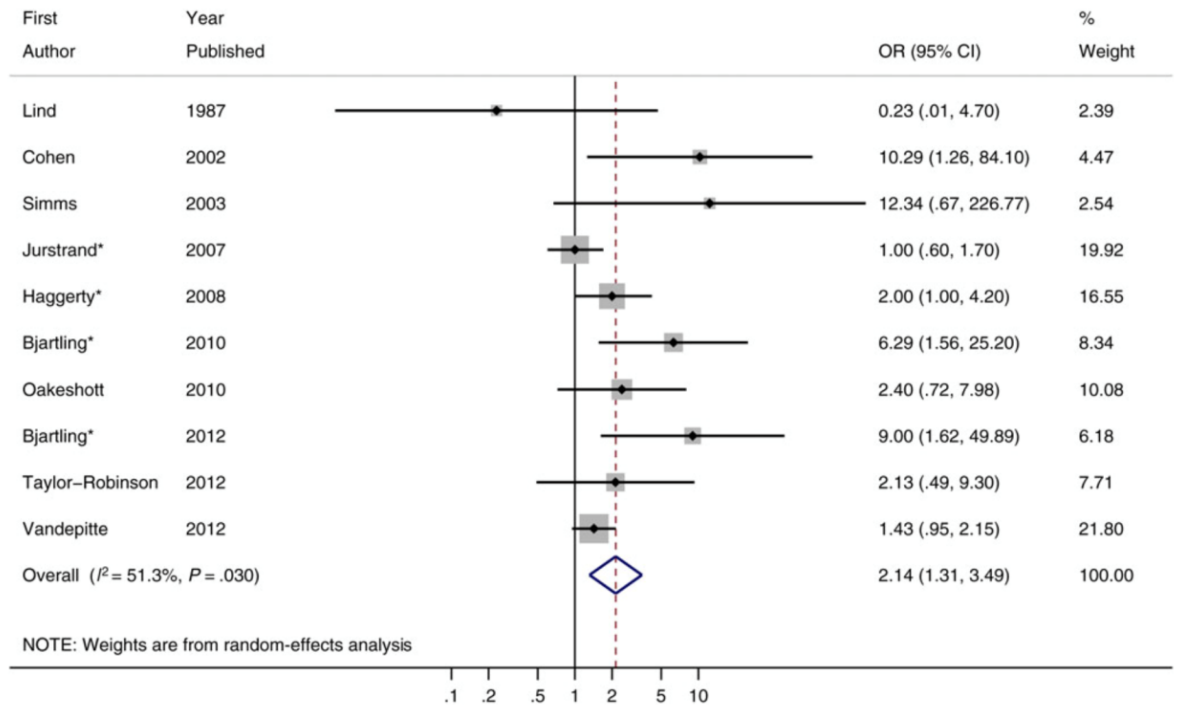

Figure 5. Forest plot of the association between *Mycoplasma genitalium* and pelvic inflammatory disease.

*Adjusted effect estimate (crude effect estimate in all other cases). *Forest plot and text from Lis et al. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis 2015*(Lis *et al.* 2015)

2.7.3.7 Complications of Pelvic Inflammatory Disease

It is important to diagnose and treat PID early due to the severity of sequelae. PID is associated with the following complications:

- Recurrent PID (Ness *et al.* 2002)
- Chronic pelvic pain (Ness *et al.* 2002): pain in the pelvic region lasting six months or longer.
- Infertility (Westrom *et al.* 1992): a couple who after twelve months of regular unprotected sexual intercourse during the fertile phase of the females menstrual cycle, have been unable to conceive.

- Ectopic pregnancy (Westrom *et al.* 1992): pregnancy developing outside of the uterus, typically in a fallopian tube.
- Tubo-ovarian abscess (Chappell *et al.* 2012): an inflammatory mass involving the fallopian tube, ovary and other adjacent pelvic organs, manifesting as an agglutination of those structures or as a collection of pus.
- Fitz-Hugh-Curtis syndrome (rare) (Litt *et al.* 1978): inflammation of the peritoneum and Glisson's capsule, causing perihepatitis. This can lead to the formation of adhesions between the peritoneum and the liver.

Complications such as ectopic pregnancy are potentially life threatening, and highlight the importance of diagnosis and treatment of PID.

Many patients have long-term sequelae following PID regardless of successful treatment of the causative pathogen. Nearly 45% of patients have been reported to go on to experience chronic pelvic pain, 20% infertility, and 2% ectopic pregnancy (Figure 7) (Ness *et al.* 2002; Ross 2014). Women with recurrent PID have even higher rates of complications than those with successfully treated PID. This is particularly relevant for *M.genitalium*-PID, as nearly half of the women are not cured of the disease following the current PID treatment guidelines (see Chapter 2.8.2.3) (Haggerty *et al.* 2008).

Women who have recurrent PID are 1.8 times more likely to report infertility. However there have been no reported differences between live births (birth outcomes) in adult women with recurrent PID compared to those who have been cured. Women with recurrent PID are 4.2 times more likely to report chronic pelvic pain (Ross 2014; Trent *et al.* 2011). In American adolescents, women aged nineteen and younger, there were no significant statistical differences in pregnancy, live birth and infertility at baseline based on recurrent PID status. However they are 5.0 times more likely to report chronic pelvic pain (Figure 8) (Ness *et al.* 2002; Trent *et al.* 2011).

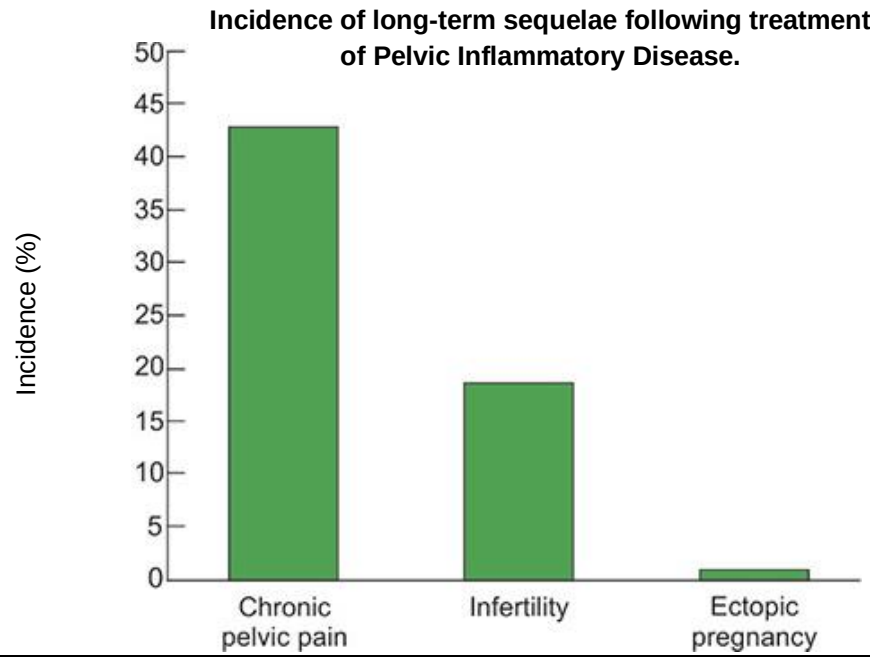

Figure 6. Incidence of long-term sequelae following treatment of Pelvic Inflammatory Disease.

From Ross JD, Pelvic inflammatory disease, Medicine: Elsevier; 2014(Ross 2014)

2.7.4 Birth Outcomes

There are very limited and conflicting studies that discuss birth outcomes in women with *M.genitalium.* Adverse birth outcomes include but are not limited to: preterm birth, spontaneous abortion (miscarriage), stillbirth, and ectopic pregnancy.

The 2015 meta-analysis by Lis *et al.* concluded there was a significant association between *M.genitalium* and preterm birth [OR 1.9 (95%CI: 1.3-2.9), and *M.genitalium* and spontaneous abortion [OR 1.8 (95%CI: 1.0-3.0)] (Lis *et al.* 2015). Lis drew from a very limited pool of sources with only 3 studies included on spontaneous abortion (Figure 8).

While studies have indicated a significant association between *M.genitalium* and preterm birth (Hitti *et al.* 2010), other studies examining *M.genitalium* in this population have concluded the prevalence of *M.genitalium* is so low, it is unlikely to be a significant contributing factor to this condition (Lu *et al.* 2001). This finding was mirrored in a study of *M.genitalium* and miscarriage, which failed to detected *M.genitalium* in any of the women enrolled in the study (Contini *et al.* 2019). A review article by Donders *et al.* concluded the prevalence of *M.genitalium* amongst pregnant women was approximately 0.5-1.0% (Donders *et al.* 2017).

Lis's meta-analysis was unable to further examine the outcomes of stillbirth or ectopic pregnancy due to limited data. There have only been two studies which have examined the association between

M.genitalium and stillbirth, with neither detecting an association (Labbe *et al.* 2002; Short *et al.* 2010). Both of these studies also examined whether *M.genitalium* was associated with premature birth, and did not detect an association (Labbe *et al.* 2002; Short *et al.* 2010).

There have been two studies examining the association between *M.genitalium* and ectopic pregnancy. In a Saudi Arabian study of tubal specimens from women undergoing a total abdominal hysterectomy, a tubectomy, or women who had had an ectopic pregnancy (n=135), *M.genitalium* was significantly more common among women who had ectopic pregnancies, compared to women who were undergoing a total abdominal hysterectomy, [COR 6.2 (95%CI: 2.3-18.1, p=0.009] (Ashshi *et al.* 2015). A Swedish study detected *M.genitalium* in 18.8% (15/82) women with ectopic pregnancy, but *M.genitalium* was not significantly associated with ectopic pregnancy (p=0.429) (Jurstrand *et al.* 2007). The data on the contribution of *M.genitalium* to ectopic pregnancy in women remains conflicting, however given ectopic pregnancy can be a complication of PID, it remains biologically plausible that *M.genitalium* may contribute to ectopic pregnancy. Further research is required to examine the association between ectopic pregnancy and *M.genitalium*, which is particularly important due to the life-threatening nature of this condition.

2.7.5 Infertility

As stated above in cha 2.8.4, infertility is defined as being present in a couple who after twelve months of regular unprotected sexual intercourse during the fertile phases of the menstrual cycle, but are unable to conceive (Evers 2002). The following studies discuss female factor infertility.

The literature has been conflicting concerning the association between tubal factor infertility and *M.genitalium*. Many past studies used serology to assess the role of *M.genitalium* in tubal factor infertility, which as mentioned previously is neither accurate nor specific: this makes it hard to study the associations with rigor.

According to the meta-analysis by Lis *et al.*, the pooled OR for *M.genitalium*-associated infertility was 3.4 (95%CI: 0.9-6.3) (Figure 9) (Lis *et al.* 2015). Although this finding was not statistically significant, it indicates a trend toward reproductive morbidity (Haggerty *et al.* 2011).

One Danish study found *M.genitalium* IgG antibodies are more likely to be found in women with tubal factor infertility (Svenstrup *et al.* 2008), whilst another study in Sweden found the opposite to be true (Idahl *et al.* 2015). *M.genitalium* IgG antibodies were more prevalent in infertile couples than couples able to spontaneously conceive (Idahl *et al.* 2015). MgPa, the major adhesin protein of *M.genitalium*, has been detected more frequently in the serum of women with tubal factor infertility than women without it (Clausen *et al.* 2001).

The data remains conflicting on the contribution of *M.genitalium* to infertility in women, however given *M.genitalium* is associated with PID, it remains plausible that *M.genitalium* may contribute to infertility in women. PID is a known cause of infertility, as the inflammation can lead to scarring of the fallopian tubes (Westrom *et al.* 1992). Further research is required to examine the association between infertility and *M.genitalium* in women.

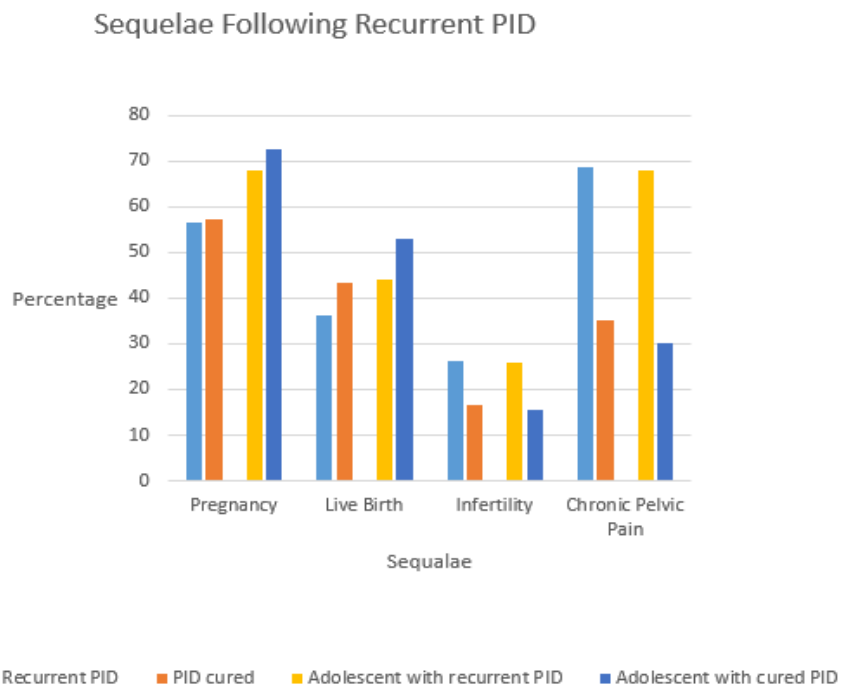

Figure 8. Sequelae following recurrent pelvic inflammatory disease.
Percentage of women experiencing sequelae following recurrent PID versus percentage of women experiencing sequelae following cured PID. *From information in the PEACH study 2002 (Ness et al. 2002) and Trent et al. Adverse adolescent reproductive health outcomes after pelvic inflammatory disease. Arch Pediatr Adolesc Med. 2011(Trent et al. 2011)*

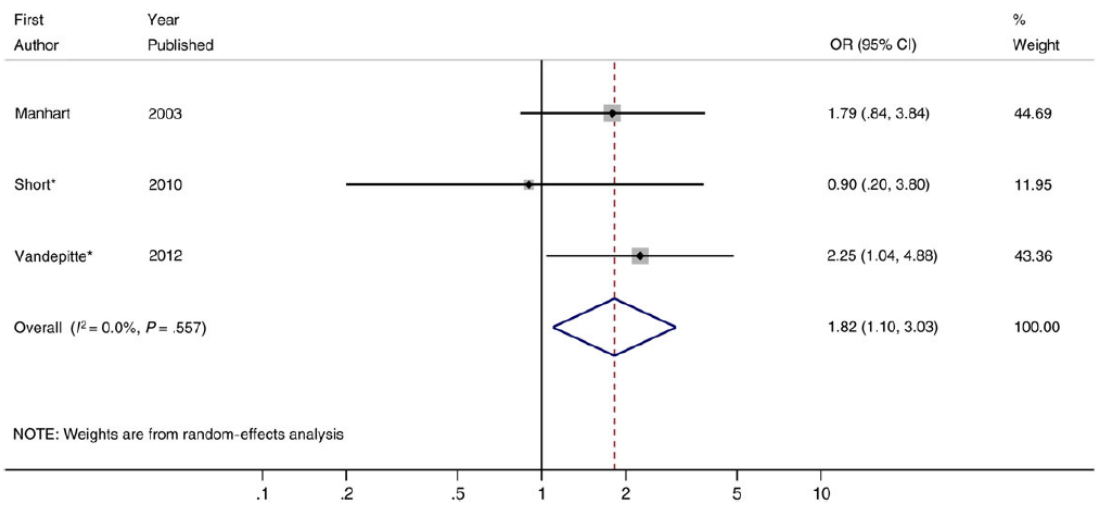

Figure 7. Forest plot of the association between *Mycoplasma genitalium* and spontaneous abortion.

*Adjusted effect estimate (crude effect estimate in all other cases). *Forest plot and text from Lis et al. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis 2015.* (Lis *et al.* 2015)

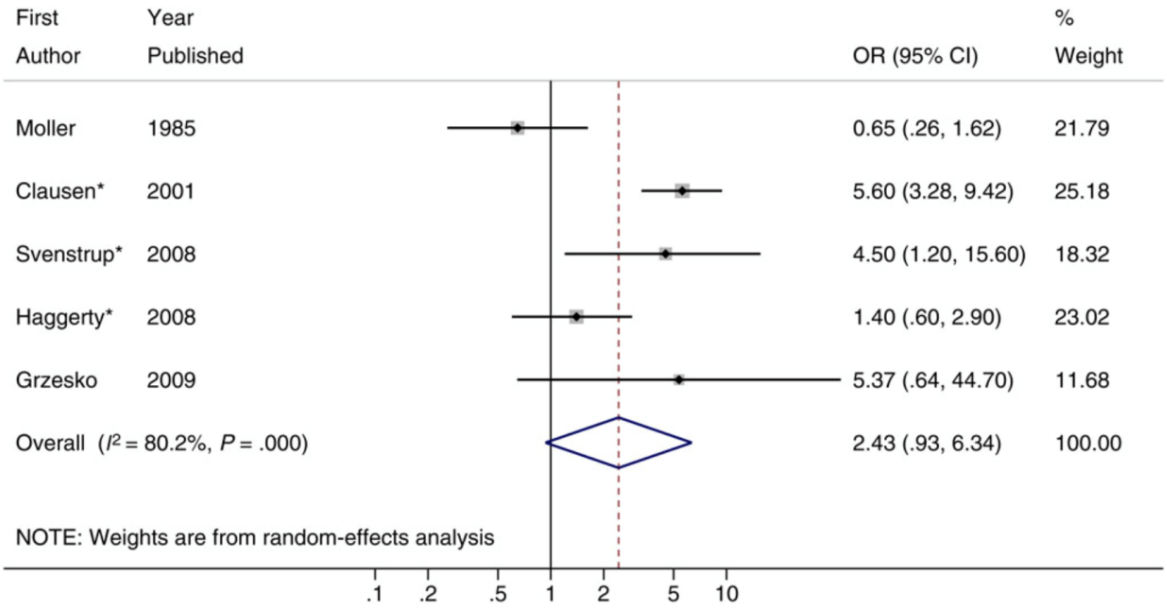

Figure 8. Forest plot of the association between *Mycoplasma genitalium* and female infertility.

*Adjusted effect estimate (crude effect estimate in all other cases). *Forest plot and text from Lis et al. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis 2015.* (Lis *et al.* 2015)

2.8 Treatment of *Mycoplasma genitalium*

2.8.1 Effective Antimicrobials

M.genitalium is treated with broad-spectrum antimicrobials, however treatment is increasingly complicated due to widespread antimicrobial resistance. As discussed in chapter 2.1 *M.genitalium* has no peptidoglycan wall, which limits the cellular targets for antimicrobials. Antibiotic targets for *M.genitalium* include antibiotics that inhibit protein synthesis and antibiotics that inhibit nucleic acid replication. Classes of antibiotics that *M.genitalium* has been shown to be susceptible to include tetracyclines, macrolides, and extended spectrum fluoroquinolones (Hamasuna *et al.* 2005; Hamasuna *et al.* 2009; Hannan 1998; Plecko *et al.* 2014). Other potential classes of antibiotics include ketolides, lincosamides, streptogramins and phenicols, but as these are rarely employed in standard clinical care and as such will not be discussed further. Beta-lactam antibiotics (including penicillins, cephalosporins, carbepenams and monobactams) are commonly employed in clinical care for common bacterial infections, but as these work at the cell wall of bacteria they are all ineffective against *M.genitalium* (Taylor-Robinson *et al.* 1997).

The World Health Organisation (WHO) recommends any drugs used in the treatment of STIs have an efficacy of 95% or greater (WHO 2003), however the majority of antimicrobials for *M.genitalium* have fallen short of this cure rate. Resistance to the drug classes most widely used against *M.genitalium* has occurred and is increasing. Transmission of *M.genitalium* mutations, which confer resistance, is discussed in chapter 2.1. The future may require tailoring treatment according to specific *M.genitalium* mutations that render certain antibiotics ineffective (Nijhuis *et al.* 2015). This chapter will discuss the antimicrobials most commonly used to treat *M.genitalium*, as well as *M.genitalium* treatment guidelines.

2.8.1.1 Tetracyclines

When *M.genitalium* was discovered, it was established as a cause of non-gonococcal urethritis in men, as discussed above. The recommended treatment for non-gonococcal urethritis is doxycycline, a member of the tetracycline family. Doxycycline was therefore the first antibiotic assessed for efficacy in treating *M.genitalium.* Doxycycline works biostatically by binding with ribosomes and inhibiting bacterial protein synthesis (Amin *et al.* 1996). Initial assessment of tetracycline efficacy against *M.genitalium* was promising, with ability proven *in vitro* (Bebear *et al.* 2000; Duffy *et al.* 2000; Renaudin *et al.* 1992). The minimum inhibitory concentrations represent the lowest concentration (in micrograms/millilitre) of an antibiotic that inhibits the growth of a given strain of bacteria, with sensitivity or susceptibility implying that a bacterium is inhibited by a drug at its usual given dose (IDEXX Lab 2017). Initial studies found that tetracyclines were effective

treatment for *M.genitalium*, with minimum inhibitory concentrations in the susceptible range (Bebear *et al.* 2000; Duffy *et al.* 2000; Renaudin *et al.* 1992). However, these initial studies only looked at the G37 strain of *M.genitalium*. When other strains were examined, *in vitro* efficacy declined and *M.genitalium* showed resistance to doxycycline (Hamasuna *et al.* 2005; Hannan 1998).

Clinical trials also showed low efficacy *in vivo*. Manhart's 2011 RCT stated that the pooled microbiological cure rate of the efficacy of doxycycline for *M.genitalium* was 41.5%, compared to 79.6% with azithromycin (Manhart *et al.* 2011). Prior to the emergence of macrolide resistance, a 1g stat dose of azithromycin was significantly more effective than a 7-day twice daily dose of doxycycline (Bjornelius *et al.* 2008; Manhart *et al.* 2011). Doxycycline is therefore not recommended for treatment of *M.genitalium*. While not curative, doxycycline does lower the bacterial load of *M.genitalium*, which may render *M.genitalium* more susceptible to treatment with subsequent antimicrobials (Read *et al.* 2017a; Read *et al.* 2018; Guschin *et al.* 2015; Read *et al.* 2019b).

2.8.1.2 Macrolides

Azithromycin has been recommended as first-line treatment for non-gonococcal urethritis, and as stated above, was proven to be more effective than doxycycline in the treatment of *M.genitalium*. Early i*n vitro* analysis confirmed azithromycin to be exceptionally promising as a treatment for *M.genitalium* (Duffy *et al.* 2000; Hamasuna *et al.* 2005), with no cases of treatment failure (Hamasuna *et al.* 2005). Azithromycin was implemented as a treatment for *M.genitalium* following these studies, and is recommended in national and international guidelines as first line therapy for *M.genitalium* (ASHA 2018; CDC 2015). Azithromycin works biostatically like doxycycline, interfering with protein synthesis by binding to the 50S subunit of the bacterial ribosome and thus inhibiting translation of messenger-RNA (Retsema *et al.* 1987).

In 2008, *M.genitalium*-associated cervicitis had a cure rate of 90.5% with azithromycin (Terada *et al.* 2012). Longer courses of treatment were shown in some Scandinavian studies to have a higher microbiological cure rate than treatment regimens of a 1g stat dose (Bjornelius *et al.* 2008). However there has been increasing resistance to macrolides, with 100% of treatment failures containing macrolide resistant mutations (Anagrius *et al.* 2011). Single nucleotide polymorphisms in domain V of the 23S rRNA gene of *M.genitalium* confer high-level macrolide resistance (Chrisment *et al.* 2012). A review performed in 2015 gave a pooled efficacy of 77.2% for azithromycin in urogenital *M.genitalium* (Lau *et al.* 2015) (Figure 10). A subgroup analysis showed that the overall efficacy decreased from 85.3% in studies conducted prior to 2009 to 67.0% in studies conducted since 2009 (Lau *et al.* 2015). A recent meta-analysis by Machalek *et al.* published

in 2020 also examined the prevalence of macrolide resistance mutations over time. Before 2010 the overall summary prevalence of mutations associated with macrolide resistance was 10.0% (95%CI: 2.6–20.1); from 2016-2017 it had increased to 51.4% (40.3–62.4) (p<0.0001) (Machalek *et al.* 2020). Changes in macrolide resistance varied by geographical region, with the greatest increases in mutation prevalence over time noted in Australia, from 18.8% before 2010, to 66.0% in 2016–17 (p<0.0001) (Machalek *et al.* 2020). Since this meta-analysis, even higher rates of resistance have been reported, with macrolide resistance rates as high as 90% reported in 2019 amongst MSM in Australia (McIver *et al.* 2019). Although azithromycin was initially highly effective in the treatment of *M.genitalium*, it is clear this efficacy is declining over time.

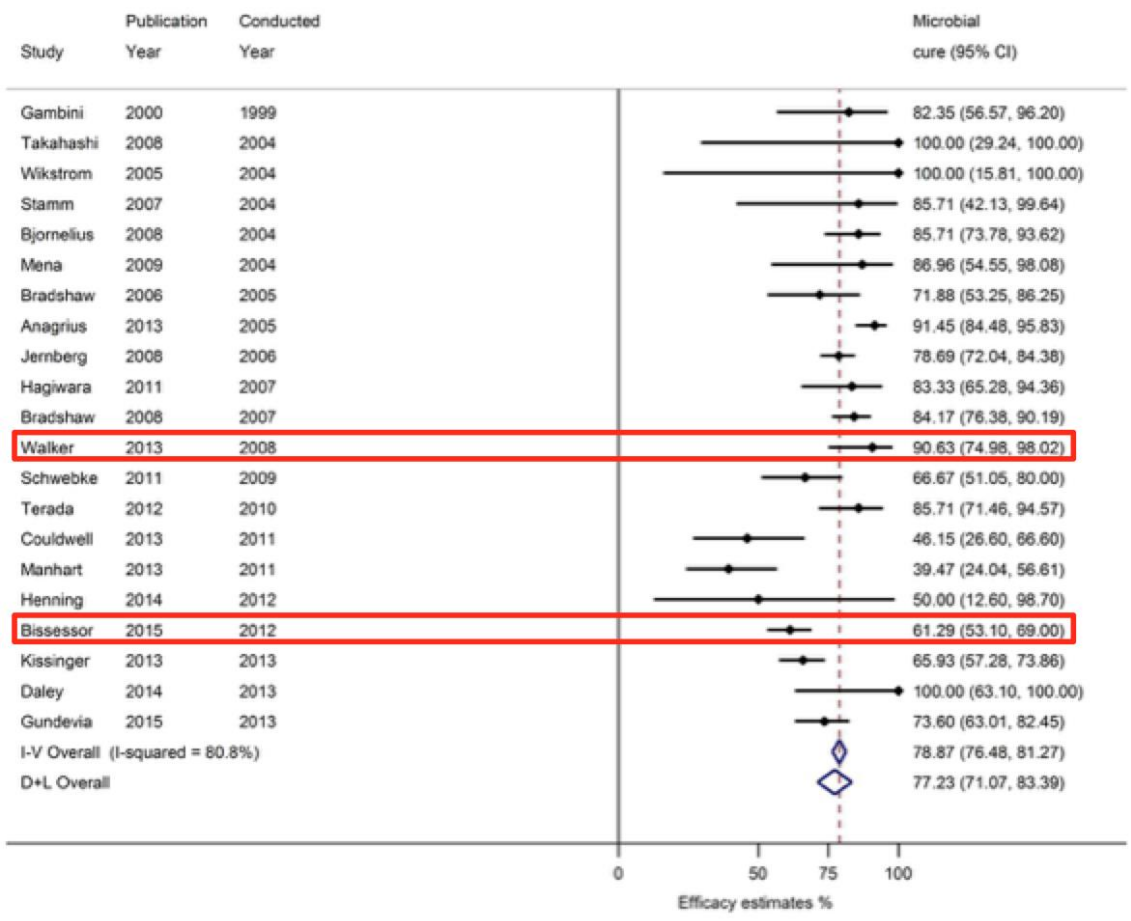

Figure 9. Microbial cure following treatment with one gram of azithromycin for urogenital *Mycoplasma genitalium* infection*(Lau et al. 2015).*

Studies performed in Melbourne are highlighted. *Forest plot from Lau et al. The Efficacy of Azithromycin for the Treatment of Genital Mycoplasma genitalium 2015. (Walker et al. 2013; Bissessor et al. 2015).*

Macrolide resistant mutations have also been detected in cases of azithromycin treatment failure that were not present prior to treatment, i.e. the mutation occurred as a direct consequence of treatment (Ito *et al.* 2011). Treatment of *M.genitalium* with 1g azithromycin has been shown to select for macrolide resistance in at least 12% of *M.genitalium* infections (Read *et al.* 2017a; Bissessor *et al.* 2015). *M.genitalium* load may contribute to macrolide resistance; patients with a lower pre-treatment load appear more effectively treated with macrolide antibiotics than those with a high pre-treatment load (Guschin *et al.* 2015; Walker *et al.* 2013).

Collectively, this data shows that despite being retained as first line therapy for *M.genitalium* by the majority of international guidelines, azithromycin is no longer a highly effective treatment for *M.genitalium* in many countries (Bissessor *et al.* 2015; Tagg *et al.* 2013).

2.8.1.3 Fluoroquinolones

Fluoroquinolones have been a more recent addition to *M.genitalium* treatment regimens, initially used as highly effective second line agents for treatment failures of azithromycin-treated non-gonococcal urethritis in 2006. Fluoroquinolones exhibit concentration-dependent bactericidal activity by inhibiting the activity of DNA gyrase and topoisomerase, enzymes essential for bacterial DNA replication (Hooper 1995). *In vitro* studies indicate *M.genitalium* has a reduced susceptibility to older generation fluoroquinolones, examples being ofloxacin and ciprofloxacin, compared to newer fourth generation fluoroquinolones (Renaudin *et al.* 1992), such as moxifloxacin. In clinical studies of the fluoroquinolones, moxifloxacin is the most effective against *M.genitalium* to date (Manhart *et al.* 2011). There have been no RCTs performed evaluating moxifloxacin as a therapy for *M.genitalium.* Moxifloxacin was reported to have a 100% microbiological cure rate when initially used to treat *M.genitalium* in 2006 (Jernberg *et al.* 2008).

Although 100% effective, moxifloxacin was not universally deployed due to the side effect profile of fluoroquinolones. Newer generation fluroquinolones have been associated with severe anaphylaxis, QTc-interval prolongation, cardiotoxicity, central nervous system side effects, and tendon rupture, in addition to the side effects usually associated with antibiotics such as diarrhoea, nausea and vomiting (Bertino *et al.* 2000; Sarro *et al.* 2001). Severe side effects of moxifloxacin specifically include peripheral neuropathy, hepatitis, *torsades de pointes*, *Clostridium difficile*-associated disease, spontaneous tendon rupture, and tendonitis (Carbon 2001). Although these side effects are rare, they are an important consideration for clinicians, when considering the cost-benefit of treatment using fluoroquinolones. Out of all the quinolones, moxifloxacin has one of the lowest risks of central nervous system side effects (Carbon 2001).

Unfortunately, fluoroquinolone resistance also appears to be emerging in the Asia-Pacific region. The first cases of fluoroquinolone failure in the treatment of *M.genitalium* were reported in Sydney in 2013 (Couldwell *et al.* 2013). The aforementioned meta-analysis by Machalek *et al.* found the summary prevalence of mutations associated with fluoroquinolone resistance to be 7.7% (95%CI: 4.5–11.4), however unlike with macrolides, they did not find the prevalence of mutations to change significantly over time (Machalek *et al.* 2020). While azithromycin has commonly been utilised internationally in the treatment of STIs, the detection of mutations and failures of fluoroquinolones is a concern given the lack of local evolutionary pressure: fluoroquinolones are not first line treatment for any STI and they are not widely used for the treatment of other infections due to their side effect profile (Couldwell *et al.* 2013). In the meta-analysis, the highest prevalence of fluoroquinolone resistance mutations occurred in Japan (29%), the region where fluoroquinolones have been used most commonly in the treatment of *M.genitalium* and non-gonococcal urethritis (Machalek *et al.* 2020; Hamasuna *et al.* 2018). This suggests that with increasing use, fluoroquinolones may face a decline in efficacy over time comparable to macrolides. In cases of treatment failure, all have had *M.genitalium* strains with fluoroquinolone associated resistant mutations detected (Bissessor *et al.* 2015).

In 2013, the first strains of *M.genitalium* harbouring both macrolide and fluoroquinolone resistant mutations were noted in Japan (Kikuchi *et al.* 2014). Machalek's meta-analysis found the summary prevalence of dual resistance was 2.8% (95%CI: 1.3–4.7), which did not change significantly over time (Machalek *et al.* 2020). In Japan, where prevalence of dual class resistance was highest, the prevalence increased from 0.0% before 2010 to 25.6% in 2016–17, although this increase was not statistically significant (p=0.22), likely due to small numbers of studies (Machalek *et al.* 2020).

Sitafloxacin, an extended spectrum fluoroquinolone, has recently been assessed as an alternative agent to *M.genitalium* strains harbouring resistance to other fluoroquinolones, and was shown to be highly effective (Deguchi *et al.* 2015).

While resistance increases, moxifloxacin is still highly effective in Europe and Scandinavia (Manhart *et al.* 2011), as the region with the lowest prevalence of mutations associated with resistance noted in Machalek's analysis (2.8%) (Machalek *et al.* 2020). It has the highest proven efficacy of any antibiotic for the treatment of *M.genitalium* in these regions and the Asia-Pacific region, in spite of resistance and side-effect profile, and will continue to be of use in the treatment of *M.genitalium* for some time to come.

2.8.2 Treatment guidelines

2.8.2.1 Treatment of Mycoplasma genitalium

The Australian STI Management Guidelines recommend the therapy for *M.genitalium* as outlined in Table 1(ASHA 2018). They advise when there is no access to resistance testing, it is reasonable to assume macrolide resistance when infections persist after azithromycin treatment, or in patients who are MSM. Both the American and British STI guidelines do not contain direction for *M.genitalium* detection, but contain information for *M.genitalium* detection within the context of clinical syndromes i.e. *M.genitalium*-associated urethritis, *M.genitalium*-associated cervicitis and *M.genitalium*-PID. These will be discussed in subsequent chapters.

2.8.2.2 Treatment of Mycoplasma genitalium associated urethritis and cervicitis

The Australian STI Management Guidelines do not contain specific direction around the treatment of *M.genitalium*-associated urethritis or *M.genitalium*-associated cervicitis. The British and US guidelines for treatment of uncomplicated urogenital infection are discussed in Table 2.

2.8.2.3 Treatment of Pelvic Inflammatory Disease

PID is treated presumptively, often prior to the pathogen being identified, due to the risk of sequalae outlined in chapter 2.9.3.6. If the CDC criteria outlined in chapter 2.9.3.4 are fulfilled, PID is diagnosed and treated. The Australian STI Management Guidelines (ASHA 2016) and the Australian Therapeutic Guidelines (Therapeuitc Guidelines eTG Nov 2014) recommend the combination of antimicrobials outlined in Table 3, which target the most common aetiological agents of PID: *C.trachomatis, N.gonorrhoeae* and anaerobes.

The Australian STI Management Guidelines recommend that patients avoid sexual intercourse for a week following treatment and to engage in contact tracing i.e. notification of previous sexual partners (ASHA 2016). This is not a legal requirement in Australia.

Current international guidelines for the presumptive treatment of PID do not contain an agent that is highly effective against *M.genitalium* (Table 4). Many of the antibiotics recommended are beta-lactams, which target the bacterial cell wall. As stated in chapter 2.1, *M.genitalium* is a mollicute which lack a bacterial cell wall, rendering these antibiotics ineffective.

Table 1. Recommended antibiotic regimens for the treatment of *Mycoplasma genitalium*

Table adapted from The Australian STI Management Guidelines 2016. (ASHA 2016)

Situation	Recommended treatment
***M.genitalium* that is known to be macrolide-susceptible**	Doxycycline 100mg orally (PO), BD for 7 days *Followed by* Azithromycin 1g stat PO, then 500mg daily for 3 days *OR* Doxycycline 100mg PO, BD for 7 days *Followed by* Azithromycin 1g stat PO
***M.genitalium* that is known or suspected to be macrolide-resistance**	Doxycycline 100mg PO, BD for 7 days *Followed by* Moxifloxacin 400mg for 7 days

Abbreviations: STI= sexually transmitted infection, mg=milligrams, PO=orally, BD= twice a day, g=grams

Table 2. Recommended antibiotic regimens for the treatment of *Mycoplasma genitalium* associated urethritis or cervicitis

Table adapted from BASHH STI Management Guidelines 2018 and Centers for Disease Control and Prevention treatment guidelines (CDC 2015; BASHH et al. 2018)

Situation	Recommended treatment- British Guidelines(BASHH *et al.* 2018)	Recommended treatment- US guidelines(CDC 2015)
***M.genitalium* that is known to be macrolide-susceptible**	Doxycycline 100mg PO, BD for 7 days *Followed by* Azithromycin 1g stat PO, then 500mg daily for 2 days	Azithromycin 500mg stat PO *Followed by* Azithromycin 250mg for 4 days
***M.genitalium* that is known to be macrolide-resistance**	Moxifloxacin 400mg PO for 10 days	Moxifloxacin 400mg PO for 7 to 10 or 14 days

Abbreviations: STI= sexually transmitted infection, mg=milligrams, PO=orally, BD= twice a day, g=grams

Data derived from the PEACH study (Ness *et al.* 2002) showed that when clinicians follow current PID treatment guidelines, 43.8% of women with *M.genitalium*-PID will test positive for *M.genitalium* following thirty days of treatment (Haggerty *et al.* 2008). In other studies, *M.genitalium* has been associated with persistent infection (up to 3 months in approximately 47.7% of women) despite the high prevalence of antibiotic use (Cohen *et al.* 2006). This is an important finding, as *M.genitalium* is not eradicated in half the women with *M.genitalium*-PID despite being treated in accordance with current treatment guidelines.

Until recently there have been no international guidelines for *M.genitalium*-PID. The Australian, UK and US guidelines now recommend considering *M.genitalium* in PID cases that do not respond to antibiotic treatment in 7-10 days (CDC 2015). A regimen of 400mg/day of moxifloxacin for 14 days has been recommended, based upon expert opinion (CDC 2015).

At present there are no studies directly examining the efficacy of antibiotics against *M.genitalium* in PID, with no RCT, and expert opinion guiding the Melbourne Sexual Health Centre and CDC antimicrobial recommendations. There has been one RCT of moxifloxacin for uncomplicated PID which showed moxifloxacin to be highly effective in uncomplicated PID of any cause, though the causes were *C.trachomatis* (n=47) and *N.gonorrhoeae* (n=35). Clinical resolution of PID occurred in 90.2% of women treated with moxifloxacin, and a negative test of cure occurred in 87.5% (Ross *et al.* 2006). This provided good evidence that moxifloxacin is a highly effective treatment for all cause PID. There were only three patients with *M.genitalium*-PID in this study; these patients had a 100% microbiological cure rate when treated with moxifloxacin (Ross *et al.* 2006). Although promising, this is an extremely small sample size. A retrospective case-notes review of moxifloxacin for uncomplicated non-*N.gonorrhoeae* PID (n=257) confirmed similar efficacy, although it is unknown how many women in this study had *M.genitalium*. In this series 70.2% of patients treated with moxifloxacin experienced significant clinical improvement; 11.4% experienced marginal clinical improvement; and 18.4% experienced no change in their symptoms (Boothby *et al.* 2010). The results of these studies were comparable to the reported efficacy of antibiotics recommended by current treatment guidelines for PID (Piyadigamage *et al.* 2005), indicating that moxifloxacin is an effective treatment for *C.trachomatis*-PID, and PID of unknown aetiology, and a potential agent in the treatment of *M.genitalium*-PID.

Table 3. Recommended antibiotic regimens for the treatment of Pelvic Inflammatory Disease

Table adapted from The Australian STI Management Guidelines 2016. (ASHA 2016)

Situation	Recommended treatment
Mild to moderate	Ceftriaxone 500mg in 2mL of 1% lignocaine intramuscular
Outpatient treatment	injection, or 500mg IV stat
	PLUS
	Metronidazole 400mg PO BD for 14 days
	PLUS
	Azithromycin 1g PO, stat
	PLUS EITHER
	Doxycycline 100mg PO, BD for 14 days
	OR
	Azithromycin 1g PO, one week later
Severe:	Ceftriaxone 2g IV, daily
Inpatient treatment	*OR*
	Cefotaxime 2g IV, three times daily
	PLUS
	Azithromycin 500mg IV, daily
	PLUS
	Metronidazole 500mg IV, BD

Abbreviations: STI= sexually transmitted infection, mg=milligrams, mL=millilitres, IV=intravenous, PO=orally, BD= twice a day, g=grams

Table 4. Pelvic Inflammatory Disease guidelines and their ineffectiveness for *Mycoplasma genitalium*

Presumptive pelvic inflammatory disease Regimens	**Reason for Ineffectiveness against *M.genitalium***
Ceftriaxone	Third generation cephalosporin (beta-lactam antibiotic), which works at the bacteria's cell wall.
Metronidazole	Belongs to the class nitroimidazoles; works on anaerobes only.
Azithromycin	Initially highly effective but now has cure rates as low as 60% in Australia and many parts of the world for *M.genitalium* (Bissessor *et al.* 2015; Tagg *et al.* 2013).
Doxycycline	Consistently low cure rate of 20-40% for *M.genitalium* (Anagrius *et al.* 2011).
Cefotaxime	Third generation cephalosporin (beta-lactam antibiotic), which works at the bacteria's cell wall.

2.9 Summary of literature review exploring *Mycoplasma genitalium*

M.genitalium is a relatively recently discovered bacterial STI, associated with urethritis, cervicitis and PID. *M.genitalium* has intrinsically limited susceptibility to many commonly used antimicrobials and a marked propensity to develop antimicrobial resistance, which has complicated its management. Due to limited information regarding its natural history and lack of readily available cheap and effective treatment options, screening for *M.genitalium* is not recommended in any populations. Testing is generally limited to those with symptoms of urethritis and PID, and in people reporting to be sexual contacts of someone with a confirmed diagnosis.

With a number of commercial diagnostic assays for *M.genitalium* on the horizon and increasing testing in the community, it is important to define the prevalence of *M.genitalium* in specific populations and by anatomical site, and understand its contribution to genital symptoms and syndromes in men and women. This is particularly important in MSM, as a population at high risk of STI, and in women, as the population most affected by the pathological consequences of STI. This thesis seeks to expand the literature around *M.genitalium* prevalence, determine *M.genitalium*'s association with symptoms and signs in women, and determine the efficacy of moxifloxacin therapy for *M.genitalium*-PID.

2.10 Hypotheses and Aims of Section A

2.10.1 Hypotheses

1) *M.genitalium* is a common STI in MSM and prevalence will vary by anatomical site, with the lowest estimate at the pharynx.
2) Rectal *M.genitalium* will be a common co-infection with rectal *C.trachomatis* and *N.gonorrhoeae*.
3) *M.genitalium* will be associated with common genital symptoms and signs in women such as abdominal pain and dysuria. *M.genitalium* will be associated with the syndromes of cervicitis and PID.
4) *M.genitalium* will present similarly to *C.trachomatis*, with similar symptoms and signs. The syndrome of PID will present similarly when caused by either *M.genitalium* or *C.trachomatis*.
5) Moxifloxacin is likely to be highly effective in the treatment of *M.genitalium*-PID.

2.10.2 Aims

1) To determine the prevalence of *M.genitalium* amongst MSM by anatomical site.
2) To determine the proportion of rectal *C.trachomatis* and rectal *N.gonorrhoeae* infections in MSM who are co-infected with rectal *M.genitalium*.
3) To determine the prevalence of *M.genitalium* in women attending a sexual health service.
4) To determine the clinical characteristics associated with *M.genitalium* infection in women.
5) To describe the clinical characteristics associated with *M.genitalium*-PID and to compare them to those associated with *C.trachomatis*-PID.
6) To determine the efficacy of moxifloxacin in the treatment of *M.genitalium*-PID.

3. The prevalence of *Mycoplasma genitalium* in men who have sex with men.

3.1 Background

M.genitalium is an STI that was first discovered in the urethra of two men in 1981 and is recognised as a cause of non-gonococcal urethritis in men (Tully *et al.* 1981). However more information is needed on the prevalence of *M.genitalium*, particularly in populations at high risk for STIs such as MSM. This is pertinent given current debate on whether or not to test or screen for *M.genitalium* in various populations. There are particularly limited data on the prevalence of *M.genitalium* in the rectum and pharynx.

This study contained in this chapter had the following aims:

1) To determine the prevalence of *M.genitalium* in the urethra of MSM..
2) To determine the prevalence of *M.genitalium* in the rectum of MSM.
3) To determine the prevalence of *M.genitalium* in the pharynx of MSM.

This findings were published in *Sexually Transmitted Infections*: Latimer RL, Shilling HS, Vodstrcil LA, et al. 'Prevalence of Mycoplasma genitalium by anatomical site in men who have sex with men: a systematic review and meta-analysis.' *Sexually Transmitted Infections* 2020; 96:563-570.

The findings were also presented as a poster at the STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019. Published: Latimer R, Shilling H, Vodstrcil L, et al. P525. Prevalence of Mycoplasma genitalium by anatomical site in men who have sex with men: a systematic review and meta-analysis. *Sexually Transmitted Infections* 2019; 95:A239-A240.

The paper has been included as text in the thesis to allow for inclusion of what was supplementary material in the published study. No alterations have been made, aside from abbreviations, figure and table numbers, for thesis consistency. Please see Appendix A for the PDF of the published study.

3.2 Abstract

3.2.1 Objective

To systematically review and appraise published data, to determine the prevalence of *M.genitalium* in MSM tested at each anatomical site, that is, at the urethra, rectum and/or pharynx.

3.2.2 Design

Systematic review and meta-analysis.

3.2.3 Data sources

Ovid Medline, PubMed, Embase were searched for articles from 1st January 1981 (the year *M.genitalium* was first identified) to 1st June 2018.

3.2.4 Data sources

Studies were eligible for inclusion if they reported *M.genitalium* prevalence in MSM tested at the urethra, rectum and/or pharynx, in at least 50 MSM, using NAAT. Data were extracted by anatomical site, symptom and HIV status. Summary estimates (95% CIs) were calculated using random- effects meta- analysis. Subgroup analyses were performed to assess heterogeneity between studies.

3.2.5 Results

Forty- six studies met inclusion criteria, with 34 reporting estimates of *M.genitalium* prevalence at the urethra (13 753 samples), 25 at the rectum (8629 samples) and 7 at the pharynx (1871 samples). *M.genitalium* prevalence was 5.0% (95% CI: 3.5 to 6.8; I^2=94.0) at the urethra; 6.2% (95% CI: 4.6 to 8.1; I^2=88.1) at the rectum and 1.0% (95% CI: 0.0 to 5.1; I^2=96.0) at the pharynx. The prevalence of *M.genitalium* was significantly higher at urethral and rectal sites in symptomatic versus asymptomatic MSM (7.1% vs 2.2%, p<0.001; and 16.1% vs 7.5%, p=0.039, respectively). *M.genitalium* prevalence at the urethra was significantly higher in HIV- positive compared with HIV- negative MSM (7.0% vs 3.4%, p=0.006).

3.2.6 Conclusion

M.genitalium was common in MSM, particularly at urethral and rectal sites (5% to 6%). *M.genitalium* was more commonly detected in symptomatic men at both sites, and more common in HIV- positive men at the urethra. *M.genitalium* was uncommonly detected in the pharynx. Site-specific estimates are similar to those for *C.trachomatis* and will be helpful in informing testing practices in MSM.

3.3 Introduction

M.genitalium is a STI, with prevalence estimates in the general community ranging from 1.3% to 3.9% (Baumann et al. 2018). The majority of published data provides estimates of urethral infection with less data available on *M.genitalium* infection in MSM, particularly for rectal and pharyngeal sites. There has been one prior meta- analysis of community-based studies (n=8) that estimated the overall prevalence of *M.genitalium* in MSM at 3.2% (95% CI 2.1 to 5.1), but clinic studies were excluded, and estimates were predominantly derived from urine samples (Baumann et al. 2018).

International guidelines recommend screening for *C.trachomatis* and *N.gonorrhoeae* in MSM, (CDC 2015; Clutterbuck *et al.* 2016; ASHA 2020) although there is ongoing debate as to the relative contribution of these STIs at extragenital sites to transmission (Hocking 2019). Guidelines do not recommend screening for *M.genitalium* at any site (CDC 2015; Clutterbuck *et al.* 2016; ASHA 2018), as the situation is more complicated than for *C.trachomatis* or *N.gonorrhoeae* due to lack of clarity around the natural history of *M.genitalium*, and increasing challenges with treatment due to antimicrobial resistance (Read *et al.* 2019a). Testing for *M.genitalium* in men with urethritis is recommended by international guidelines (CDC 2015; BASHH *et al.* 2018), as its pathogenic role in this syndrome is well established (Anagrius *et al.* 2005). *M.genitalium*'s association with rectal symptoms and the syndrome of proctitis has been inconsistent across published studies (Read *et al.* 2019a; Bissessor *et al.* 2016), with UK and Australian guidelines recommending consideration of testing for *M.genitalium* in men with sexually acquired proctitis (ASHA 2018; BASHH *et al.* 2018). Site-specific prevalence estimates in MSM are needed to understand the contribution of each anatomical site to *M.genitalium* transmission, and to inform testing practices in MSM.

We undertook a systematic review and meta- analysis of published studies in order to determine the prevalence of *M.genitalium* at the urethra, rectum and pharynx of MSM tested for infection at each site, and to examine the association with symptoms, HIV status and other factors on site-specific prevalence, to inform testing and clinical practice in MSM.

3.4 Methods

The study protocol was registered on Prospero (ID CRD42017058326).

3.4.1 Search Strategy and selection criteria

This systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (Table 5). Two authors (R.L.L, H.S.S) independently searched for published peer-reviewed studies reporting the prevalence of *M.genitalium* in MSM by anatomical site, from 1st January 1981 (the year *M.genitalium* was first identified) to 1st August 2017, with the search updated on the 1st June 2018.

We included cross-sectional, longitudinal and cohort studies, and RCTs where baseline *M.genitalium* prevalence was reported, and conference abstracts from major STI conferences between 1st January 2015 and 1st June 2018 for studies that may not have been published yet. The search was performed using Ovid Medline, PubMed and Embase using search terms [('mycoplasma genitalium' or 'mycoplasma infections' or 'mycoplasmosis') and/or ('men adj3 sex' or 'males adj3 sex' or 'homosexual' or 'homosexuality')] (Table 6). Medical Subject Headings were used where possible. Reference lists of included studies were reviewed to identify other relevant studies. Studies were eligible for inclusion if they were published in English and reported *M.genitalium* prevalence at the urethra, rectum and/or pharynx, in at least 50 MSM, to reduce small study bias. Prevalence at each anatomical site was defined as the prevalence among MSM tested for *M.genitalium* at each anatomical site. Definition of MSM varied between studies. Participants were assumed to be MSM if studies stated that men either had male sexual partners, or if men had a rectal swab collected (Callander *et al.* 2015; Templeton *et al.* 2014)

Two reviewers (RLL, HSS) independently screened studies for eligibility using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). Abstracts and titles were screened, and then articles reviewed. Differences were resolved by discussion with other reviewers (CSB, LAV and/or DAM). When multiple articles reported on the same study population, we either included the most comprehensive article or the original article published.

Table 5. Preferred Reporting Items for Systematic Reviews and Meta-analyses.			
Section/topic	**#**	**Checklist item**	**Reported on page #**
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	79
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	80
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	81
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	86
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	82
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	82
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	82
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	84
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	82 and Figure 11.
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	86
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	86 + 87
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	87

Table 5. cont.			
Section/topic	**#**	**Checklist item**	**Reported on page #**
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	87
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	87

Table 6. Search terms for the systematic review.

Embase

1. mycoplasma genitalium/
2. mycoplasma genitalium.mp.
3. genitalium.mp.
4. 1 or 2 or 3
5. mycoplasmosis/
6. (men adj3 sex).mp.
7. (males adj3 sex).mp.
8. homosexual*.mp.
9. 6 or 7 or 8
10. 5 and 9
11. 4 or 10
12. Limit 11 to (english language and yr=’1981 – current’)

OVID Medline

1. Mycoplasma genitalium/
2. Genitalium.mp.
3. mycoplasma genitalium.mp.
4. 1 or 2 or 3
5. Mycoplasma Infections/
6. Homosexuality, Male/
7. (men adj3 sex).mp.
8. (males adj3 sex).mp.
9. homosexual*.mp.
10. 6 or 7 or 8 or 9
11. 5 and 10
12. 4 or 11
13. limit 12 to yr=’1981 –current’

limit 12 to english language

Medical subject headings were used where possible.
Abbreviations and symbols:
/ = Medical subject heading, .mp. = search as keyword, Adj3 = proximity operator, words found within three words of each other, * = used for truncation i.e. permitting any letter, symbol or space

Table 7. Adapted Risk of Bias Tool.

Adapted from Hoy et al.(Hoy *et al.* 2012)

Name of authors:

Year of publication:

Study title:

Risk of bias items	Risk of bias levels	Points scored
1. Was the study's target population a close representation of the national population in relation to relevant variables, e.g. age, occupation?	**Yes (LOW RISK):** The study's target population was a close representation of the national population.	0
	No (HIGH RISK): The study's target population was clearly NOT representative of the national population.	1
2. Was the sampling frame a true or close representation of the MSM population?	**Yes (LOW RISK):** The sampling frame was a true or close representation of the target population.	0
	No (HIGH RISK): The sampling frame was NOT a true or close representation of the target population. i.e. Selected HIV patients or symptomatic patients.	1
3. Was some form of random selection used to select the sample, OR, was a census undertaken?	Yes **(LOW RISK):** A census was undertaken, OR, some form of random selection was used to select the sample (e.g. simple random sampling, stratified random sampling, cluster sampling, systematic sampling).	0
	No (HIGH RISK): A census was NOT undertaken, AND some form of random selection was NOT used to select the sample.	1
4. Was an acceptable case definition of MSM used in the study?	**Yes (LOW RISK):** MSM was clearly defined as a male having penetrative sex in the last year; or questionnaire where patients identified sex with men	0
	Yes (MODERATE RISK): MSM were recruited; not defined how MSM were identified, states MSM in paper though	1
	No (HIGH RISK): MSM was based off male rectal swabs only	2
5. Was the same mode of data collection used for all subjects?	**Yes (LOW RISK):** The same mode of data collection was used for all subjects.	0
	No (HIGH RISK): The same mode of data collection was NOT used for all subjects, some had self-collected swabs/some by clinician etc.	1
6. Were the numerator(s) and denominator(s) for the parameter of interest appropriate	**Yes (LOW RISK):** The paper presented appropriate numerator(s) AND denominator(s) for the parameter of interest.	0
	No (HIGH RISK): The paper did present numerator(s) AND denominator(s) for the parameter of interest – presented percentages or did not present number of swabs excluded.	1

7. Sample size	**Yes (LOW RISK):** Greater than 100 MSM	0
	No (HIGH RISK): Less than 100 MSM included	1
8. Was a sample size calculation reported? Was the achieved sample size at least as good as the sample size calculation?	**Yes (LOW RISK):** The paper presented appropriate calculations AND the sample size was the same as the calculation	0
	No (HIGH RISK): The paper did not present a sample size calculation OR the sample size shown was smaller than the sample size calculation required.	1
Summary on the overall risk of study bias	**LOW RISK**	0-3
	MODERATE RISK	4-6
	HIGH RISK	7-9

3.4.2 Data Extraction

Variables extracted included: name of first author, publication year, study design, geographical location [using WHO regions ('Regional Offices')], study period, study setting, median or mean age, HIV-status, symptom status, symptom location, clinical diagnoses, reason for testing, number of participants, laboratory testing methods, and number positive for *M.genitalium* by specimen type (urethral swab, first-void urine, rectal swab, and/or pharyngeal swab). Reported prevalence estimates with 95% CIs were also extracted, and where CIs were not reported, these were calculated using binomial methods. One reviewer (R.L.L.) extracted the data, and a second reviewer (H.S.S.) checked extracted data for transcription errors. Any disagreements were discussed between the two reviewers, and where required, consultation with a third reviewer (C.S.B.) was sought until consensus was reached. Authors were contacted for prevalence data where estimates were not stratified by anatomical site, HIV or symptom status, or if the study design suggested that additional information was available.

3.4.3 Primary Outcome

The primary outcome was the prevalence of *M.genitalium* in MSM at the urethra, rectum and/or pharynx. This was calculated as the number of men who tested positive for *M.genitalium* at each site (defined as a positive test using NAAT) divided by the total number tested for *M.genitalium* at the site.

3.4.4 Secondary Outcome

Secondary outcomes included the prevalence of *M.genitalium* either by symptom status (asymptomatic vs symptomatic), HIV-status (HIV-negative vs HIV-positive), or recruitment setting or location. Analyses were dependent on sufficient data being available.

3.4.5 Analysis

Summary prevalence estimates were calculated using random-effects meta-analysis with Freeman-Tukey double arcsine transformation, and study specific 95% CIs computed using score method (Nyaga *et al.* 2014). Included studies were examined using forest plots. I^2 statistics were calculated to assess between-study heterogeneity when more than two studies were included, with values of <25%, 25-75% and >75% representing low, medium and high heterogeneity, respectively. The χ^2 statistic was used to assess the strength of the evidence for heterogeneity.

We undertook subgroup analyses and univariable random-effects meta-regression by symptom status, HIV-status, and broad WHO geographic region ('Regional Offices'), to investigate potential sources of heterogeneity. Sensitivity analyses were conducted to determine the effect on summary estimates of removal of either: i) outlier studies, or ii) studies at high risk of being non-representative of MSM. Studies were considered outliers if they fell outside the overall pattern of distribution for prevalence at each site. Studies were considered at risk of not representing the wider MSM community if a) the sample was not a true representation of the broader MSM population, for example select HIV patients, or b) MSM were not clearly defined as men who had penetrative sex in the past year with another man. Data were analysed using Stata (V.14.0, StataCorp, Austin, Texas, USA).

3.4.6 Assessment of Bias and Quality

The potential presence of publication and small study bias was assessed using funnel plots of proportions against study samples size and the Egger test (Egger *et al.* 1997). To evaluate within study bias, we adapted an instrument designed by Hoy et al., which examines both the internal and external validity of the selected studies (Table 7) (Hoy et al. 2012). The tool consisted of eight questions and two reviewers (R.L.L., H.S.S.) independently assessed each study as being low, medium or high risk of bias for each item, with differences resolved by discussion with C.S.B.

3.5 Results

3.5.1 Study Selection

The search process identified 4518 records and 2595 duplicates were removed. Titles and abstracts of 1923 records were screened and 1586 excluded, leaving 337 records for full- text screening. Forty-six records were included in the final analysis (Figure 10, included studies described in Table 8).

3.5.2 Study characteristics

Of the 46 included studies, 34 reported *M.genitalium* prevalence at the urethra (n=13753 samples), 25 at the rectum (n=8629 samples), and seven at the pharynx (n=1871 samples) (Table 9). Sample sizes ranged from 51 to 6293 (Table 8). Nineteen studies provided information on the presence of symptoms (Bissessor *et al.* 2016; Bradshaw *et al.* 2006b; Clarivet *et al.* 2014; Cosentino *et al.* 2012; Couldwell *et al.* 2018; da Costa *et al.* 2010; Francis *et al.* 2008; Gottesman *et al.* 2017; Gratrix *et al.* 2017; Hakre *et al.* 2017; Libois *et al.* 2018; Manhart *et al.* 2013; Mezzini *et al.* 2013; Moi *et al.* 2009b; Ong *et al.* 2018a; Rane *et al.* 2014; Read *et al.* 2017b; Reynolds-Wright *et al.* 2016; van der Veer *et al.* 2016), and 29 on HIV-status (Bissessor *et al.* 2016; Chen *et al.* 2015; Clarivet *et al.* 2014; Cosentino *et al.* 2012; Couldwell *et al.* 2018; da Costa *et al.* 2010; Dionne-Odom *et al.* 2018; Edouard *et al.* 2017; Francis *et al.* 2008; Fuchs *et al.* 2016; Gratrix *et al.* 2017; Hakre *et al.* 2017; Ham *et al.* 2015; Homfray *et al.* 2015; Jian-Ru *et al.* 2012; Lefebvre *et al.* 2017; Libois *et al.* 2018; Manhart *et al.* 2013; Mezzini *et al.* 2013; Moi *et al.* 2009b; Ong *et al.* 2018a; Peters *et al.* 2017; Philibert *et al.* 2014; Read *et al.* 2017b; Reynolds-Wright *et al.* 2016; Ruutel *et al.* 2015; Soni *et al.* 2010; Wu *et al.* 2013; Zheng *et al.* 2014). Most studies [n=20 (42%)] were from Europe (Clarivet *et al.* 2014; Cox *et al.* 2017; de Jong *et al.* 2016; Edouard *et al.* 2017; Homfray *et al.* 2015; Fuchs *et al.* 2016; Lallemand *et al.* 2015; Lefebvre *et al.* 2017; Libois *et al.* 2018; Moi *et al.* 2009b; Pereyre *et al.* 2017; Philibert *et al.* 2014; Plaas *et al.* 2015; Reinton *et al.* 2013; Reynolds-Wright *et al.* 2016; Ruutel *et al.* 2015; Soni *et al.* 2010; Unemo *et al.* 2018; van der Veer *et al.* 2016; Gottesman *et al.* 2017), 13 (28%) were from the Western Pacific, although all were from Australia and China (Bissessor *et al.* 2016; Bradshaw *et al.* 2009; Bradshaw *et al.* 2006b; Couldwell *et al.* 2018; Mezzini *et al.* 2013; Ong *et al.* 2018a; Rane *et al.* 2014; Read *et al.* 2017b; Chen *et al.* 2015; Jian-Ru *et al.* 2012; Jiang *et al.* 2015; Zheng *et al.* 2014; Wu *et al.* 2013), 12 (26%) were from the Americas, predominately the USA (Cosentino *et al.* 2012; Creswell *et al.* 2012; da Costa *et al.* 2010; Dionne-Odom *et al.* 2018; Francis *et al.* 2008; Gratrix *et al.* 2017; Hakre *et al.* 2017; Ham *et al.* 2015; Kriesel *et al.* 2016; Manhart *et al.* 2013; Munson *et al.* 2017; Xiao *et al.* 2018), and the WHO Africa region contained only one South African tudy (2%) (Peters *et al.* 2017) (Table 8 &

Table 9). Overall, 36 (78%) studies were cross-sectional (Bissessor *et al.* 2016; Bradshaw *et al.* 2009; Bradshaw *et al.* 2006b; Chen *et al.* 2015; Clarivet *et al.* 2014; Cosentino *et al.* 2012; Couldwell *et al.* 2018; Cox *et al.* 2017; Creswell *et al.* 2012; da Costa *et al.* 2010; Dionne-Odom *et al.* 2018; Edouard *et al.* 2017; Francis *et al.* 2008; Gottesman *et al.* 2017; Gratrix *et al.* 2017; Hakre *et al.* 2017; Ham *et al.* 2015; Homfray *et al.* 2015; Jian-Ru *et al.* 2012; Jiang *et al.* 2015; Kriesel *et al.* 2016; Lallemand *et al.* 2015; Lefebvre *et al.* 2017; Libois *et al.* 2018; Mezzini *et al.* 2013; Pereyre *et al.* 2017; Philibert *et al.* 2014; Plaas *et al.* 2015; Reinton *et al.* 2013; Reynolds-Wright *et al.* 2016; Ruutel *et al.* 2015; Soni *et al.* 2010; Unemo *et al.* 2018; Xiao *et al.* 2018; Zheng *et al.* 2014; Wu *et al.* 2013), six (13%) were retrospective cohorts (de Jong *et al.* 2016; Moi *et al.* 2009b; Munson *et al.* 2017; Ong *et al.* 2018a; Rane *et al.* 2014; van der Veer *et al.* 2016), and four (9%) were prospective cohort studies (Fuchs *et al.* 2016; Manhart *et al.* 2013; Peters *et al.* 2017; Read *et al.* 2017b) (Table 8 & Table 9).

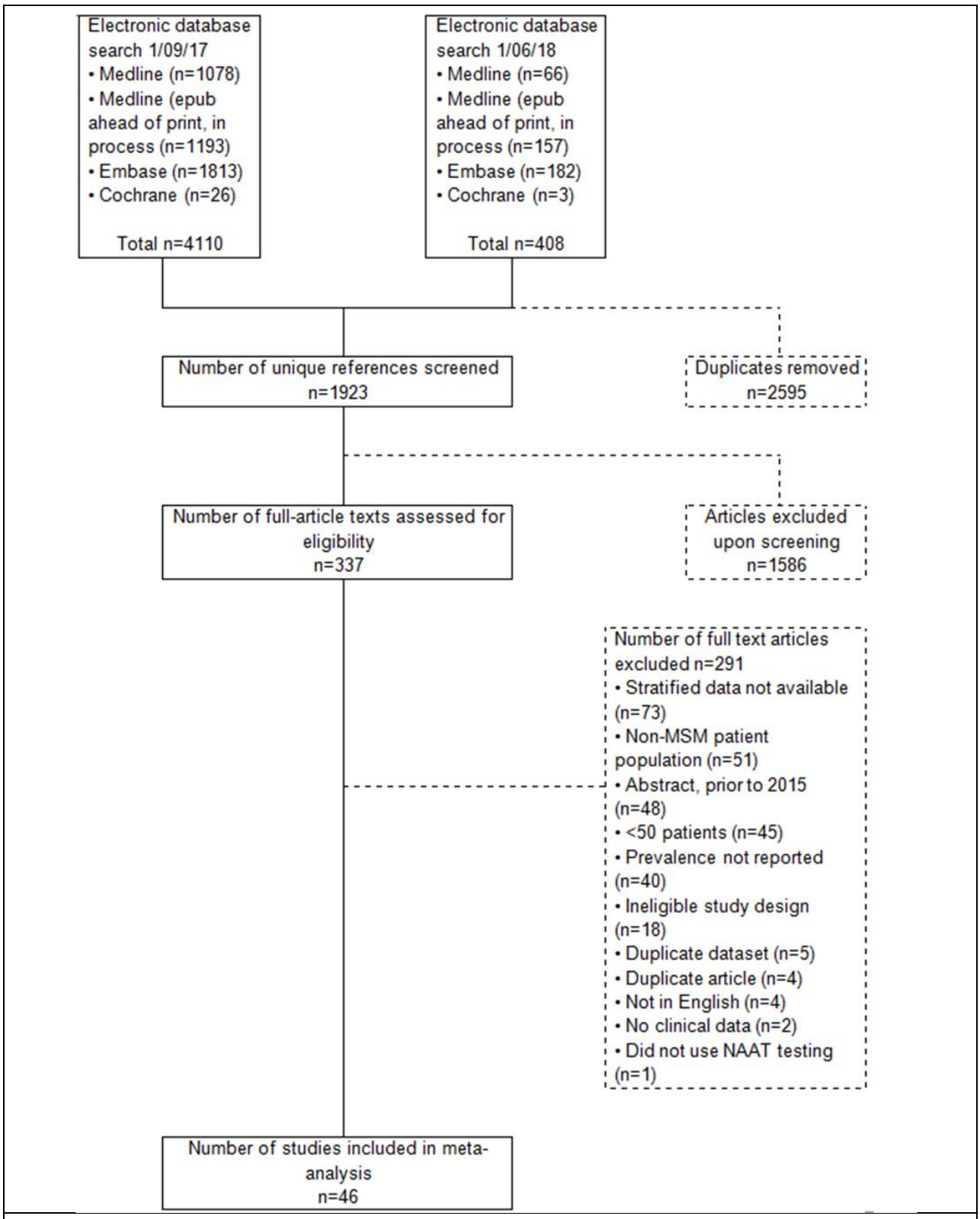

Figure 10. Flow diagram of studies included in Meta-Analysis of the Prevalence of Mycoplasma Genitalium amongst Men Who have Sex With Men

Abbreviations: e pub, electronic publication; n, number; NAAT, nucleic acid amplification testing

Table 8. Characteristics of the studies included in the meta-analysis (n=46)

Author	Year	Country; WHO Region	Study period	Setting	Recruitment methods and population	Detection assay used	Demographic characteristics	HIV-status	Symptom status	MG prevalence n/N (%, 95% CI)
Bissessor (Bissessor *et al.* 2016)	2016	Australia; Western Pacific	May 2012-August 2013	Clinic	Conducted at a walk-in STI clinic where MSM with symptomatic proctitis were tested for rectal MG All MSM with proctitis presenting during study period were recruited	Quantitative PCR	Median age 38 (range 22-58)	All	Symptomatic	Rectum: 18/154 (11.7; 7.1-17.8)
								Positive	Symptomatic	Rectum: 10/48 (20.8; 10.5–35.0)
								Negative	Symptomatic	Rectum: 8/106 (7.5; 3.3–14.3)
Bradshaw (Bradshaw *et al.* 2009)	2009	Australia; Western Pacific	October 2001-September 2002	Community	Male sexual health nurses systematically approached MSM attending SOPVs; 76% refusal rate. Participants were invited to provide a rectal, FPU, and pharyngeal sample	Real-time PCR (LightCycler, Roche Molecular Biochemicals)	SOPV patrons; median age 39 (range 18-85)	Unknown	All	FPU: 3/510 (0.6, 0.1-1.7) Rectum: 8/497 (1.6, 0.7-3.1) Pharynx: 0/515 (0; 0.0-0.7) Pooled: (2.1%; 95% CI 1.1-3.6%)
Bradshaw (Bradshaw *et al.* 2006b)	2006	Australia; Western Pacific	March 2004-March 2005	Clinic	Walk-in STI clinic where men with NGU were tested for MG with retrospective recruitment of case matched controls	PCR using automated MagNA Pure LC (Roche)	Mean age 32.3 +/- 9.1 years	Unknown	All	FPU: 8/184 (4.3; 2.0-8.4)
								Unknown	Asymptomatic	FPU: 0/75 (0; 0.0-4.8)
								Unknown	Symptomatic	FPU: 8/109 (7.3; 3.2-14.0)
Chen[a] (Chen *et al.* 2015)	2015	China; Western Pacific	2009-2011 (4 surveys at 8month intervals)	Community	Male HIV/AIDS patients >18 years of age from 13 cities in the Jiangsu Province; recruited through Jiangsu Provincial Center for Disease Prevention and Control	Nested PCR	Median age 39.14 (range 18-70); 44.8% MSM with AIDS	Positive	Unknown	FPU: 110/617 (17.8; 14.9-21.1)
Clarivet[a] (Clarivet *et al.* 2014)	2014	France; Europe	April 2009-August 2009	Clinic	All sexually active STI clinic attendees <30 years of age in Montpellier, excluding those with a current STI including HIV	In-house PCR	Mean age 22 (SD 3)	Negative	Asymptomatic	FPU: 1/85 (1.2; 0.0-6.4)
Cosentino[a] (Cosentino *et al.* 2012)	2012	USA; The Americas	May 2009-March 2010	Clinic	All attendees at three medical centres who reported having had at least one lifetime episode of receptive anal intercourse	Research-use-only Aptima TMA	Median age 40 (range 18-63)	All	All	Rectum: 25/225 (11.1; 7.3-16.0)
								All	Asymptomatic	Rectum: 23/196 (11.7; 7.6-17.1)
								All	Symptomatic	Rectum: 2/29 (6.9; 0.8-22.8)
								Positive	All	Rectum: 11/125 (8.8; 4.5-15.2)
								Negative	All	Rectum: 14/100 (14.0; 7.8-22.4)
Couldwell (Couldwell *et al.* 2018)	2018	Australia; Western Pacific	February 2017-May 2017	Clinic	Consecutive MSM attending a Sydney STI clinic, 91.5% of eligible men included in study	ResistancePlus MG assay (SpeeDx)-multiplex quantitative (qPCR) assay	Mean age 33.2 (SD not reported)	All	All	FPU: 24/508 (4.7; 3.1-6.9) Rectum: 45/505 (8.9; 6.6-11.7) Pharynx: 0/508 (0; 0.0-0.7) Pooled: 68/508 (13.4; 10.5-16.7)
								Positive	All	Pooled: 3/30 (10.0; 2.1-26.5
								Negative	All	Pooled: 65/478 (13.6; 10.7-17.0)
								All	Asymptomatic	FPU: 19/472 (4.0; 2.4-6.2) Rectum: 42/493 (8.5; 6.2-11.3)

								All	Symptomatic	FPU: 5/36 (13.9; 4.7-29.5) Rectum: 3/12 (25.0; 5.5-57.2)
Cox[a] (Cox *et al.* 2017)	2017	UK; Europe	Not described. 12 month duration.	Clinic	Anonymised residual rectal specimens submitted for STI testing from STI clinic. Could contain TOC or repeat samples. Samples with co-infection were excluded. All rectal samples submitted by males were considered MSM for the purposes of this study	1X Platinum Quantitative PCR (Life Technologies, Paisley, UK)	Mean age 35.3 (SD 12); median age 33	Unknown	Unknown	Rectum: 10/107 (9.3; 4.6-16.5)
Creswell (Creswell *et al.* 2012)	2012	El Salvador; The Americas	March 2008-September 2008	Community	Respondent driven sampling of MSM 18 years of age or older in San Salvador and San Miguel	PCR	61% under the age of 24; 13.4% HIV+ve	All	Unknown	FPU: 16/647 (2.5; 1.4-4.0)
da Costa (da Costa *et al.* 2010)	2010	Brazil; The Americas	Not reported.	Clinic	Patients presenting to two HIV clinics for screening were recruited	Realtime PCR	MG+ve mean age 43 (SD not reported); MG-ve mean age 44 (SD not reported)	Positive	Asymptomatic	FPU: 10/119 (8.4; 4.1-14.9)
de Jong[a] (de Jong *et al.* 2016)	2016	Netherlands ; Europe	June 2013-July 2013, December 2013-February 2014	Laboratory	Stored samples, predominately from STI clinics and general practitioners, sent to diagnostic centres in the Netherlands for STI testing. All males providing rectal swabs assumed to be MSM	qPCR using S-Diamg kit	Median age 28, mean 32.5 (SD 12.7)	Unknown	Unknown	Rectum: 3/105 (2.9; 0.6-8.1)
Dionne-Odom (Dionne-Odom *et al.* 2018)	2018	USA; The Americas	December 2014-November 2016	Clinic	HIV-infected MSM presenting to a HIV primary care clinic in Birmingham, Alabama were enrolled	LightCycler 480 Probes Master kit	Median age 34 (IQR 29-46); 66% African American	Positive	All	FPU: 17/157 (10.8; 6.4-16.8) Rectum: 10/157 (6.4; 3.1-11.4) Pooled 27/157 (17.2; 11.6-24.0)
Edouard (Edouard *et al.* 2017)	2017	France; Europe	January 2014-December 2015	Clinic	HIV-infected patients from a HIV outpatient clinic in Marseille, France were voluntarily enrolled	in house qPCR using the QuantiTect Probe PCR Kit (Qiagen)	Full cohort 118 HIV-positive patients, 53% MSM; median age 34 (IQR 29-46)	Positive	All	Rectum: 7/62 (11.3; 4.7-21.9)
Francis (Francis *et al.* 2008)	2008	USA; The Americas	November 2005-January 2006	Clinic	Consecutive rectal specimens collected from MSM attending a San Francisco STI clinic	Research TMA assay	All <35 years; 54% Caucasian	All	All	Rectum: 27/500 (5.4; 3.6-7.8)
								Positive	All	Rectum: 14/133 (10.5; 5.9-17.0)
								Negative	All	Rectum: 13/359 (3.6; 1.9-6.1)
								All	Asymptomatic	Rectum: 22/460 (4.8; 3.0-7.2)
								All	Symptomatic	Rectum: 5/40 (12.5; 4.2-26.8)
								All	Asymptomatic	Rectum: 24/474 (5.1; 3.3-7.4)
								All	Symptomatic	Rectum: 3/26 (11.5; 2.4-30.2)
Fuchs (Fuchs *et al.* 2016)	2016	Germany; Europe	September 2012-	Clinic	Intra-anal swabs collected from 511 HIV+ve MSM attending an anal cancer screening program	Real-time PCR	Mean age 45.4 (SD 10.9)	Positive	All	Rectum 18/433 (4.2; 2.5-6.5)

			October 2014							
Gottesman (Gottesman *et al*. 2017)	2017	Israel; Europe	November 2008-November 2010	Clinic	Successive male patients presenting to STI clinic in Tel Aviv	Seeplex STD6 ACE Detection kit (Seegene, Inc., Seoul, Korea)	60% MSM; median age 30 (range 17-65)	Unknown	All	FPU: 5/156 (3.2; 1.0-7.3)
								Unknown	Asymptomatic	FPU: 0/96 (0; 0-3.8)
								Unknown	Symptomatic	FPU: 5/60 (8.3; 2.8-18.4)
Gratrix[a] (Gratrix *et al*. 2017)	2017	Canada; The Americas	January 2016-April 2016	Clinic	Sequential specimens of all attendees over the age of 18 presenting to two Alberta STI clinics	TMA-research use only (Hologic Inc, San Diego, California)	53% male, 29% of men MSM; median age 30 (IQR 25-37)	All	All	FPU: 23/349 (6.6; 4.2-9.7)
								Positive	All	FPU: 2/16 (12.5; 1.6-38.3)
								Negative	All	FPU: 16/236 (6.8; 3.9-10.8)
								Unknown	All	FPU: 5/97 (5.2; 1.7-11.6)
								All	Asymptomatic	FPU: 14/281 (5.0; 2.8-8.2)
								All	Symptomatic	FPU: 9/67 (13.4; 6.3-24.0)
Hakre[a] (Hakre *et al*. 2017)	2017	USA; The Americas	16th May 2016-30th September 2016	Clinic	All HIV+ve US Air Force members attending the air force HIV clinic at Joint Base San Antonio, Texas	Aptima MG research-use-only	73% of all patients MSM; median age 31 (IQR 26-36)	Positive	All	FPU: 9/63 (14.3; 6.7-25.4) Rectum: 10/66 (15.2; 7.5-26.1) Pharynx: 1/57 (1.8; 0.0-9.4) Pooled: 18/74 (24.3; 15.1-35.7)
								Positive	Asymptomatic	Pooled: 12/57 (21.1; 11.4-33.9)
								Positive	Symptomatic	Pooled: 6/17 (35.3; 14.2-61.7)
Ham[a] (Ham *et al*. 2015)	2015	Honduras; The Americas	2012	Community	Standardized behavioural and biological surveillance survey administered in 4 countries: El Salvador (2007-2008), Guatemala (2012), Honduras (2012), and Nicaragua (2009-2010). Participants recruited using respondent driven sampling	In-house realtime multiplex CDC	2727 MSM, with 12% identifying as MTFTG; median age 23 for non-transgender MSM (no range given)	All	All	FPU: 87/2206 (3.9; 3.2-4.8)
								Positive	All	FPU: 13/225 (5.8; 3.1-9.7)
								Negative	All	FPU: 73/1911 (3.8; 3.0-4.8)
Homfray/ NATSAL-3[ab] (Homfray *et al*. 2015)	2015	Britain; Europe	2010-2012	Community	Stratified probability sample survey of 15162 men and women, 57.7% response rate. 4828 asked to provide urine samples. 4507 tested for MG	In-house realtime PCR; positive results confirmed with GenProbe Mycoplasma test	89 MSM in those who provided urine samples, age range 16-44	All	All	FPU: 1/89 (1.1; 0-6.1)
								Positive	All	FPU: 0/4 (0; 0-60.2)
								Negative	All	FPU: 1/84 (1.2; 0-6.5)
Jian-Ru (Jian-Ru *et al*. 2012)	2012	China; Western Pacific	2009	Clinic	497 HIV-1 infected men recruited from Jiangsu Centers for Diseases Prevention and Control	Nested PCR	29% of total cohort MSM; mean age 37 (range 5-75)	Positive	Unknown	FPU: 34/144 (23.6; 16.9-31.4)
Jiang (Jiang *et al*. 2015)	2015	China; Western Pacific	September 2007-November 2008	Community	388 MSM approached at gay bars in 5 cities of China	PCR. No further detail provided	388 MSM. No further detail provided	Unknown	All	FPU: 67/388 (17.3; 13.6-21.4) Rectum: 46/388 (11.9; 8.8-15.5) Pharynx: 52/388 (13.4; 10.2-17.2)

Kriesel[a] (Kriesel *et al.* 2016)	2016	USA; The Americas	April 2009-February 2012	Clinic	STI clinic attendees seeking screening and/or treatment were invited to participate in a study via a flyer	FilmArray STI panel (BioFire Diagnostics, LLC, Salt Lake City, Utah)	STI clinic attendees, self-identified MSM, median age 31 years (no range given)	Unknown	All	FPU: 8/66 (12.1; 5.4-22.5) Rectum: 0/16 (0; 0-20.6) Urethral swab: 0/2 (0; 0-84.2) Pooled: 8/106 (7.5; 3.3-14.3)
Lallemand[a] (Lallemand *et al.* 2015)	2015	Germany; Europe	Missing	Clinic	Patients presenting for HIV screening at 18 local public health authorities in North Rhine-Westphalia	Aptima MG (Hologic)	17% of total cohort MSM; median age 30 (25-38)	All	All	FPU: 11/549 (2.0; 1.0-3.6)
Lefebvre[a] (Lefebvre *et al.* 2017)	2016	France; Europe	2014	Clinic	All patients presenting for STI screening at Nantes STI Reference Centre, France	Diangenode S-DiamgTV (Diagnode ST, Liege, Belgium)	651 patients, 357 men; 14% of total cohort MSM; mean age 28 (SD 5.8)	All	All	FPU: 2/92 (2.2; 0.3-7.6)
								Positive	All	FPU: 0/2 (0; 0.0-84.2)
								Negative	All	FPU: 2/90 (2.2; 0.3-7.8)
Libois[a] (Libois *et al.* 2018)	2018	Belgium; Europe	October 2012-February 2014	Clinic	Men presenting with symptoms of urethritis to emergency unit or STI clinic of University Saint-Pierre hospital	In-house realtime PCR	Total cohort 187 men, 37% MSM; median age 30 (IQR 26-38)	All	Symptomatic	FPU: 7/55 (12.7; 5.3-24.5)
								Positive	Symptomatic	FPU: 1/12 (8.3; 0.2-38.5)
								Negative	Symptomatic	FPU: 6/43 (14.0; 5.3-27.9)
Manhart[a] (Manhart *et al.* 2013)	2013	USA; The Americas	January 2007- July 2011	Clinic	Men with NGU attending a STI clinic in Seattle, Washington were prospectively recruited for a RCT	NAAT	All English speaking >16 years of age; total cohort 606 men; 33% MSM; no age data for MSM	All	Symptomatic	FPU: 22/199 (11.1; 7.1-16.3)
								Positive	Symptomatic	FPU: 1/14 (7.1; 0.2-33.9)
								Negative	Symptomatic	FPU: 21/171 (12.3; 12.3-18.2)
Mezzini[a] (Mezzini *et al.* 2013)	2013	Australia; Western Pacific	May 2007-June 2011	Clinic	Men presenting with symptoms of urethritis to STI clinic of Royal Adelaide Hospital	TaqMan real-time PCR	1957 men tested, 31% <25 years of age; 16.3% MSM	All	Symptomatic	FPU: 6/193 (3.1; 1.1-6.6)
								Negative	Symptomatic	FPU: 6/187 (3.2; 1.2-6.9)
Moi[a] (Moi *et al.* 2009b)	2009	Norway; Europe	November 2005-December 2007	Clinic	Retrospective analysis of men at high risk of a STI, voluntarily presenting to a STI clinic	Real-time PCR	8468 men included, mean age 31.3 (+/-8.9); 10.1% MSM	All	All	FPU: 10/859 (11.6; 0.6-2.1)
								Positive	All	FPU: 0/6 (0; 0-45.9)
								Negative	All	FPU: 7/678 (1.0; 0.4-2.1)
								All	Asymptomatic	FPU: 4/694 (0.6; 0.2-1.5)
								All	Symptomatic	FPU: 5/165 (3.0; 1.0-6.9)
Munson (Munson *et al.* 2017)	2017	USA; The Americas	2014-2015	Clinic	Retrospective audit of screening practices for men presenting to a STI clinic. Could contain TOC or repeat samples. All rectal samples submitted by males were considered MSM for the purposes of this study	TMA-based analyte-specific reagent (Hologic).	1493 men included, 55% with rectal swabs. No clinical data collected including age	Unknown	Unknown	Rectum: 48/823 (5.8; 4.3-7.7)
Ong[a] (Ong *et al.* 2018a)	2018	Australia; Western Pacific	2012-2016	Clinic	Retrospective analysis of 201 MSM presenting to a STI clinic, diagnosed with proctitis	In-house real-timer PCR	No overall age data reported, men with MG aged 33 (IQR 28-38)	All	Symptomatic	Rectum: 60/201 (29.9; 23.6-36.7)
								Positive	Symptomatic	Rectum: 17/25 (68.0; 46.5-85.1)
								Negative	Symptomatic	Rectum: 43/115 (37.4; 28.5-46.9)

Pereyre (Pereyre *et al.* 2017)	2017	France; Europe	September 2014-January 2015	Laboratory	Prospective collection of 2594 consecutive urogenital specimens submitted to 16 medical diagnostic units. Could contain TOC or repeat samples. All rectal samples submitted by males were considered MSM for the purposes of this study	In-house PCR	2594 patients, 51 rectal swabs from men; median age 25 (overall range 1-90)	Unknown	Unknown	Rectum: 3/51 (5.9; 1.2-16.2)
Peters[a] (Peters *et al.* 2017)	2017	South Africa; Africa	Dates not reported	Clinic	Prospective cohort of 78 MSM presenting with urethral discharge to two primary healthcare clinics	States 'molecular microbiological investigations'.	MSM with urethral discharge; age data not reported	All	Symptomatic	FPU: 1/78 (1.3; 0-6.9) Rectum: 0/78 (0; 0-4.6) Pooled: 1/78 (1.3; 0-6.9)
								Positive	Symptomatic	FPU: 0/43 (0; 0-8.2) Rectum: 0/43 (0; 0-8.2) Pooled: 0/43 (0; 0-8.2)
								Negative	Symptomatic	FPU: 1/32 (3.1; 0-16.2) Rectum: 0/32 (3.1; 0-16.2) Pooled: 1/32 (3.1; 0-16.2)
Philibert (Philibert *et al.* 2014)	2014	France; Europe	2012	Clinic	116 consecutive MSM presenting to an urban public health clinic for STI diagnosis and/or treatment	Cobas 4800 (Roche)	96% Caucasian; mean age 46.4 (+/- 9.4)	All	All	FPU: 0/116 (0; 0-3.1) Rectum: 1/116 (0; 0-4.7) Pharynx: 0/116 (0; 0-3.1)
								Positive	All	FPU: 0/99 (0; 0-3.7) Rectum: 1/99 (1.0; 0-5.5) Pharynx: 0/99 (0; 0-3.7)
								Negative	All	FPU: 0/17 (0; 0-19.5) Rectum: 1/17 (5.9; 0.1-28.7) Pharynx: 0/17 (0; 0-19.5)
Plaas (Plaas *et al.* 2015)	2015	Estonia; Europe	August 2014-February 2015	Community	Samples collected via Internet-based self-testing	NAAT on Luminex xMAP platform	233 Estonian MSM; age data not reported	Unknown	Unknown	FPU: 6/233 (2.6; 1.0-5.5) Rectum: 6/233 (2.6; 1.0-5.5) Pharynx: 0/233 (0; 0-1.6)
Rane (Rane *et al.* 2014)	2014	Australia; Western Pacific	January 2006-December 2011	Clinic	Retrospective review of men with acute NGU (first presentation)	ProbeTec-ETCT amplified DNA assay (Beckton, Dickinson)	5452 males with NGU, 344 exclusively MSM; median age 31 (IQR 26-41)	Unknown	Symptomatic	FPU: 23/344 (6.7; 4.3-9.9)
Read[a] (Read *et al.* 2017b)	2017	Australia; Western Pacific	August 2016-September 2017	Clinic	Consecutive MSM presenting to a STI clinic for screening	ResistancePlus MG test (SpeeDx Australia)	Asymptomatic MSM; median age 28.8 (IQR 24.3-34.1)	All	Asymptomatic	FPU: 27/1001 (2.7; 1.8-3.9) Rectum: 70/1001 (7.0; 5.5-8.8) Pharynx: 1/54 (1.9; 0.0-9.9) Pooled: 95/1001 (9.5; 7.7-11.5)
								Positive	Asymptomatic	Pooled: 5/107 (4.7; 1.5-10.6)
								Negative	Asymptomatic	Pooled: 90/894 (10.1; 8.2-12.2)
								All	Symptomatic	Pooled: 83/1019 (8.1; 0.9-10.0)
								All	Symptomatic	Pooled: 20/355 (5.6; 3.5-8.6)
Reinton (Reinton *et al.* 2013)	2013	Norway; Europe	January 2009-May 2011	Clinic	Samples from consecutive men who had submitted an anorectal swab. Samples from STI clinics sent to laboratory	Cobas TaqMan CT Test, v2.0 (Roche)	2289 Norwegian MSM; median age 32.9 (range 15.7-81.5)	Unknown	Unknown	FPU: 26/1778 (1.5; 1.0-2.1) Rectum: 69/1778 (3.9; 3.0-4.9) Pooled: 91/1778 (5.1; 4.1-6.2);
	2016	England;		Clinic				All	Symptomatic	FPU: 16/136 (11.8; 6.9-18.4)

Reynolds-Wright[a] (Reynolds-Wright *et al.* 2016)		Europe	September 2015- January 2016		Samples from consecutive men who had NGU, presenting to a STI clinic	Fast Track Diagnostics™ urethritis PCR	77.6% white ethnicity; 8.2% HIV-positive; mean age 33.2	Positive	Symptomatic	FPU: 4/32 (12.5; 3.5-29.0)
								Negative	Symptomatic	FPU: 12/104 (11.5; 6.1-19.3)
Ruutel (Ruutel *et al.* 2015)	2015	Estonia; Europe	April 2013- September 2013	Community	Online questionnaire promoted on Estonian gay social media pages. STI testing optional, with questionnaire linked to samples	PCR	Estonian MSM >18years; median age 31 (range 18-67)	All	Unknown	FPU: 0/65 (0; 0-5.5)
								Positive	Unknown	FPU: 0/3 (0; 0-70.1)
								Negative	Unknown	FPU: 0/40 (0;0-8.8)
Soni (Soni *et al.* 2010)	2010	England; Europe	February 2008- July 2008	Clinic	MSM attending STI clinic in Brighton, UK, for STI screening	MG Pa real-time PCR	438 MSM; 94.4% white ethnicity; mean age 36 (range 16-81)	All	All	FPU: 11/438 (2.5; 1.3-4.4) Rectum 20/412 (4.9; 3.0-7.4)
								Positive	All	FPU: 7/90 (7.8; 3.2-15.4) Rectum 12/83 (14.5; 7.7-23.9)
								Negative	All	FPU: 7/329 (2.1; 0.9-4.3) Rectum 5/348 (1.4; 0.5-3.3)
Unemo (Unemo *et al.* 2018)	2018	Denmark; Europe	March 2016- June 2016	Clinic	Consecutive male and female subjects attending the STI clinics in Denmark, Norway and Sweden	MG Alt TMA-1 (Hologic)	5269 males total; only Denmark tested rectal swabs - 766 males in Denmark; Denmark overall median age 29; males median age 30 (range 15-79)	Unknown	Unknown	Rectum: 15/237 (6.3; 3.6-10.2)
van der Veer[a] (van der Veer *et al.* 2016)	2016	Netherlands; Europe	March 2014- October 2014	Clinic	Data from routine patient care retrospectively analysed	MGPa real-time PCR	1204 men, 678 MSM; median age 41 (IQR 31-51)	All	All	FPU: 17/678 (2.5; 1.5-4.0)
								All	Asymptomatic	FPU: 15/626 (2.4; 1.3-3.9)
								All	Symptomatic	FPU: 2/52 (3.8; 0.5-13.2)
Wu (Wu *et al.* 2013)	2013	China; Western Pacific	March 2009- May 2010	Clinic	Patients recruited from HIV clinic at Jiangsu Centers for Diseases Prevention and Control	Nested PCR	243 HIV-infected MSM; mean age 35 (range 19-68)	Positive	Unknown	FPU: 62/243 (25.5; 20.2-31.5)
Xiao[a] (Xiao *et al.* 2018)	2018	USA; The Americas	February 2015- March 2017	Clinic	Specimens submitted from a STI clinic for testing	Cobas 4800 (Roche)	530 specimens, 79 rectal swabs from men; no age data reported	Unknown	Unknown	Rectum: 2/79 (2.5; 0.3-8.8)
Zheng (Zheng *et al.* 2014)	2014	USA; The Americas	January 2010- May 2010	Clinic	Participants were recruited from a MSM voluntary counselling and testing clinic in Shenzhen, China	MGPa real-time PCR	409 MSM; 96% Han ethnicity; mean age 30.5 (range 18-64)	All	All	FPU: 14/406 (3.4; 1.9-5.7) Rectum: 22/405 (5.4; 3.4-8.1)
								Positive	All	Pooled: 3/16 (18.8; 4.0-45.6)
								Negative	All	Pooled: 19/389 (4.9; 3.0-7.5)

Abbreviations: HIV, human immunodeficiency virus; MG, *Mycoplasma genitalium*; n, number positive for *M.genitalium*; N, total number of samples tested for *M.genitalium*; CI, confidence interval; STI, sexually transmitted infection; MSM, men who have sex with men; PCR, polymerase chain reaction; SOPV, sex on premises venue; FPU, first pass urine; NGU, nongonococcal urethritis; AIDS, acquired immune deficiency syndrome; SD, standard deviation; TMA, transmediated amplification assay; TOC, test of cure; qPCR, quantitative PCR; +ve, positive; -ve, negative; IQR, interquartile range; MTFTG, male to female transgendered; RCT, randomised controlled trial; NAAT, nucleic acid amplification testing. **Notes:** [a]Additional data was provided by the study authors; [b]Estimates from Britain's third National Survey of Sexual Attitudes and Lifestyles (Natsal-3)

Table 9. Summary of the characteristics of the included studies in the meta-analysis (n=46)

	All n=46	Urethra n=34, (total sample= 13753[a])	Rectum n=25 (total sample= 8629)	Pharynx n=7 (total sample= 1871)
Publication year (range)	2006-2018	2006-2018	2008-2018	2009-2018
Period of sample collection[b]	2002-2017	2002-2017	2002-2017	2002-2017
Symptom status				
Asymptomatic	12	9 (3449)	4 (2150)	1 (54)
Symptomatic	18	12 (1494)	5 (436)	0 (0)
HIV-status				
Negative	21	14 (4026)	7 (1058)	1 (17)
Positive	27	18 (1889)	11 (1274)	2 (156)
WHO Geographic region				
Africa	1	1 (78)	1 (78)	0 (0)
Europe	20	14 (5329)	10 (3534)	2 (349)
The Americas	12	8 (3808)	7 (1866)	1 (57)
Western Pacific	13	11 (4538)	7 (3151)	4 (1465)
Recruitment setting				
Laboratory	2	0 (0)	2 (156)	0 (0)
Community[c]	8	9 (4245)	3 (1054)	2 (621)
Clinic[d]	36	26 (8998)	8 (7355)	4 (735)
Type of study				
Cross-sectional	36	28 (10594)	19 (5988)	6 (1817)
Prospective cohort	4	3 (1278)	3 (1512)	1 (54)
Retrospective cohort	6	3 (1881)	3 (1129)	0 (0)

Abbreviations: n, number; WHO, World Health Organisation; STI, sexually transmitted infection; HIV, human immunodeficiency virus

Notes: [a]Includes 13751 first pass urines and 2 urethral smears; [b]Five studies missing date of sample collection; [c]Includes one study conducted at a sex on premises venue; [d]Includes 28 studies which recruited from STI clinics, 5 studies which recruited from HIV clinics, 1 mixed methods study (Cosentino) which predominately recruited from a HIV clinic, 1 study which recruited HIV-positive men in an anal cancer screening program (Fuchs) and 1 study conducted at primary health care centres which target MSM (Peters).

Table 10. Analyses assessing prevalence of *Mycoplasma genitalium* by site.

	Overall					Urethra[a]					Rectum					Pharynx				
Analysis	No	SE, %(95% CI)		I^2%[b]	p-value[c]	No	SE, %(95% CI)		I^2%[b]	p-value[c]	No	SE, %(95% CI)		I^2%[b]	p-value[c]	No	SE, %(95% CI)		I^2%[b]	p-value[c]
Overall prevalence	46	5.8	(4.5-7.3)	95.0		34	4.6	(3.0-6.4)	94.4		25	6.1	(4.5-7.9)	89.0		7	1.0	(0.0-5.1)	96.0	
Symptom status																				
Asymptomatic	12	4.0	(1.5-3.9)	90.7		9	2.2	(1.0-3.7)	81.8		4	7.5	(5.4-10.0)	72.5		1	1.9	(0.0-7.8)	-	
Symptomatic	18	9.2	(6.2-12.7)	87.3	0.003	12	7.1	(4.7-9.7)	67.2	<0.001	5	16.1	(7.2-27.5)	82.9	0.039	0	-	-	-	-
HIV-status																				
Negative	21	5.7	(3.5-8.2)	93.1		14	3.4	(1.8-5.5)	82.6		7	6.8	(1.2-15.8)	94.4		1	0.0	(0.0-18.4)		
Positive	27	9.0	(5.2-13.4)	90.7	0.019	18	7.0	(3.0-12.2)	86.3	0.006	11	10.6	(5.5-17.0)	89.4	0.456	2	0.3	(0.0-2.2)		0.819
WHO Geographic region																				
Africa	1	0.6	(0.1-3.5)	-	Ref[d]	1	1.3	(0.2-6.9)	-	Ref[d]	1	0.0	(0.0-4.7)	-	Ref[de]	0	-	-	-	-
Europe	20	3.1	(2.1-4.2)	80.2	0.034	14	2.2	(1.3-3.3)	75.3	0.698	10	4.3	(3.1-5.7)	56.0	0.006	2	0.0	(0.0-5.2)	-	Ref[d]
The Americas	12	6.6	(4.9-8.5)	83.0	<0.001	8	7.4	(4.8-10.6)	86.5	0.021	7	6.3	(4.1-8.9)	65.0	0.001	1	1.8	(0.3-9.3)	-	0.086
Western Pacific	13	9.9	(6.1-14.5)	97.8	<0.001	11	8.2	(4.0-13.6)	97.1	0.030	7	9.5	(5.1-15.1)	95.5	<0.001	4	1.8	(0.0-10.3)	97.9	0.273
Recruitment setting																				
Laboratory	2	3.6	(1.1-7.4)	-	Ref[d]	-	-	-	-	-	2	3.6	(1.1-7.4)	-	Ref[d]	-	-	-	-	-
Community[f]	8	3.9	(1.0-8.4)	98.2	0.935	8	4.2	(1.2-8.9)	97.2		3	4.5	(0.4-12.3)	-	0.915	3	1.9	(0.0-13.7)	-	-
Clinic[g]	36	6.3	(4.9-7.9)	93.1	0.235	26	5.4	(3.7-7.5)	92.7	0.580	20	6.9	(4.8-8.6)	88.6	0.184	4	0.2	(0.0-1.7)	57.8	0.599

Abbreviations: No, number of studies; SE, Summary Estimate; CI, confidence interval; HIV, human immunodeficiency virus; WHO, World Health Organisation; Ref= reference group

Notes: [a]Includes 13751 first pass urines and 2 urethral smears; [b]I^2 statistic to estimate the proportion of total variability in summary estimates attributed to heterogeneity other than that due to chance. All I^2 statistic values had a p-value<0.001. [c]p-values reflect random-effects meta-analyses to determine differences in pooled summary estimates between subgroups; [d]Reference refers to reference group for statistical comparison between subsequent groups; [e]Meta-analysis unable to run with naught value in reference group, Peters et. Al paper prevalence altered to 0.5%; [f]Includes one study conducted at a sex on premises venue; [g]Includes 28 studies which recruited from STI clinics, 5 studies which recruited from HIV clinics, 1 mixed methods study (Cosentino) which predominately recruited from a HIV clinic, 1 study which recruited HIV-positive men in an anal cancer screening program (Fuchs) and 1 study conducted at primary health care centres which target MSM (Peters).

3.5.3 *Mycoplasma genitalium* prevalence at the urethra

Prevalence of *M.genitalium* at the urethra (n=34 studies) ranged from 0% to 25.5%, with a summary estimate of 4.6% (95%CI: 3.0-6.4; I^2=94.4%) (Table 10).

Of the 15 studies reporting symptom status at the urethra, 12 included symptomatic men and 9 asymptomatic men. Prevalence of *M.genitalium* was significantly higher among men with urethral symptoms [7.1% (95%CI: 4.7-9.7;I^2=67.2)] than among asymptomatic men [2.2% (95%CI: 1.0-3.7; I^2=81.8)](p<0.001)(Table 10, Figure 11).

Twenty studies examining prevalence of *M.genitalium* at the urethra reported HIV status; 18 studies included men living with HIV and 14 included HIV-negative men. Urethral prevalence of *M.genitalium* was significantly higher among men living with HIV [7.0% (95%CI: 3.0-12.2;I^2=86.3)] than among HIV-negative men [3.4% (95%CI: 1.8-5.5; I^2=82.6)](p=0.006) (Table 10, Figure 12).

Prevalence of urethral *M.genitalium* varied across geographical regions (Table 10), although assessment of geographical regions was impacted by the limited number of countries providing data in each region. Prevalence of urethral *M.genitalium* was 1.3% (95%CI 0.2-6.9) in the single African study, 2.2% (95%CI: 1.3-3.3; I^2=75.3) across European studies (n=14 studies), 7.4% (95%CI: 4.8-10.6; I^2=86.5) in studies from the Americas (n=8 studies), and 8.2% (95%CI: 4.0-13.6; I^2=97.1) across studies from the Western Pacific (n=11 studies). Prevalence of *M.genitalium* did not differ by recruitment setting (Table 10).

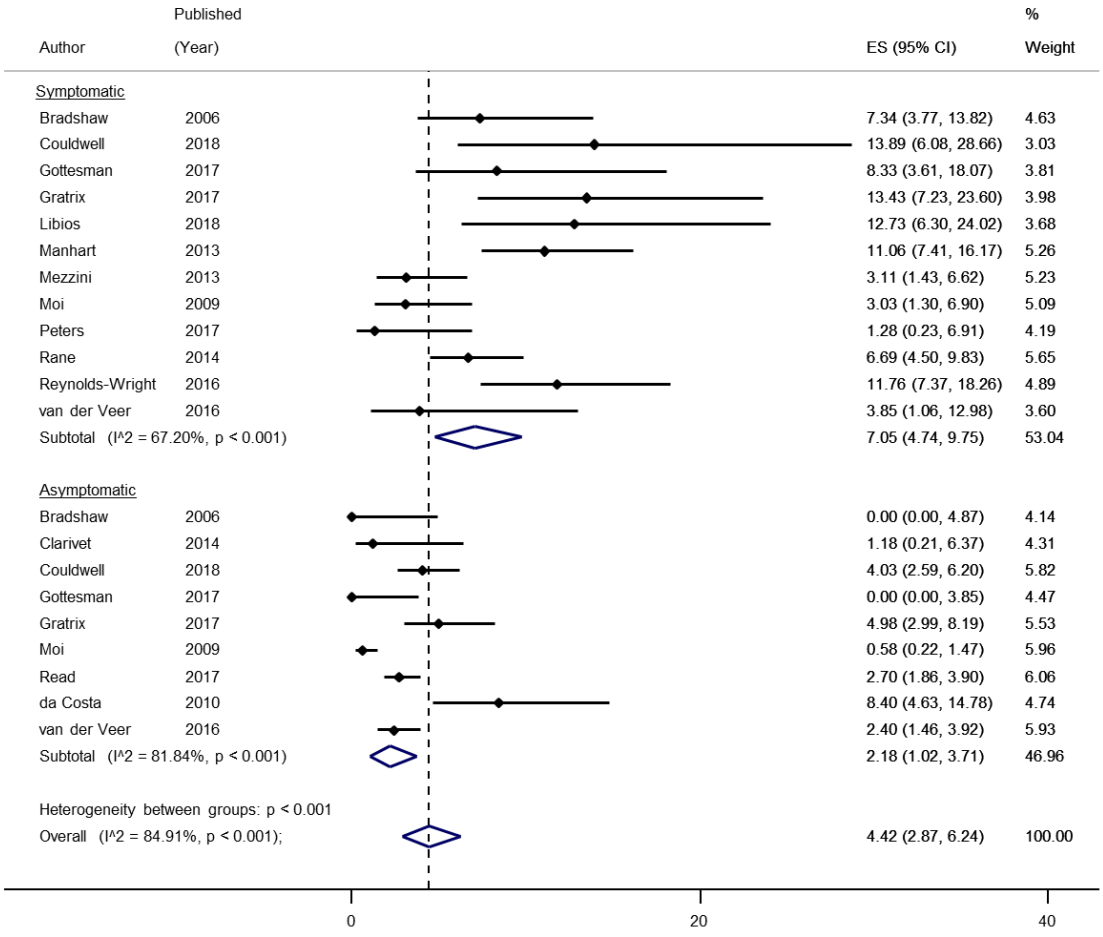

Figure 11. Prevalence of *Mycoplasma genitalium* in the urethra of MSM, by symptom status.

Abbreviations: CI=confidence interval

Notes: Symptomatic refers to studies included containing a study population described as symptomatic at the urethra; asymptomatic refers to studies included containing a study population described as asymptomatic at the urethra. Studies that did not expressly state the population was symptomatic or asymptomatic were excluded from the analysis (n=19).

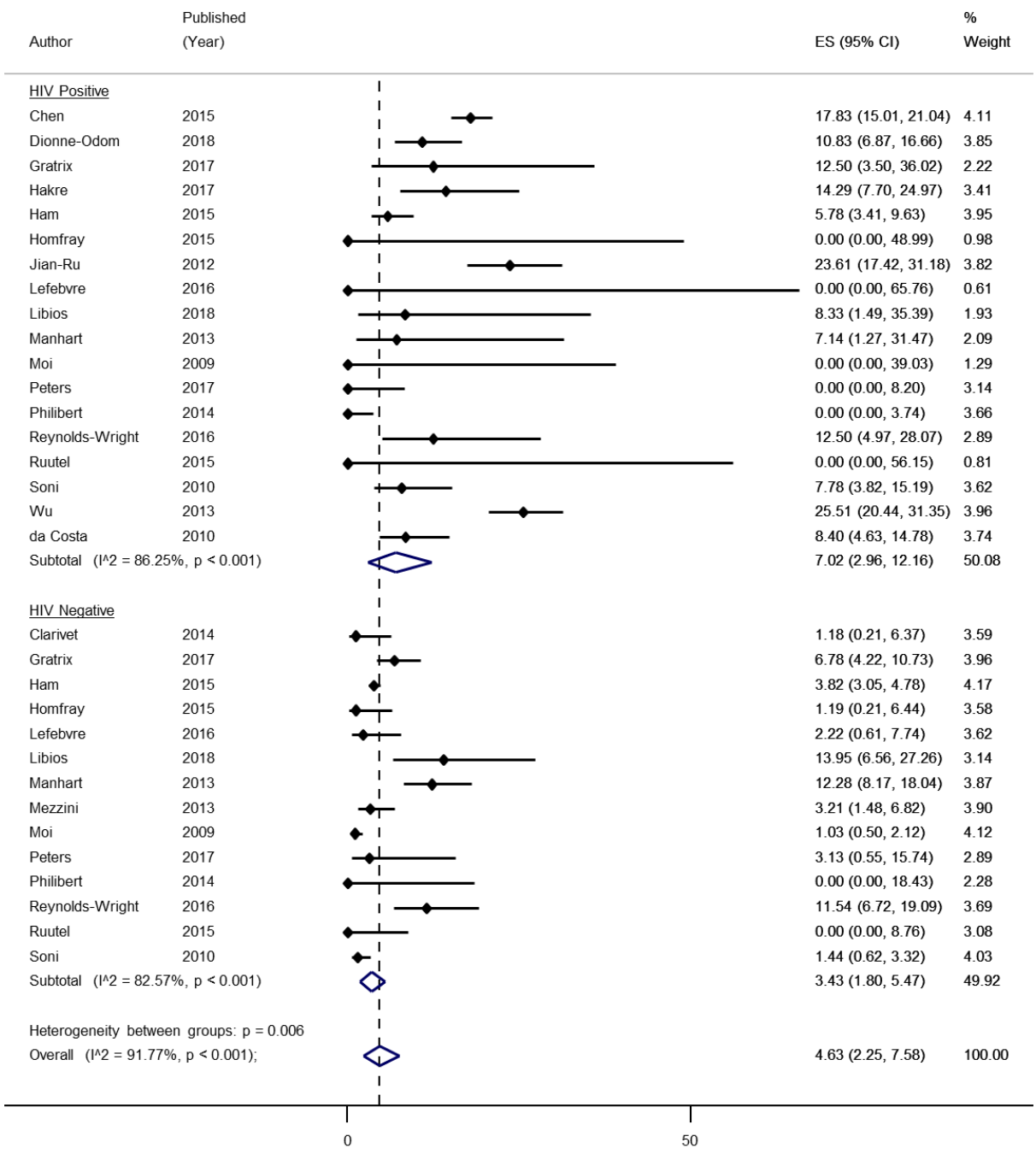

Figure 12. Prevalence of *Mycoplasma genitalium* in the urethra of MSM, by HIV-status.

Abbreviations: CI=confidence interval; HIV= human immunodeficiency virus

Notes: Studies that did not expressly state the population was HIV-positive or HIV-negative were excluded from the analysis (n=14).

3.5.4 *Mycoplasma genitalium* prevalence at the rectum

Prevalence of *M.genitalium* at the rectum (n=25 studies) ranged from 0% to 29.9%, with a summary estimate of 6.1% (95%CI: 4.5-7.9; I2=89.0) (Table 10). One outlier study by Ong et. al. reported a prevalence of 29.9% (Ong et al. 2018a). A sensitivity analysis excluding this study resulted in a summary estimate of 5.4% (95%CI: 4.2-6.8; I^2=81.9) (Table 11).

Of the seven studies reporting symptom status at the rectum, five included symptomatic men and four asymptomatic men. Prevalence of *M.genitalium* was higher among men with rectal symptoms [16.1% (95%CI: 7.2-27.5; I^2=82.9)] than asymptomatic men [7.5% (95%CI: 5.4-10.0; I^2=72.5)] (p=0.039), (Table 10, Figure 13t).

Eleven studies examining prevalence of *M.genitalium* at the rectum reported HIV status; all included men living with HIV, with seven also including HIV-negative men. Rectal prevalence of *M.genitalium* was not different among men living with HIV [10.6% (95%CI: 5.5-17.0; I^2=89.4)] compared to HIV-negative men [6.8% (95%CI: 1.2-15.8; I^2=94.4)](p=0.456), (Table 10, Figure 14).

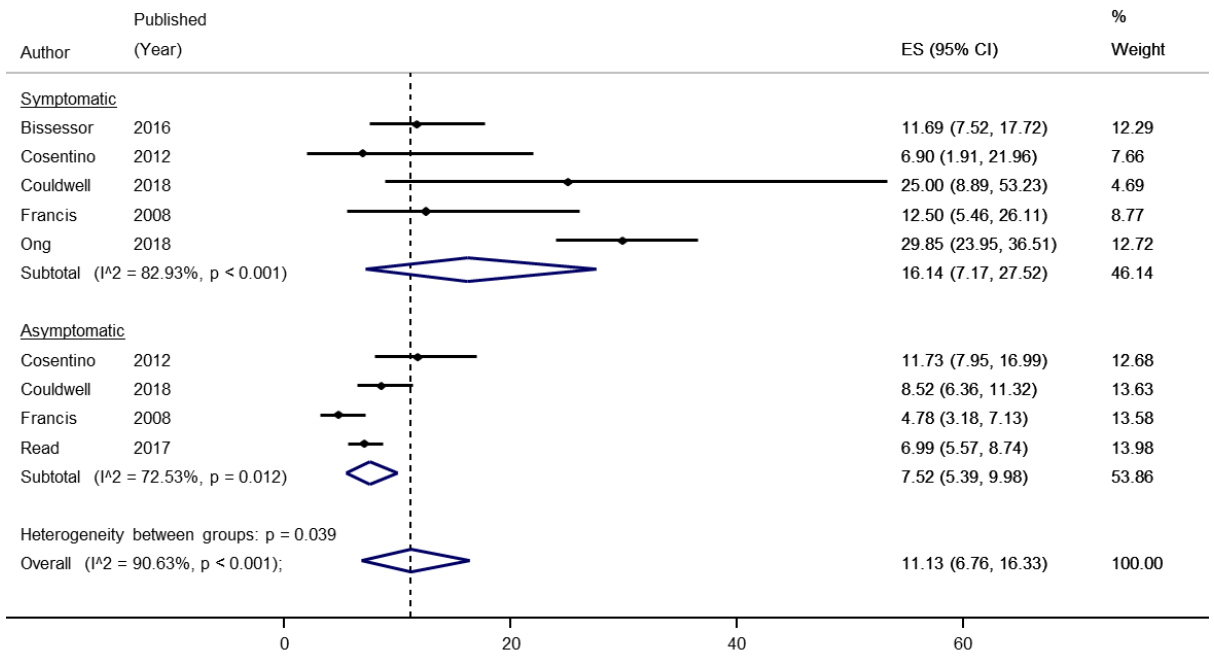

Figure 13. Prevalence of *Mycoplasma genitalium* in the rectum of MSM, by symptom status.

Abbreviations: CI=confidence interval

Notes: Symptomatic refers to studies included containing a study population described as symptomatic at the rectum; asymptomatic refers to studies included containing a study population described as asymptomatic at the rectum. Studies that did not expressly state the population was symptomatic or asymptomatic were excluded from the analysis (n=18).

The prevalence of rectal *M.genitalium* varied across geographical regions (Table 10). Prevalence of rectal *M.genitalium* was 0.0% (95%CI: 0.0-4.7) in the single African study, 4.3% (95%CI: 3.1-5.7; I^2=56.0) across European studies (n=10 studies), 6.3% (95%CI: 4.1-8.9; I^2=65.0) in studies from the Americas (n=7 studies) and 9.5% (95%CI: 5.1-15.1; I^2=95.5) across studies from the Western Pacific (n=7 studies). Prevalence of *M.genitalium* did not differ by recruitment setting (Table 10).

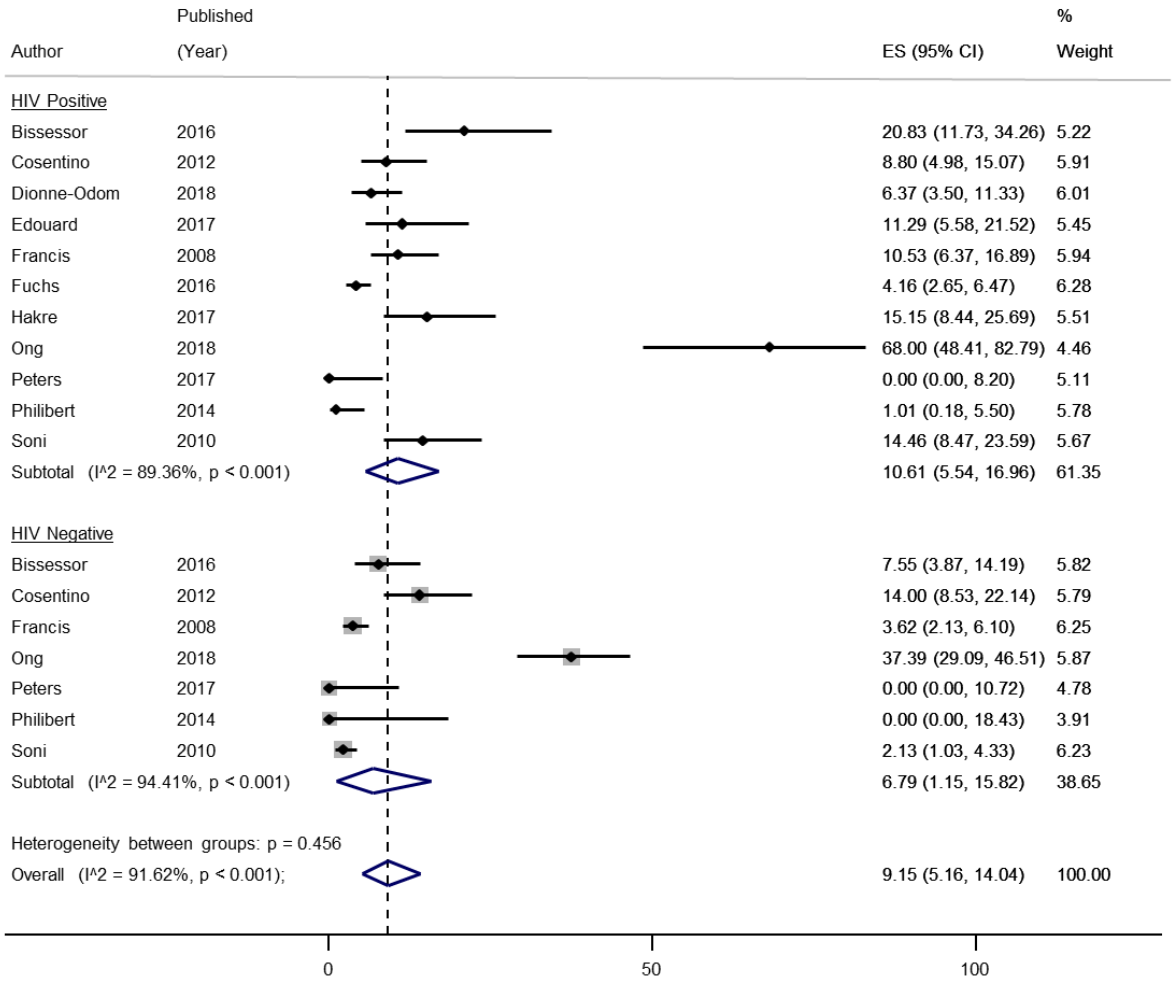

Figure 14. Prevalence of *Mycoplasma genitalium* in the rectum of MSM, by HIV-status.

Abbreviations: CI=confidence interval; HIV= human immunodeficiency virus

Notes: Studies that did not expressly state the population was HIV-positive or HIV-negative were excluded from the (n=14).

3.5.5 *Mycoplasma genitalium* prevalence at the pharynx

Prevalence of *M.genitalium* at the pharynx (n=7 studies) ranged from 0% to 13.4%, with a summary estimate of 1.0% (95%CI: 0.0-5.1;I2=96.0)(Table 10, Figure 15). Six of the seven studies reported pharyngeal prevalence between 0% and 2%. One outlier study by Jiang *et al* reported a prevalence of 13.4% (Jiang et al. 2015). A sensitivity analysis excluding this study resulted in a summary estimate of 0.0% (95% CI 0.0 to 0.3; I^2=36.6) (Table 11) . As symptom status was only reported in one study (Read *et al.* 2017b), subgroup analysis by symptoms was not performed.

Two studies examining prevalence of *M.genitalium* at the pharynx reported HIV status. One study included MSM living with HIV only, and the other included both men living with HIV and HIV-negative MSM, with no difference in the prevalence of pharyngeal *M.genitalium* by HIV-status (p=0.891) (Table 10).

The prevalence of pharyngeal *M.genitalium* did not differ by geographical region, ranging from 0.0% (95%CI: 0.0-5.2) across European studies (n=2 studies), to 1.8% (95%CI: 0.3-9.3) in studies from the Americas (n=1 study) and 1.8% (95%CI: 0.0-10.3) across studies from the Western Pacific (n=4 studies) (Table 10). Prevalence of *M.genitalium* did not differ by recruitment setting (Table 10).

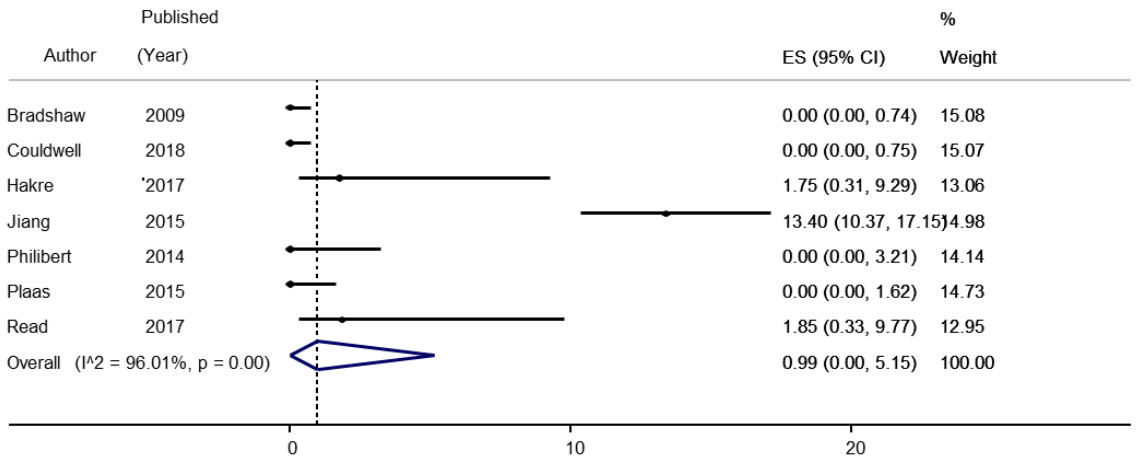

Figure 15. Prevalence of *Mycoplasma genitalium* in the pharynx of MSM.

Abbreviations: CI, confidence interval

3.5.6 Between-Study Bias

Assessment of funnel plots indicated no small study effects (Figure 16, Figure 17 & Figure 18), although inclusion criteria required studies to contain ≥50 MSM. There was no evidence of publication bias by the Egger test, with a co-efficient of 0.23 (95%CI: -0.65-1.11; p=0.573).

3.5.7 Within-Study Bias

Table 12 provides a summary of risk of bias for included studies, using the adapted tool from Hoy *et al.* (Table 7). Twenty-one studies (46%) were deemed to be at low risk of bias, twenty-three (50%) at medium risk, and two studies (4%) at high risk of bias (Munson *et al.* 2017; Xiao *et al.* 2018). Only one study was considered as low risk of bias across all domains (Homfray *et al.* 2015). Thirteen studies randomly or consecutively selected participants or specimens (Bissessor *et al.* 2016; Clarivet *et al.* 2014; Cox *et al.* 2017; Francis *et al.* 2008; Gottesman *et al.* 2017; Gratrix *et al.* 2017; Hakre *et al.* 2017; Homfray *et al.* 2015; Pereyre *et al.* 2017; Philibert *et al.* 2014; Read *et al.* 2017b; Unemo *et al.* 2018). Seven studies did not state the study population to be MSM but used rectal samples from men (Cox *et al.* 2017; de Jong *et al.* 2016; Munson *et al.* 2017; Pereyre *et al.* 2017; Reinton *et al.* 2013; Unemo *et al.* 2018; Xiao *et al.* 2018), which for the purpose of this meta-analysis were assumed to be from MSM (Callander *et al.* 2015; Templeton *et al.* 2014). Four papers did not provide a numerator or denominator, instead presenting percentage data (Jian-Ru *et al.* 2012; Jiang *et al.* 2015; Munson *et al.* 2017; Plaas *et al.* 2015). Sensitivity analyses which removed studies deemed to be at high risk of representative bias or outlier studies did not show any major shift in summary estimates (Table 11 & Table 13).

Table 11. Sensitivity analyses excluding studies at high risk of bias or outlier studies

	Urethra			Rectum			Pharynx		
	No	SE, %(95% CI)	I^2%[a]	No	SE, %(95% CI)	I^2%[a]	No	SE, %(95% CI)	I^2%[a]
Reported summary estimate (Table 9)	34	4.6 (3.0-6.4)	94.4	25	6.1 (4.5-7.9)	89.0	7	1.0 (0.0-5.1)	96.0
Criteria 2: high risk studies excluded[bc]	27	5.5 (3.6-7.6)	95.2	12	6.1 (4.3-8.3)	87.7	6	1.3 (0.0-6.4)	96.6
Criteria 4: high risk studies excluded[bc]	33	5.3 (3.7-7.3)	94.2	18	6.5 (4.3-9.1)	91.2	7	1.0 (0.0-5.1)	96
Criteria 4: high and moderate risk studies excluded[bc]	23	4.7 (3.0-6.8)	93.5	12	7.1 (4.2-10.8)	92.9	5	1.9 (0.0-10.1)	96.6
Outlier rectal study excluded[d]	-	- -	-	24	5.4 (4.2-6.8)	81.9			
Outlier pharyngeal study excluded[d]	-	- -	-				6	0.0 (0.0-0.3)	36.6

Abbreviations: No, number of studies; SE, Summary Estimate; CI, confidence interval

Notes: [a]I^2 statistic to estimate the proportion of total variability in summary estimates attributed to heterogeneity other than that due to chance. All I^2 statistic values had a p-value<0.001; [b]Criteria 2 and criteria 4 refer to questions 2 and 4 from Table 7 (Adapted from (Hoy *et al.* 2012)); [c]Please refer to Table 12 for studies classified as low, medium or high risk of bias in criteria 2 or 4; [d]Outlier paper refers to papers that fell outside the overall pattern of distribution for prevalence at each site. There were no studies at the urethra were this occurred. At the rectum, (Ong *et al.* 2018a) was excluded, and at the pharynx, (Jiang *et al.* 2015) was excluded.

Table 12. Summary of risk of bias and overall bias scores for included studies.[ab]

Author	Year of publication	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8	Summary Grade[c]
Bissessor (Bissessor *et al.* 2016)	2016	1	0	0	1	0	0	0	0	Low
Bradshaw (Bradshaw *et al.* 2009)	2009	1	0	1	0	0	0	0	1	Low
Bradshaw (Bradshaw *et al.* 2006b)	2006	1	0	1	0	0	0	0	0	Low
Chen (Chen *et al.* 2015)	2015	1	0	1	0	0	0	0	1	Low
Clarivet (Clarivet *et al.* 2014)	2014	1	0	0	0	0	0	1	1	Low
Cosentino (Cosentino *et al.* 2012)	2012	1	1	1	0	1	0	0	1	Medium
Couldwell (Couldwell *et al.* 2018)	2018	1	0	0	1	1	0	0	0	Low
Cox (Cox *et al.* 2017)	2017	1	1	0	2	1	0	0	1	Medium
Creswell (Creswell *et al.* 2012)	2012	0	0	1	0	0	0	0	1	Low
da Costa(da Costa *et al.* 2010)	2010	1	1	1	0	1	0	0	1	Medium
de Jong (de Jong *et al.* 2016)	2016	1	0	1	2	1	0	0	1	Medium
Dionne-Odom (Dionne-Odom *et al.* 2018)	2018	1	0	1	0	0	0	0	1	Low
Edouard (Edouard *et al.* 2017)	2017	1	1	1	1	0	0	1	1	Medium
Francis (Francis *et al.* 2008)	2008	1	1	0	0	1	0	0	1	Medium
Fuchs (Fuchs *et al.* 2016)	2016	1	1	1	1	0	0	0	1	Medium
Gottesman (Gottesman *et al.* 2017)	2017	1	0	0	0	0	0	0	1	Low
Gratrix (Gratrix *et al.* 2017)	2017	1	0	0	0	1	0	0	0	Low
Hakre (Hakre *et al.* 2017)	2017	1	0	0	0	0	0	1	1	Low
Ham (Ham *et al.* 2015)	2015	0	0	1	0	1	0	0	1	Low
Homfray (Homfray *et al.* 2015)	2015	0	0	0	0	0	0	0	0	Low

Jian-Ru (Jian-Ru *et al.* 2012)	2012	1	0	1	1	0	1	0	1	Medium
Jiang (Jiang *et al.* 2015)	2015	1	0	1	0	1	1	0	1	Medium
Kriesel (Kriesel *et al.* 2016)	2016	1	1	1	0	0	0	0	1	Medium
Lallemand (Lallemand *et al.* 2015)	2015	0	0	1	0	1	0	0	1	Low
Lefebvre (Lefebvre *et al.* 2017)	2016	1	0	1	1	0	0	1	1	Medium
Libois (Libois *et al.* 2018)	2018	1	0	1	1	0	0	1	1	Medium
Manhart (Manhart *et al.* 2013)	2013	1	0	1	0	0	0	0	1	Low
Mezzini (Mezzini *et al.* 2013)	2013	1	0	1	0	0	0	0	1	Low
Moi (Moi *et al.* 2009b)	2009	1	1	1	1	0	0	0	1	Medium
Munson (Munson *et al.* 2017)	2017	1	1	1	2	1	1	0	1	High
Ong (Ong *et al.* 2018a)	2018	1	1	1	0	1	0	0	1	Medium
Pereyre (Pereyre *et al.* 2017)	2017	0	1	0	2	1	0	1	1	Medium
Peters (Peters *et al.* 2017)	2017	1	1	1	1	0	0	1	1	Medium
Philibert (Philibert *et al.* 2014)	2014	1	1	0	0	1	0	0	1	Medium
Plaas (Plaas *et al.* 2015)	2015	1	0	1	1	0	1	0	1	Medium
Rane (Rane *et al.* 2014)	2014	1	0	1	0	0	0	0	1	Low
Read (Read *et al.* 2017b)	2017	1	0	0	0	0	0	0	1	Low
Reinton (Reinton *et al.* 2013)	2013	1	0	1	2	1	0	0	1	Medium
Reynolds-Wright (Reynolds-Wright *et al.* 2016)	2016	1	1	1	1	0	0	0	1	Medium
Ruutel (Ruutel *et al.* 2015)	2015	0	0	1	0	0	0	1	0	Low
Soni (Soni *et al.* 2010)	2010	1	0	1	0	1	0	0	1	Medium
Unemo (Unemo *et al.* 2018)	2018	1	0	0	2	1	0	0	1	Medium
van der Veer (van der Veer *et al.* 2016)	2016	1	0	1	1	0	0	0	0	Low

Wu (Wu *et al.* 2013)	2014	1	0	1	1	0	0	0	1	Medium
Xiao (Xiao *et al.* 2018)	2018	1	1	1	2	1	0	1	1	High
Zheng (Zheng *et al.* 2014)	2014	1	1	1	0	0	0	0	0	Low

Notes: [a]To evaluate within study bias, we adapted an instrument designed by Hoy *et al.* (13) which examines both the internal and external validity of the selected studies (Table 7); [b]Risk of bias was based on the published article plus any additional data supplied by the authors; [c]A score of 0 to 3 was classified as low risk of bias; 4 to 6 as medium risk of bias; and 7 to 9 as high risk of bias.

Table 13. Meta-regression comparing studies at low and high risk for non-representativeness of the men who have sex with men study population

	Urethra				Rectum			
	No	SE, %(95% CI)	I^2%[a]	p-value[b]	No	SE, %(95% CI)	I^2%[a]	p-value[b]
Criteria 2 low risk studies[c]	27	5.5 (3.6-7.6)	95.2		12	6.1 (4.3-8.3)	87.7	
Criteria 2 high risk studies[c]	7	4 (1.3-7.9)	89.4	0.501	13	5.9 (3.2-9.4)	90.5	0.943

Abbreviations: No, number of studies; SE, Summary Estimate; CI, confidence interval

Notes: [a]I^2 statistic to estimate the proportion of total variability in summary estimates attributed to heterogeneity other than that due to chance. All I^2 statistic values had a p-value<0.001; [b]p-values reflect random-effects meta-analyses to determine differences in pooled summary estimates between those at low risk and those at high risk of bias. [a]I^2 statistic to estimate the proportion of total variability in summary estimates attributed to heterogeneity other than that due to chance. All I^2 statistic values had a p-value<0.001; [c]Criteria 2 refers to question 2 from Table 7 (Adapted from (Hoy *et al.* 2012)). Please refer to Table 12 for studies classified as low or high risk of bias in criteria 2.

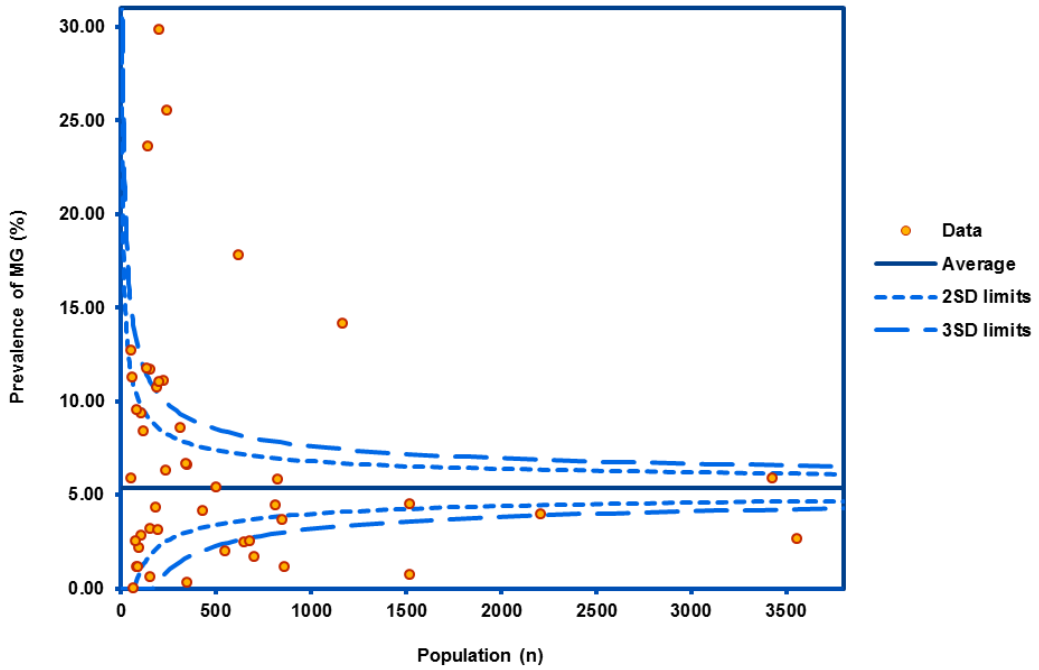

Figure 16. Prevalence of *Mycoplasma genitalium* by size of population studied.

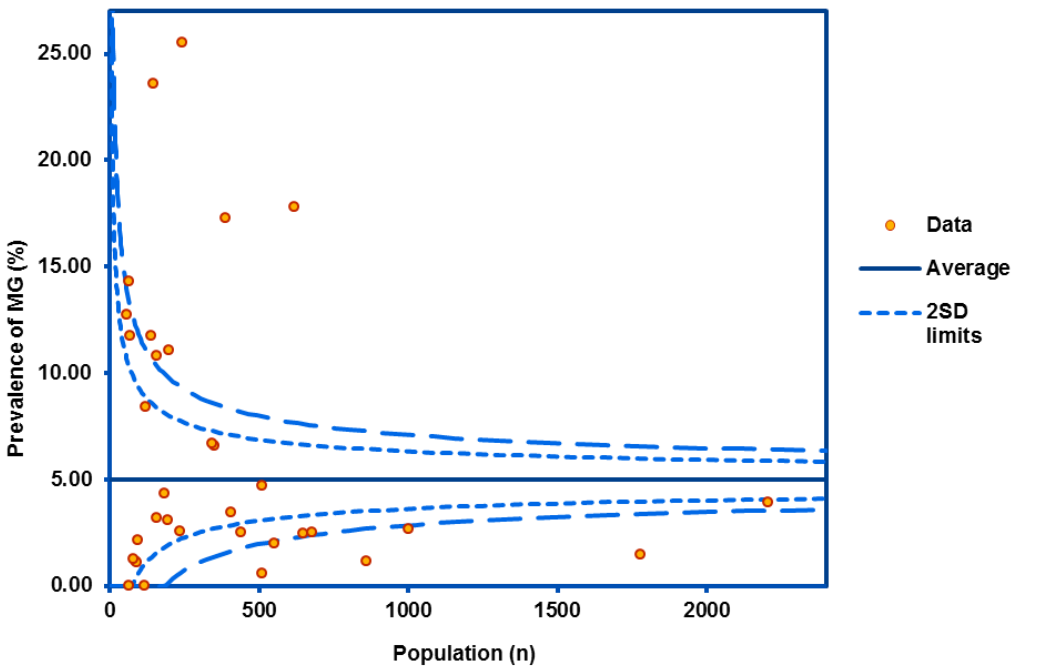

Figure 17. Prevalence of *Mycoplasma genitalium* at the urethra, by size of population studied.

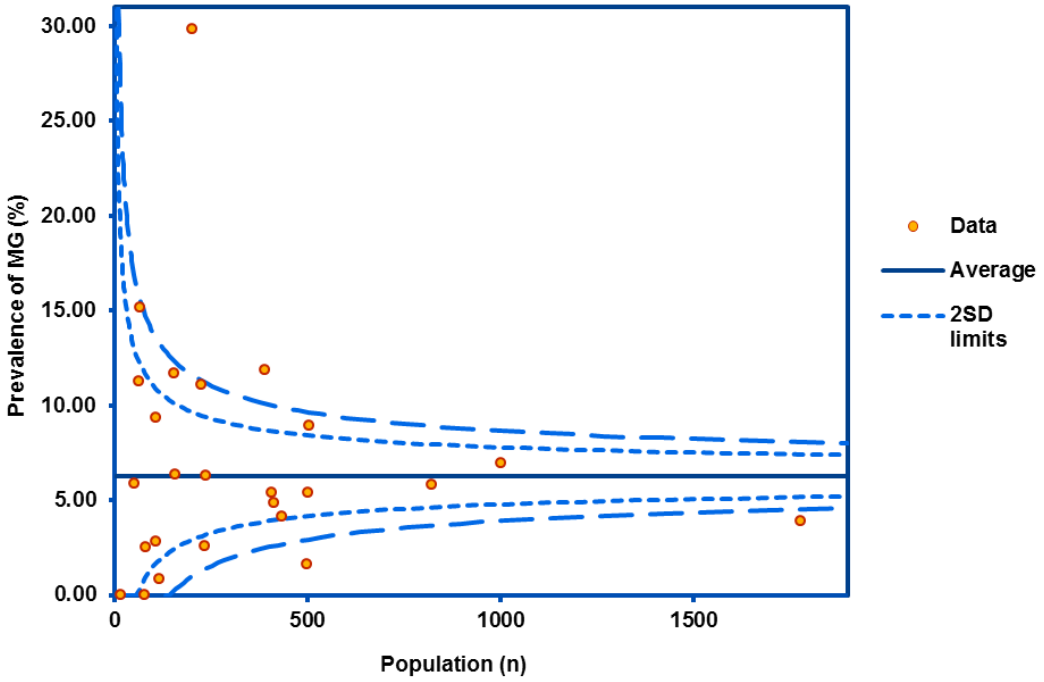

Figure 18. Prevalence of *Mycoplasma genitalium* at the rectum, by size of population studied.

3.6 Discussion

To our knowledge, this is the first meta-analysis to determine the prevalence of *M.genitalium* by anatomical site in MSM tested for infection at each site, and to explore the association between *M.genitalium* and specific factors such as symptom and HIV status. We combined data from 46 studies, representing nearly 24 000 samples. Prevalence of *M.genitalium* in MSM ranged from 0% to 29.9%, with summary estimates of 6.2% (95%CI: 4.6-8.1; I^2=88.1) at the rectum, 5.0% (95%CI: 3.5-6.8; I^2=94.0) in the urethra and 1.0% (95%CI: 0.0-5.1; I^2=96.0) in the pharynx, but estimates were limited by high heterogeneity. *M.genitalium* was more common in MSM with symptoms at the urethra or rectum and more common at the urethra in MSM living with HIV.

The prevalence of *M.genitalium* in this study was similar to *C.trachomatis* prevalence estimates in MSM, which have been reported in the order of 3.5%–3.7% at the urethra, 5.6% (95%CI: 4.8 to 6.3) at the rectum and 0.5% (95%CI: 0.2 to 0.9) at the pharynx (Vodstrcil *et al.* 2011; Lallemand *et al.* 2016). Screening for *C.trachomatis* and *N.gonorrhoeae* at urethral, rectal and pharyngeal sites in MSM is recommended in many countries (CDC 2015; Clutterbuck *et al.* 2016; ASHA 2020). However, WHO states a number of key principles must be satisfied when screening for an infection (Wilson *et al.* 1968), including: 1) the natural history of the condition should be understood and 2) the cost-benefit of finding and treating cases should be balanced (Wilson *et al.* 1968). *M.genitalium* meets neither criteria, with limited knowledge of the natural history of asymptomatic *M.genitalium* infection, particularly in MSM, and increasing antimicrobial resistance resulting in greater use of costly antimicrobials that can be associated with serious side effects (Read *et al.* 2019a).

Our meta- analysis confirmed *M.genitalium* to be uncommonly detected in the pharynx of MSM (1%), although estimates were limited by small numbers of included studies. When the outlier study by Jiang *et al.* was excluded, the overall estimate declined to 0.0% (95%CI: 0.0-0.3; I^2=36.6) (Jiang et al. 2015). In contrast to *N.gonorrhoeae*, where pharyngeal prevalence is 5.5%–8.3% (Morris *et al.* 2006; Cornelisse *et al.* 2017), pooled estimates of *M.genitalium* appear similar to those for *C.trachomatis* at 0.5% (Lewis *et al.* 2012). These data suggest the pharynx does not play a significant role in transmission of *M.genitalium*.

While *M.genitalium* is an established cause of urethritis, its contribution to proctitis has been less clear. Several studies have reported no association between rectal *M.genitalium* and proctitis (Read *et al.* 2019a; Francis *et al.* 2008; Soni *et al.* 2010). However in this meta-analysis *M.genitalium* was associated with symptoms at both the urethra and rectum. These data support recommendations that *M.genitalium* testing should be undertaken in men with urethral symptoms (BASHH *et al.* 2018; CDC 2015), and suggest that *M.genitalium* is associated with rectal symptoms in MSM,

supporting UK and Australian guidelines that recommend consideration of *M.genitalium* testing in men with proctitis (BASHH *et al.* 2018; ASHA 2018).

Our meta-analysis found the prevalence of *M.genitalium* to be higher at the urethra among men living with HIV compared with HIV-negative men. The pattern was similar at the rectum, but there were fewer studies, limiting this comparison. A prior meta- analysis reported an association between *M.genitalium* and HIV (OR=2.01, 95% CI 1.44 to 2.79)(Mavedzenge *et al.* 2009) , but only two studies with data specific to MSM were included (Mavedzenge *et al.* 2009). While our data suggests *M.genitalium* may be more common at the urethra and rectum in HIV- positive men, specific recommendations around testing for *M.genitalium* in men living with HIV cannot be inferred from these results due to the high heterogeneity and limited number of studies. Overall however, these data add plausibility to a relationship between *M.genitalium* and HIV, as has been previously reported (Mavedzenge *et al.* 2009). There were several important limitations to this study. Random- effects meta-analysis takes into consideration real or larger heterogeneity between studies, but despite this we recorded high heterogeneity in most of the summary estimates. Symptoms and HIV status were only known for a subset of studies, reducing precision around these estimates. Assessment of the influence of geographical region on *M.genitalium* prevalence was greatly impacted by the limited number of countries providing regional data. While the prevalence of *M.genitalium* appeared highest in the Western Pacific, studies from this region only originated from Australia and China. Similarly, only one South African study contributed to African estimates, and there were no studies from South East Asian or Eastern Mediterranean regions. High heterogeneity may have resulted from a combination of all three of the risk factors explored (ie, symptoms, HIV status and geographical location), but the small number of studies available for each end point limited our ability to perform multivariable meta- regression analyses (Nyaga *et al.* 2014; Westfall *et al.* 1993). As information on potentially important factors such as age and risk behaviour were not consistently reported, we could not assess the impact of these on heterogeneity. There was limited power to look at the influence of time (ie, year of study) within different subgroups. Few of the included studies were from systematically sampled populations, as screening is not recommended for *M.genitalium*, and testing has generally been limited to symptomatic individuals and specialist services with access to research assays, resulting in over-representation of STI clinics compared with other sites. Thus, estimates may represent a select population, contributing to the significant heterogeneity and limited generalisability of the findings. Finally, this review was limited to studies published in English only, which also limits the generalisability of our findings; however, only four studies were excluded on this basis.

Our meta- analysis found the prevalence of *M.genitalium* to be similar to that of *C.trachomatis* in MSM tested across all three anatomical sites, with associations seen between symptoms and HIV-positivity in subgroup analyses. This meta- analysis provides site- specific estimates for *M.genitalium* in MSM that, despite high heterogeneity, represent biologically plausible patterns of infection. These data provide an evidence base to inform testing and clinical practice, and highlight the need for further research in this population to understand the pathogenic role and natural history of *M.genitalium* in MSM.

4. *Mycoplasma genitalium* pharyngeal infection and rectal co-infection in men who have sex with men.

4.1 Background

Azithromycin is used in the treatment of the two most common sexually transmitted bacterial infections, *C.trachomatis* and *N.gonorrhoeae*. A single dose of one gram is used alone for *C.trachomatis* infections, and in combination with ceftriaxone for *N.gonorrhoeae* infections (ASHA 2020). *M.genitalium* testing is not commonly performed alongside *C.trachomatis* and *N.gonorrhoeae* testing, however a proportion of patients likely to be co-infected with *M.genitalium*. Treatment with azithromycin can result in a macrolide resistant *M.genitalium* infection, with treatment of *M.genitalium* infection with azithromycin leading to the selection of macrolide resistance in at least 12% of infections (Read *et al.* 2017a; Bissessor *et al.* 2015). It is therefore likely that *M.genitalium* infections in MSM are being inadvertently exposed to azithromycin through treatment of *C.trachomatis* and *N.gonorrhoeae* coinfections, which may further induce resistance.

As shown in Chapter Three, there are very limited studies examining the prevalence of *M.genitalium* pharyngeal infection. It is important to determine the prevalence of *M.genitalium* infection at the pharynx, to determine whether the oropharynx contributes to the ongoing transmission of *M.genitalium*.

This study contained in this chapter had the following aims:

1) To determine the proportion of rectal *C.trachomatis* and rectal *N.gonorrhoeae* infections in MSM who are co-infected with rectal *M.genitalium*.
2) To determine the proportion of MSM who are infected with pharyngeal *M.genitalium*.

The findings of the study were published in *Sexually Transmitted Infections*: Latimer RL, Vodstrcil L, De Petra, et. Al. 'Extragenital Mycoplasma genitalium infections among men who have sex with men.' *Sexually Transmitted Infections*. 2019 Jun 19. pii: sextrans-2019-054058. doi: 10.1136/sextrans-2019-054058.

The findings were also presented as an oral presentation at the STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019. Published: Latimer R, Vodstrcil L, Read T, et al. O02.6 Extragenital mycoplasma genitalium infections amongst men who have sex with men. *Sexually Transmitted Infections* 2019; 95:A41.

The paper has been included as text in the thesis to allow for thesis cohesion. No alterations have been made, aside from abbreviations and table numbers, for thesis consistency. Please see Appendix B for the PDF of the published study.

4.2 Abstract

4.2.1 Objectives

There are limited data on the prevalence of *M.genitalium* coinfection with rectal *C.trachomatis* and rectal *N.gonorrhoeae* infections and few studies examining the prevalence of pharyngeal *M.genitalium* in MSM. Using transcription mediated amplification (TMA) assay, this study aimed to determine the proportion of rectal *C.trachomatis* and rectal *N.gonorrhoeae* infections in MSM that are co-infected with rectal *M.genitalium*, and the proportion of MSM with *M.genitalium* detected in the pharynx in order to inform clinical practice.

4.2.2 Methods

This was a cross-sectional study conducted at Melbourne Sexual Health Centre in Australia. Consecutively collected rectal swabs from MSM, that tested positive for *C.trachomatis* (N=212) or *N.gonorrhoeae* (N=212), and consecutively collected pharyngeal samples (N=480) from MSM were tested for *M.genitalium* using the Aptima *Mycoplasma genitalium* Assay (Hologic, San Diego). Samples were linked to demographic data and symptom status.

4.2.3 Results

Rectal *M.genitalium* was co-detected in 27/212 rectal *C.trachomatis* (13%, 95%CI 9-18) and in 29/212 rectal *N.gonorrhoeae* (14%, 95%CI 9-19) samples, with no difference in the proportion positive for *M.genitalium*. MSM with rectal *C.trachomatis*/*M.genitalium* co-infection had more sexual partners than those with rectal *C.trachomatis* monoinfection (mean 6 vs 11, p=0.06). MSM with rectal *N.gonorrhoeae*/*M.genitalium* co-infection were more likely to be HIV positive than those with rectal *N.gonorrhoeae* monoinfection (OR=2.96,95%CI:1.21-7.26, p=0.023). MSM with rectal *C.trachomatis*/*M.genitalium* co-infection were more likely to be using HIV pre-exposure prophylaxis (PrEP) than MSM with rectal *N.gonorrhoeae*/*M.genitalium* co-infection (OR 0.25,95%CI:0.10-0.65, p=0.002). Pharyngeal *M.genitalium* was uncommon and detected in 8 of 464 samples (2%, 95%CI: 1-3%). Pharyngeal *M.genitalium* was associated with having a rectal STI (OR=10.61, 95%CI: 2.30-48.87, p=0.002) and there was a borderline association with being HIV positive (p=0.079).

4.2.4 Conclusion

These data indicate one in seven MSM treated for rectal *C.trachomatis* or rectal *N.gonorrhoeae* will have undiagnosed *M.genitalium* that is potentially exposed to azithromycin during treatment of these STIs. Rectal *M.genitalium* coinfection was associated with specific risk factors which may inform testing practices. Pharyngeal *M.genitalium* was uncommon.

4.3 Introduction

M.genitalium is an established cause of urethritis (Jensen 2004), however there are limited data on the prevalence of *M.genitalium* at anatomical sites other than the urethra. A recent meta-analysis estimated the prevalence of *M.genitalium* in MSM in the community at 3.2% (95%CI: 2.1-5.1) (Baumann *et al.* 2018). Studies in urban STI clinics in Melbourne and Sydney estimate the overall prevalence of *M.genitalium* in MSM between 9.5% and 13.4%, respectively, with a prevalence of 7.0%-8.9% in the rectum, and 2.7%-4.9% in the urethra (Couldwell *et al.* 2018; Read *et al.* 2019a). There are limited data on the prevalence of *M.genitalium* coinfection with the two most common STIs, *C.trachomatis* and *N.gonorrhoeae*. In a study of clinical samples collected at STI clinics in London, *M.genitalium* was found to be a coinfection in 13.0% of *C.trachomatis* and 2.4% of *N.gonorrhoeae* infections in all people at the clinic, including women, using PCR (Broad *et al.* 2017). Screening and/or testing for *C.trachomatis* and *N.gonorrhoeae* at extragenital sites is commonly performed in MSM in line with international guidelines (CDC 2015); however, this is not currently recommended for *M.genitalium.*

Both *C.trachomatis* and *N.gonorrhoeae* have been commonly treated with macrolides, in accordance with international treatment guidelines (CDC 2015; ASHA 2020). Britain has recently moved away from azithromycin for first-line treatment of *C.trachomatis* and *N.gonorrhoeae* due to growing concerns over macrolide resistance (Fifer *et al.* 2019; BASHH 2018). One gram (1g) of azithromycin has also commonly been recommended for treatment of *M.genitalium* (ASHA 2018; CDC 2015); however, macrolide resistance is now detected in at least 40-60% of *M.genitalium* infections in many countries (Gesink *et al.* 2016; Dumke *et al.* 2016), and treatment failure following azithromycin currently exceeds 60% in Melbourne, Australia. Treatment with 1g azithromycin leads to the selection of macrolide resistance in at least 12% of infections (Read *et al.* 2017a; Bissessor *et al.* 2015). The inadvertent exposure of *M.genitalium* to azithromycin during treatment of *C.trachomatis* or *N.gonorrhoeae* infections may lead to the selection of macrolide resistance and contribute to the rising rates of macrolide resistance seen in *M.genitalium* globally over the past decade (Read *et al.* 2017a).

Screening for *N.gonorrhoeae* and *C.trachomatis* in the pharynx is widely recommended in MSM (Clutterbuck *et al.* 2016). There are few studies on the prevalence of *M.genitalium* at the pharynx, with conflicting data from published studies. In two Australian studies *M.genitalium* was not detected at the pharynx in MSM (Couldwell *et al.* 2018; Bradshaw *et al.* 2009). However another study presented at the STI & HIV World Congress in 2015 showed a high prevalence of pharyngeal *M.genitalium* (13.5%) among 388 MSM recruited from gay bars in five cities across China (Jiang *et*

al. 2015). All studies to date have used PCR-based assays. We used a highly sensitive TMA assay to determine the prevalence of *M.genitalium* in MSM attending Melbourne Sexual Health Centre, given the high background prevalence of rectal (7%) and urethral (3%) *M.genitalium* infection in asymptomatic MSM at Melbourne Sexual Health Centre (Read *et al.* 2019a).

This study aimed to determine the proportion of rectal *C.trachomatis* and *N.gonorrhoeae* infections in MSM co-infected with rectal *M.genitalium* and predictors of coinfection, as well as the proportion of MSM infected with *M.genitalium* at the pharynx and risk factors associated with pharyngeal *M.genitalium*.

4.4 Materials and Methods

This was a cross-sectional study among MSM attending Melbourne Sexual Health Centre in Victoria, Australia, between May 2017 and January 2018. Patients presenting to Melbourne Sexual Health Centre routinely complete a computer assisted self-interview about their sexual history including gender, number of partners and condom use over the last 3 months, HIV status, and use of PrEP for HIV. MSM were identified as men attending Melbourne Sexual Health Centre who reported having anal sex with another man in the previous year via the computer assisted self-interview. Melbourne Sexual Health Centre is the largest public sexual health service in Victoria, Australia. The centre provides around 50,000 consultations every year, with 36% of consultations for MSM.

We examined the prevalence of *M.genitalium* rectal co-infection with either *C.trachomatis* or *N.gonorrhoeae,* and the prevalence of *M.genitalium* pharyngeal infection, in serially collected specimens. Clients attending Melbourne Sexual Health Centre are offered free screening for STIs, including rectal and pharyngeal *C.trachomatis* and *N.gonorrhoeae,* with swabs tested using Aptima Combo 2 TMA assay (Hologic, San Diego, California, USA).

4.4.1 Rectal co-infection

Consecutive swabs that tested positive for *C.trachomatis* or *N.gonorrhoeae* were stored at room temperature in Aptima specimen transport tubes until further testing, and samples obtained from MSM were identified. Rectal samples that tested positive for *C.trachomatis* from HIV-positive patients at Melbourne Sexual Health Centre were sent to an external laboratory for testing for Lymphogranuloma venereum (LGV) testing and were therefore not available for this study. Samples from MSM were stored until batches of 100 samples were formed. Samples were stored and tested within 60 days. Samples were assessed prior to further testing to determine if there was sufficient remaining buffer; a minimum of 1.7mL of buffer was required for *M.genitalium* testing and samples without sufficient buffer were discarded. The remaining samples were tested for *M.genitalium* using the Aptima *Mycoplasma genitalium* Assay (Hologic, San Diego). Equal numbers of sequential *C.trachomatis* and *N.gonorrhoeae* positive samples were selected.

4.4.2 Pharyngeal infection

All consecutive pharyngeal swabs with sufficient remaining buffer following testing for *C.trachomatis* and *N.gonorrhoeae* were stored at room temperature, and swabs from MSM were identified. Samples from MSM were stored until batches of 100 samples were formed. Samples

were stored and tested within 60 days. Samples determined to have adequate buffer remaining were tested for *M.genitalium* using the Aptima *Mycoplasma genitalium* Assay (Hologic, San Diego).

4.4.3 Data extraction

Samples were linked to demographic and epidemiological data (e.g., age, number of male partners, condom and PrEP use, and HIV status), as well as clinical diagnosis, presence of any symptoms and reason for presentation and then irreversibly de-identified.

4.4.4 Statistical analysis

All analyses were performed using Stata IC version 14 (StataCorp, College Station, Texas). We estimated that if 5% of 225 *C.trachomatis* or *N.gonorrhoeae* samples were positive for *M.genitalium* this would yield CIs of 2.5% to 8.6%. We used univariable and multivariable logistic regression analyses to determine risk factors associated with rectal coinfection with *C.trachomatis*/*M.genitalium* and rectal coinfection with with *N.gonorrhoeae*/*M.genitalium*, and pharyngeal *M.genitalium* monoinfection. Variables were included in multivariable models if the p-value was ≤0.05; strongly correlated variables were excluded from multivariable analyses. Ninety-five percent binomial CIs were calculated for all proportions.

4.5 Results

4.5.1 Characteristics of rectal samples examined for co-infection

Four hundred and eighty rectal samples, positive for either *C.trachomatis* or *N.gonorrhoeae*, were selected for testing for *M.genitalium*. Fifty-five (11%) samples yielded invalid results due to insufficient buffer despite careful selection. Valid results were obtained for 212 rectal samples that were positive for *C.trachomatis* and 212 rectal samples that were positive for *N.gonorrhoeae*. One sample which was positive for both *C.trachomatis* and *N.gonorrhoeae* and excluded from analyses tested negative for *M.genitalium*.

4.5.2 Characteristics of cases with rectal *Chlamydia trachomatis* compared to rectal *Neisserria gonorrhoeae*

Compared with MSM with rectal *N.gonorrhoeae*, MSM with rectal *C.trachomatis* were more likely to be presenting to Melbourne Sexual Health Centre as a contact with partners diagnosed with an STI (p=0.07), and to be using PrEP (p=0.084), although these did not reach significance (Table14). MSM with rectal *N.gonorrhoeae* were more likely to be symptomatic compared with those with rectal *C.trachomatis* (OR=2.54, 95%CI: 1.71-3.77, p<0.001, Table14). MSM with rectal *N.gonorrhoeae* were more likely to be HIV-positive compared to men with rectal *C.trachomatis* (OR=5.40, 95%CI: 2.32-12.52, p<0.001) (Table14), although this finding is likely to have been influenced by the removal of a significant proportion of rectal *C.trachomatis* samples from HIV-positive men for LGV testing. Both groups were similar in terms of age, number of sexual partners and condom use.

A relatively high proportion of MSM with rectal *C.trachomatis*, also had urethral *C.trachomatis* [41/211 (19%, 95%CI:14-25)], and pharyngeal *C.trachomatis* [25/212 (12%, 95%CI:8-17) (Table14)]. In patients who had rectal *N.gonorrhoeae*, 66/208 (32%, 95%CI:25-39) also had urethral *N.gonorrhoeae*, and 85/209 (41%, 95%CI:34-48) had pharyngeal *N.gonorrhoeae* (Table14).

4.5.3 Characteristics of rectal *Chlamydia trachomatis* and rectal *Neisseria gonorrhoeae* cases that were co-infected with rectal Mycoplasma genitalium

Overall, *M.genitalium* was detected in 27 of 212 rectal *C.trachomatis* samples (13%, 95%CI: 9-18), and in 29 of 212 rectal *N.gonorrhoeae* samples (14%, 95%CI:9-19) (Table14), with no difference in the proportion of cases with *M.genitalium* coinfection (p=0.774) (Table14).

MSM with rectal *C.trachomatis*/*M.genitalium* co-infection had more sexual partners than those who had rectal *C.trachomatis* monoinfection (mean 6 vs 11, p=0.06); however this was of borderline

significance (Table15). There were no significant differences in terms of age, condom use in the last three months, symptom status, whether they were a STI contact, or STIs at other anatomical sites (Table15).

MSM with rectal *N.gonorrhoeae*/*M.genitalium* co-infection were more likely to be HIV-positive than those with rectal *N.gonorrhoeae* monoinfection (OR=2.96, 95%CI:1.21-7.26, p=0.023) (Table15). There were no significant differences in terms of age, number of sexual partners, PrEP usage, symptom status, or reason for presentation (Table15).

Overall MSM with rectal *N.gonorrhoeae*/*M.genitalium* co-infection were less likely to be using PrEP than MSM with rectal *C.trachomatis*/*M.genitalium* co-infection (OR 0.25, 95%CI: 0.10-0.65, p=0.002, Table16).

4.5.4 Pharyngeal *Mycoplasma genitalium* infection

Four hundred and eighty pharyngeal samples from MSM were tested for *M.genitalium* during the course of the study. Fourteen samples yielded invalid results due to insufficient buffer, with valid results obtained for 464 pharyngeal samples. Eight of 464 samples were positive for *M.genitalium* (2%, 95%CI: 1-3%), 7 of 464 were positive for *C.trachomatis* (2%, 95%CI: 1-3%), and 23 of 464 were positive of *N.gonorrhoeae* (5%, 95%CI: 3-7%).

Pharyngeal *M.genitalium* was associated by univariable analyses with being HIV-positive (OR=5.13, 95%CI: 1.19-22.12), having *M.genitalium* detected at the rectum (OR=60.14, 95%CI: 3.41-1061.47), and having either rectal *C.trachomatis* or rectal *N.gonorrhoeae* (OR=12.99, 95%CI:3.01-56.06) (Table17). Using a composite variable of any rectal STI, in multivariable analyses, MSM with pharyngeal *M.genitalium* were more likely to have a rectal STI detected (OR=10.61, 95%CI:2.30-48.87, Table17) and have a borderline association with being HIV-positive. Although MSM with pharyngeal *M.genitalium* were more likely to have either rectal *C.trachomatis* and/or rectal *N.gonorrhoeae*, none of the eight patients had a STI detected in their urethra, or a co-infection detected in their pharynx. Of note, no male with pharyngeal *M.genitalium* had pharyngeal symptoms.

As for pharyngeal *M.genitalium*, men who tested positive for *C.trachomatis* or *N.gonorrhoeae* in the pharynx were also more likely to have *C.trachomatis* or *N.gonorrhoeae* detected at the rectum. Five of 7 pharyngeal *C.trachomatis* infections were associated with concurrent rectal *C.trachomatis* (71%, 95%CI: 29-96, OR=34.14, 95%CI: 6.35-183.65), and 10 of 23 pharyngeal *N.gonorrhoeae* infections were associated with concurrent rectal *N.gonorrhoeae* (43%, 95%CI: 23-66, OR=28.29, 95%CI: 10.14-78.90).

Table 14. Demographics and epidemiological characteristics of *Neisseria gonorrhoeae* cases compared to *Chlamydia trachomatis* cases (N=424)

		***Chlamydia trachomatis* n=212 (%, 95% CI) or mean (range)**		***Neisseria gonorrhoeae* n=212 (%, 95% CI) or mean (range)**		**Odds Ratio (95% CI)**		**p-value[a]**
Age								
		33	(20-84)	32	(21-59)	0.99	(0.97-1.01)	0.360
Number of male sexual partners in the last 3 mo								
		6	(0-100)	9	(1-270)	1.01	(0.99-1.03)	0.213
Condom use with male partners in the last 3 mo								
	Not always	169	(84, 78-89)	157	(81, 75-86)	1		
	Always	32	(16, 11-22)	37	(19, 14-25)	1.24	(0.74-2.09)	0.410
HIV status[b]								
	Negative	204	(97, 93-97)	178	(84, 79-89)	1		
	Positive	7	(3, 1-7)	33	(16, 11-21)	5.40	(2.32-12.52)	**<0.001**
Using PrEP[c]								
	No	139	(68, 62-75)	136	(76, 69-82)	1		
	Yes	64	(32, 25-38)	42	(24, 18-31)	0.67	(0.43-1.06)	0.084
Symptomatic[d]								
	No	144	(68, 61-74)	96	(46, 39-53)	1		
	Yes	68	(32, 26-39)	115	(54, 47-61)	2.54	(1.71-3.77)	**<0.001**
Anal symptoms[e]								
	No	201	(95, 91-97)	180	(85, 79-89)	1		
	Yes	11	(5, 3-9)	32	(15, 11-21)	3.25	(1.59-6.63)	**0.001**
STI contact								
	No	176	(83, 77-88)	189	(89, 84-93)	1		
	Yes	36	(17, 12-23)	23	(11, 7-16)	0.59	(0.33-1.04)	0.070
***Mycoplasma genitalium* detected in the rectum**								
	Negative	185	(87, 82-91)	183	(86, 81-91)	1		
	Positive	27	(13, 9-18)	29	(14, 9-19)	1.09	(0.62-1.91)	0.774
***Chlamydia trachomatis* detected in the urethra**								
	No	170	(81, 75-86)	199	(96, 92-98)	1		

Yes	41	(19, 14-25)	9	(4, 2-8)	0.19	(0.09-0.40)	**<0.001**
***Neisseria gonorrhoeae* detected in the urethra**							
No	209	(99, 97-100)	142	(68, 61-75)	1		
Yes	2	(1, 0-3)	66	(32, 25-39)	48.57	(11.71-201.51)	**<0.001**
***Chlamydia trachomatis* detected in the pharynx**							
No	187	(88, 83-92)	207	(99, 97-100)	1		
Yes	25	(12, 8-17)	2	(1, 0-3)	0.07	(0.02-0.31)	**<0.001**
***Neisseria gonorrhoeae* detected in the pharynx**							
No	197	(93, 89-96)	124	(59, 52-66)	1		
Yes	15	(7, 4-11)	85	(41, 34-48)	9.00	(4.97-16.29)	**<0.001**

Abbreviations: n=number; CI=confidence interval; mo=months; HIV=human immunodeficiency virus; PrEP=HIV pre-exposure prophylaxis

Notes: [a]p-value calculated using logistic regression and bold indicates significant findings; [b]*C.trachomatis* samples from HIV positive patients were removed for LGV testing; [c]Individuals with HIV were excluded for this variable; [d]Symptomatic was defined as the presence of any symptom, including symptoms at sites other than the rectum; [e]Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus.

Data missing for up to 8% of condom data, up to 2% of *C.trachomatis* and *N.gonorrhoeae* detected at other sites, and up to 1% of all other variables (excluding age).

Table 15. Characteristics of cases co-infected with *Mycoplasma genitalium* compared to rectal *Chlamydia trachomatis* or rectal *Neisseria gonorrhoeae* mono-infections (N=424)

	Chlamydia trachomatis rectal infections (n=212)[a]					
	***Chlamydia trachomatis* cases only n=185 (%, 95% CI) or mean (range)**		***Chlamydia trachomatis* cases with *Mycoplasma genitalium* detected n=27 (%, 95% CI) or mean (range)**		**Odds Ratio (95% CI)**	**p-value[b]**
Age	33	(20-84)	32	(22-52)	0.99 (0.95-1.03)	0.658
Number of male sexual partners in the last 3 mo						
	6	(0-30)	11	(1-100)	1.04 (1.00-1.09)	0.064
Condom use with male partners in the last 3 mo						
Not always	145	(83, 76-88)	24	(92, 75-99)	1	
Always	30	(17, 12-24)	2	(8, 1-25)	0.40 (0.09-1.80)	0.233
Using PrEP[c]						
No	124	(70, 63-77)	15	(56, 35-75)		
Yes	52	(30, 23-37)	12	(44, 35-65)	1.91 (0.84-4.35)	0.125
STI detected in the urethra[d]						
No	146	(79, 73-85)	22	(81, 62-94)	1	
Yes	38	(21, 15-27)	5	(19, 6-38)	0.87 (0.31-2.46)	0.797
STI detected in the pharynx[e]						
No	152	(82, 76-87)	22	(81, 62-94)	1	
Yes	33	(18, 13-24)	5	(19, 6-38)	1.05 (0.37-2.97)	0.931
Symptomatic[f]						
No	125	(68, 60-74)	19	(70, 50-86)	1	
Yes	60	(32, 26-40)	8	(30, 14-50)	0.88 (0.36-2.12)	0.771
Anal symptoms[g]						
No	176	(95, 91-98)	25	(93, 76-99)	1	
Yes	9	(5, 2-9)	2	(7, 1-24)	1.56 (0.32-7.66)	0.597

STI contact							
	No	153	(83, 76-88)	23	(85, 66-96)	1	
	Yes	32	(17, 12-24)	4	(15, 4-34)	0.83 (0.27-2.57)	0.749

***Neisseria gonorrhoeae* rectal infections (n=212)**

		***Neisseria gonorrhoeae* cases only n=183 (%, 95% CI) or mean (range)**		***Neisseria gonorrhoeae* cases with *Mycoplasma genitalium* detected n=29 (%, 95% CI) or mean (range)**		**Odds Ratio (95% CI)**	**p-value[a]**
Age		32	(21-59)	31	(21-56)	0.98 (0.92-1.03)	0.351
Number of male sexual partners in the last 3 mo							
		9	(1-270)	8	(1-25)	1 (0.98-1.02)	0.872
Condom use with male partners in the last 3 mo							
	Not always	136	(80, 74-86)	21	(84, 64-95)		
	Always	33	(20, 14-26)	4	(16, 5-36)	0.78 (0.25-2.44)	0.669
HIV status							
	Negative	158	(87, 81-91)	20	(69, 49-85)		
	Positive	24	(13, 9-19)	9	(31, 15-51)	2.96 (1.21-7.26)	**0.023**
Using PrEP[c]							
	No	122	(77, 70-84)	14	(70, 46, 88)		
	Yes	36	(23, 16-30)	6	(30, 12-54)	1.45 (0.52-4.05)	0.485
STI detected in the urethra[h]							
	No	116	(64, 57-71)	19	(68, 48-84)	1	
	Yes	64	(36, 29-43)	9	(32, 16-52)	0.86 (0.37-2.01)	0.725
STI detected in the pharynx[i]							
	No	106	(59, 51-66)	16	(57, 37-76)	1	
	Yes	75	(41, 34-49)	12	(43, 24-63)	1.06 (0.47-2.37)	0.887
Symptomatic[f]							
	No	81	(45, 37-52)	15	(52, 33-71)	1	
	Yes	101	(55, 48-63)	14	(48, 29-67)	0.75 (0.34-1.64)	0.469

Anal symptoms[g]							
	No	156	(85, 79-90)	24	(83, 64-94)	1	
	Yes	27	(15, 10-21)	5	(17, 6-36)	1.2	(0.42-3.43)	0.732
STI contact							
	No	164	(90, 84-94)	25	(86, 68-96)	1	
	Yes	19	(10, 6-16)	4	(14, 4-32)	1.38	(0.43-4.39)	0.585

Abbreviations: n=number; CI=confidence interval; mo=months; HIV=human immunodeficiency virus; PrEP=HIV pre-exposure prophylaxis

Notes: [a]Due to the removal of the majority of *C.trachomatis* positive samples from individuals with HIV, the association between *C.trachomatis*, *C.trachomatis*/*M.genitalium* and HIV could not be assessed [b]p-value calculated using logistic regression and bold indicates significant findings; [c]Individuals with HIV were excluded for this variable; [d] *C.trachomatis* monoinfection cases, infections in urethra: *C.trachomatis*=37, *N.gonorrhoeae*=1, *M.genitalium* =1. *C.trachomatis* and *M.genitalium* coinfection cases, infections in urethra: *C.trachomatis* = 4, *N.gonorrhoeae*=1, *M.genitalium* =1; [e]*C.trachomatis* monoinfection cases, infections in pharynx: *C.trachomatis*=21, *N.gonorrhoeae*=14. *C.trachomatis* and *M.genitalium* coinfection cases, infections in pharynx: *C.trachomatis*=4, *N.gonorrhoeae*=1; [f]Symptomatic was defined as the presence of any symptoms, including symptoms at sites other than the rectum; [g]Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus; [h] *N.gonorrhoeae* mono-infection cases, infections in urethra: *C.trachomatis*=9, *N.gonorrhoeae*=58, *M.genitalium* =1. *N.gonorrhoeae* and *M.genitalium* coinfection cases, infections in urethra: *C.trachomatis*=1, *N.gonorrhoeae*=7, *M.genitalium* =2; [i] *N.gonorrhoeae* mono-infection cases, infections in pharynx: *C.trachomatis*=2, *N.gonorrhoeae*=73. *C.trachomatis* and *M.genitalium* coinfection cases, infections in pharynx: *C.trachomatis*=0, *N.gonorrhoeae*=12.

C.trachomatis rectal infections, data missing for up to: 5% condom use data; 1% of HIV status, PrEP use, and STI detected in the urethra data. Gonorrhoea rectal infections, data missing for up to: 14% of condom use data; 3% of STI detected at the urethra and or pharynx; and 1% of HIV, PrEP and symptom status data.

Table 16. Demographics and epidemiological features of *Chlamydia trachomatis* cases co-infected with *Mycoplasma genitalium*, compared to *Neisseria gonorrhoeae* cases co-infected with *Mycoplasma genitalium* respectively (N=56)

		Chlamydia trachomatis cases with *Mycoplasma genitalium* detected n=27 (%, 95% CI) or mean (range)		*Neisseria gonorrhoeae* cases with *Mycoplasma genitalium* detected n=29 (%, 95% CI) or mean (range)		Odds Ratio (95% CI)		p-value[a]
Age		32	(22-51)	30	(20-56)	0.98	(0.92-1.05)	0.556
Number of male sexual partners in the last 3mo								
		10.5	(1-100)	8.08	(1-25)	0.99	(0.95-1.03	0.567
Condom use with male partners in the last 3 mo								
	Not always	24	(92, 75-99)	21	(84, 64-95)	1		
	Always	2	(8, 1-25)	4	(16, 5-36)	2.29	(0.38-13.77)	0.367
Using PrEP[b]								
	No	15	(56, 35-75)	14	(70, 46, 88)	1		
	Yes	12	(44, 35-65)	6	(30, 12-54)	0.25	(0.10-0.65)	**0.002**
Symptomatic[c]								
	No	19	(70, 50-86)	15	(52, 33-71)	1		
	Yes	8	(30, 14-50)	14	(48, 29-67)	2.22	(0.74-6.67)	0.157
Anal symptoms[d]								
	No	25	(93, 76-99)	24	(83, 64-94)	1		
	Yes	2	(7, 1-24)	5	(17, 6-36)	2.60	(0.46-14.73)	0.258
STI contact								
	No	23	(85, 66-96)	25	(86, 68-96)	1		
	Yes	4	(15, 4-34)	4	(14, 4-32)	0.92	(0.21-4.11)	0.913

Abbreviations: n=number; CI=confidence interval; mo=months; PrEP=HIV pre-exposure prophylaxis

Notes: [a]p-value calculated using logistic regression and bold indicates significant findings; [b]Individuals with HIV were excluded for this variable; [c]Symptomatic was defined as the presence of any symptoms, including symptoms at sites other than the rectum; [d]Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus.

Data missing for up to 14% of condom use in gonorrhoea samples, and 4% of condom use in *C.trachomatis* samples

Table 17. Demographics and epidemiological features of *Mycoplasma genitalium* negative pharyngeal samples versus *Mycoplasma genitalium* positive pharyngeal samples from men who have sex with men (N=466)

		***Mycoplasma genitalium* negative n=458 (%, 95% CI) or mean (range)**		***Mycoplasma genitalium* positive n=8 (%, 95% CI) or mean (range)**		**Odds Ratio (95% CI)**		**p-value[a]**	**Adjusted Odds Ratio (95% CI)[b]**		**p-value**
Age		34	(18-87)	30	(22-38)	0.96	(0.88-1.04)	0.323			
Number of male sexual partners in the last 3 mo											
		5	(0-100)	3	(0-8)	0.90	(0.67-1.21)	0.484			
HIV status											
	Negative	410	(90, 86, 92)	5	(62.5, 24-91)	1					
	Positive	48	(10, 8-14)	3	(37.5, 9-76)	5.13	(1.19-22.12)	**0.028**	4.19	(0.85-20.81)	0.079
Using PrEP[c]											
	No	315	(82, 78-86)	4	(80, 28-99)	1					
	Yes	69	(18, 14-22)	1	(20, 1-72)	1.14	(0.13-10.36)	0.907			
STI detected in the rectum											
	No	374	(98, 96-99)	3	(37.5, 9-76)	1					
	Yes	7	(2, 1-4)	5	(62.5, 24-91)	12.99	(3.01-56.06)	**0.001**	10.61	(2.30-48.87)	**0.002**
STI contact											
	No	402	(88, 64-91)	7	(87.5, 47-100)	1					
	Yes	56	(12, 9-16)	1	(12.5, 0-53)	1.03	(0.12-8.49)	0.981			

Abbreviations: n=number; CI=confidence interval; mo=months; HIV=human immunodeficiency virus; PrEP=HIV pre-exposure prophylaxis

Notes: [a]p-value calculated using logistic regression and bold indicates significant findings; [b]variables were included in adjusted analysis if the p-value was less than 0.05; [c]Individuals with HIV were excluded for this variable.

Data missing for up to 6% of PrEP use, and 17% of MSM were not tested for a STI at the rectum.

4.6 Discussion

This study examined co-infection of *M.genitalium* with *C.trachomatis* and *N.gonorrhoeae* in the rectum of MSM, as well as the prevalence of pharyngeal *M.genitalium* in MSM being screened for *C.trachomatis* and *N.gonorrhoeae* at the largest urban sexual health centre in Australia. We found high rates of coinfection, with *M.genitalium* present in 13%-14% of MSM with rectal *C.trachomatis* or rectal *N.gonorrhoeae*. Pharyngeal *M.genitalium* was uncommon and detected in 2% of MSM and most cases were associated with rectal *C.trachomatis* or *N.gonorrhoeae* infection. These findings indicate 1 in 7 MSM infected with *C.trachomatis* or *N.gonorrhoeae* at the rectum are co-infected with *M.genitalium* and that a substantial number of undiagnosed rectal *M.genitalium* infections are being inadvertently exposed to macrolide antibiotics, which is likely to be contributing to increasing macrolide resistance in *M.genitalium*.

A recent meta-analysis, including five studies mostly testing urine, estimated the prevalence of *M.genitalium* in MSM in the community at 3.2% (95%CI: 2.1-5.1) at any site (Baumann *et al.* 2018). Studies have shown *M.genitalium* to be most commonly detected at the rectum in MSM, with 40.7% of contacts of the infection positive in rectal sites (Slifirski *et al.* 2017). *M.genitalium* was detected in 7% of rectal samples from asymptomatic MSM in a recent study of 1001 asymptomatic MSM at Melbourne Sexual Health Centre (Read *et al.* 2019a), and 9% of consecutive asymptomatic and symptomatic anorectal samples from 505 MSM in Sydney (Couldwell *et al.* 2018). The Melbourne study also found that of 89 MSM with *M.genitalium* at any site, 17% were co-infected with *C.trachomatis* or *N.gonorrhoeae*, and in 143 MSM diagnosed with *C.trachomatis* or *N.gonorrhoeae* at Melbourne Sexual Health Centre over the same period, 11% were co-infected with *M.genitalium* (Read *et al.* 2019a). Notably a recent Sydney study reported rectal-CT to be independently associated with anorectal rectal *M.genitalium* (OR=5.0, 95%CI: 2.1 to 11.8, p<0.001) (Couldwell *et al.* 2018). A study at our centre did not show this association at the rectum, but that *C.trachomatis* and *N.gonorrhoeae* were associated with urethral *M.genitalium* (p=0.03 and p=0.002, respectively) (Read *et al.* 2019a).

Globally for over a decade *C.trachomatis* and *N.gonorrhoeae* have commonly been treated with regimens that include 1g azithromycin. Recent studies in Australia have shown resistance to azithromycin in *M.genitalium* in MSM now exceeds 80% (Bissessor *et al.* 2015; Tagg *et al.* 2013; Couldwell *et al.* 2018). A meta-analysis and published data from our group has shown *de novo* resistance occurs in 12% of *M.genitalium* infections following use of 1g azithromycin (Read *et al.* 2017a; Horner *et al.* 2017a). Data on the effect of extended azithromycin regimens on *de novo* resistance is less clear. A recent meta-analysis containing 82 patients who received 1.5g

azithromycin (without preceding doxycycline) found *de novo* resistance to be 3.7% (95% CI 0.8% to 10.3%), while a prospective study of 106 *M.genitalium* infected patients treated with 1.5g azithromycin found *de novo* resistance to be similar to 1g (12%, 95%CI:3-27%) (Read *et al.* 2017a; Horner *et al.* 2017a). The rising levels of macrolide resistance seen in *M.genitalium* is likely to be due to azithromycin use, particularly 1g in the treatment of *M.genitalium*, as well as inadvertent exposure during treatment of *C.trachomatis* and *N.gonorrhoeae*. Macrolide resistance is also rising in gonorrhoea and syphilis (Unemo *et al.* 2014; Lukehart *et al.* 2004). Collectively, these data adds to a growing body of evidence that suggests azithromycin use should be reduced in the STI field (BASHH 2018). However, while limited safe and available treatment options exist for macrolide-susceptible *M.genitalium*, it seems sensible to use an extended azithromycin regimen with preceding doxycycline as published data suggests this may be associated with low levels of *de novo* resistance [2.6% (95%CI: 0.3%-9.2%)] (Read *et al.* 2019b).

Co-infection with *M.genitalium* in MSM with either rectal *C.trachomatis* or *N.gonorrhoeae* was associated with specific risk factors. Compared to having rectal *N.gonorrhoeae* alone, rectal *M.genitalium*/*N.gonorrhoeae* co-infection was more common amongst individuals with HIV. This association could not be assessed amongst patients with *C.trachomatis* due to the removal of samples for LGV testing. A recent meta-analysis showed an association between HIV and *M.genitalium* in MSM, predominantly reflecting cross-sectional observational data (Mavedzenge *et al.* 2009). Prospective studies have suggested an association between *M.genitalium* and HIV infection in women in Africa (Mavedzenge *et al.* 2009), but there are no prospective studies examining this relationship in MSM, and the nature of this relationship remains to be established. Co-infection with *M.genitalium* in individuals with either rectal *C.trachomatis* or rectal *N.gonorrhoeae* was associated with factors that largely reflect increased risk - current use of PrEP, HIV infection and having more male partners. While individually not particularly useful, collectively these risk factors may indicate which individuals are more likely to be rectally co-infected with *M.genitalium*. *M.genitalium* has been associated with current use of PrEP in previous studies (Couldwell *et al.* 2018), and the majority of studies have found younger age and increased number of sexual partners to be associated with *M.genitalium* (Andersen *et al.* 2007). Neither co-infection with *M.genitalium* at the rectum or infection at the pharynx were associated with site-specific symptoms.

M.genitalium has been rarely reported in the oropharynx in MSM. Previous studies in Sydney and Melbourne, of 508 and 515 men respectively, both failed to detect pharyngeal *M.genitalium* using PCR. TMA has a higher analytical sensitivity than PCR for *M.genitalium* (Anagrius *et al.* 2005; Tabrizi *et al.* 2016), although the Aptima *M.genitalium* Assay (Hologic, San Diego) has yet to be

validated for detection of pharyngeal *M.genitalium*. Given the organism load of *M.genitalium* is up to 100 times lower than that of *C.trachomatis* (Walker *et al.* 2011), and may be at particularly low loads in the pharynx, TMA may be more likely to detect pharyngeal infections. We detected *M.genitalium* in 2% of pharyngeal samples, which is similar to the prevalence of *C.trachomatis* at the pharynx in MSM attending our clinic (Ong *et al.* 2018b). Pharyngeal *M.genitalium* was associated with having a concomitant rectal STI (either *C.trachomatis* or *N.gonorrhoeae*), although a recent study at our centre found only 1.9% of 54 rectal *M.genitalium* infections had pharyngeal *M.genitalium* by PCR (Read *et al.* 2019a). Other pharyngeal STIs commonly occur concurrently with rectal infections, with 71% and 43% of *C.trachomatis* and *N.gonorrhoeae* pharyngeal infections found to have a concurrent rectal infection respectively. MSM who were HIV positive were also more likely to have pharyngeal *M.genitalium* detected than HIV negative men. As mentioned previously, there is a known association between HIV and *M.genitalium* (Mavedzenge *et al.* 2009), and the clearance rate of *M.genitalium* appears to be slower amongst HIV positive people (Vandepitte *et al.* 2013), however this association with HIV has not been previously reported with pharyngeal *M.genitalium*. There was no difference in the proportion of MSM reporting to be STI contacts between those with *M.genitalium* in the pharynx and those without. The transmissibility of pharyngeal *M.genitalium* is not known as it is not clear if this low positivity in the pharynx reflects passive infection/deposition, rather than active infection as has been hypothesized for pharyngeal *C.trachomatis*. Overall these and other data indicate the pharynx is unlikely to be a significant source of *M.genitalium* transmission.

There were several limitations with this study. The Aptima buffer evaporates from the stored rectal and pharyngeal samples when stored at room temperature, which left insufficient remnant buffer for testing for many specimens. Subsequently, the samples were resealed with parafilm, which reduced evaporation. Rectal and pharyngeal samples that were positive for both *C.trachomatis* and *N.gonorrhoeae* were more likely to have insufficient buffer remaining for additional *M.genitalium* testing, impacting on our ability in this study to examine cases with triple (*M.genitalium*, *C.trachomatis* and *N.gonorrhoeae*) infection. Removal of *C.trachomatis* positive rectal samples from HIV positive men being tested for LGV impacted on our ability to examine the association between HIV and rectal *C.trachomatis*. This study was conducted at a single sexual health clinic, and so is likely to reflect a higher risk population that may impact on prevalence estimates.

We have demonstrated a high prevalence of co-infection of *M.genitalium* with *C.trachomatis* and *N.gonorrhoeae* in the rectum of MSM attending our service, which highlights how commonly *M.genitalium* is being inadvertently exposed to antibiotics in the treatment of other STIs. While the prevalence of *M.genitalium* in men with other rectal STIs is high, lack of clarity around the natural

history of *M.genitalium* in the rectum and concerns around issues of cost, toxicity, and antimicrobial resistance in the treatment of macrolide-resistant *M.genitalium* makes the issue of screening for *M.genitalium* in MSM far more complex than it is for *C.trachomatis* and *N.gonorrhoeae*. Few papers have investigated pharyngeal *M.genitalium* in MSM. Our findings are in line with other publications and show *M.genitalium* is not commonly detected in the pharynx of MSM being screened for *C.trachomatis* and *N.gonorrhoeae,* indicating it is not a common site of infection.

5. The clinical indications for testing women for *Mycoplasma genitalium*

5.1 Background

M.genitalium is a recognised cause of urethritis in men, however it has taken much longer for its role in women to become apparent. *M.genitalium* is now an established cause of cervicitis and PID in women, with less evidence for associations with infertility, spontaneous abortion and premature birth (Taylor-Robinson *et al.* 2011; Lis *et al.* 2015). Guidelines do not recommend screening for *M.genitalium* in any individuals (CDC 2015), however testing has been recommended for patients presenting with symptoms indicative of a genital tract infection or high-risk patients (Oakeshott *et al.* 2010a; Andersen *et al.* 2007; Walker *et al.* 2013). Although *M.genitalium* is a common infection of the genitourinary tract, its pathogenic effects have not been well described in women. Limited evidence exists to suggest *M.genitalium* may be associated with symptoms of post-coital bleeding (Bjartling *et al.* 2012), abnormal vaginal discharge (Vandepitte *et al.* 2012; Walker *et al.* 2011) and dysuria (Vandepitte *et al.* 2012). If women are to be tested for *M.genitalium* based on the presence of symptoms, it is important to have robust evidence underpinning which symptoms are significantly associated with *M.genitalium* infection in women.

This chapter had the following aims:

1) To determine the clinical characteristics of *M.genitalium* in women.
2) To determine the relative contributions of *M.genitalium* , *C.trachomatis*, other common genital infections, and to common genitourinary symptoms in women.
3) To determine the risk factors for *M.genitalium* in sexually active women to inform screening guidelines.

This paper was accepted for publication in May 20201 in *Sexually Transmitted Infections*, pending publication at the time of submission of this thesis.

This study has been presented as an oral presentation at the 2020 Joint Australasian HIV&AIDS and Sexual Health Conferences: Virtual, November 16th-20th 2020 (Oral Presentation O182), and at the Central Clinical School Graduate Research Symposium, Melbourne, October 7th 2019.

This study has been presented as a poster at the STI & HIV World Congress, Vancouver, Canada, July 14th-17th 2019. Published: Latimer R, Vodstrcil L, Read T, et al. P606 Oh MG! the symptoms of mycoplasma genitalium in women. *Sexually Transmitted Infections* 2019; 95:A268.

The submitted paper has been included as text in the thesis to allow for inclusion of what was supplementary material in the submitted study, and a further appendix. No alterations have been made aside from abbreviations, and figure and table numbers, for thesis consistency.

5.2 Abstract

5.2.1 Background

While the contribution of *M.genitalium* to symptoms in men is well described, less is known about its association with common genital symptoms in women. We aimed to determine the prevalence of *M.genitalium* and macrolide-resistance, and its association with common genital symptoms in women attending a sexual health service, to inform indications for testing and clinical practice.

5.2.2 Methods

We undertook a cross-sectional study of symptomatic and asymptomatic women attending Melbourne Sexual Health Centre, between April 2017-April 2019. Women were tested for *M.genitalium* and macrolide-resistance, *C.trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis*, bacterial vaginosis and vulvovaginal candidiasis. Women completed a questionnaire on symptoms, and symptomatic women underwent examination. The prevalence of *M.genitalium* (and macrolide-resistance) and other genital infections was calculated with 95%CI, and associations between these outcomes and specific genital symptoms were examined using logistic regression.

5.2.3 Results

Of 1,318 women, 83 (6%, 95%CI: 5%–8%) had *M.genitalium*, of which 39 (48%, 95%CI: 36-59) had macrolide-resistant *M.genitalium*; 103 (8%, 95%CI: 6-9%) women had *C.trachomatis*. *M.genitalium* prevalence was similar in asymptomatic (10/195; 5%) and symptomatic (73/1108; 7%) women, p=0.506. *M.genitalium* was associated with mucopurulent cervicitis on examination [AOR= 4.38, 95%CI: 1.69-11.33, p=0.002], but was not associated with other specific genital symptoms or signs.

5.2.4 Conclusions

M.genitalium was as common as *C.trachomatis* amongst women attending Melbourne Sexual Health Centre. *M.genitalium* was not associated with genital symptoms, but like *C.trachomatis*, was significantly associated with cervicitis. These data provide evidence that routine testing for *M.genitalium* in women with common genital symptoms is not indicated. The presence of macrolide-resistance in 48% of women supports use of resistance-guided therapy.

5.3 Introduction

M.genitalium is a recognised cause of urethritis in men (Horner *et al.* 2017b), but in women an association with syndromes and sequelae has been less consistently observed . However, a large cross-sectional study of 5000 women attending an emergency gynecological hospital by Bjartling *et al.* found *M.genitalium* was significantly associated with both cervicitis (OR 3.8, 95%CI: 2.1-7.0) and pelvic inflammatory disease (OR 9.0, 95%CI: 1.6-49.9) (Bjartling *et al.* 2012), and a meta-analysis in 2015 by Lis *et al*. reported *M.genitalium* to be associated with an increased odds of both cervicitis and PID in women (Lis *et al.* 2015). A recent synthesis of cohort study data indicated 5% of *M.genitalium* infections progress to PID (Lewis *et al.* 2020). Based on these findings, a UK and Australian guidelines recommend testing for *M.genitalium* in women with cervicitis and PID (BASHH 2018; ASHA 2018).

While there is a substantial body of evidence supporting the association between *M.genitalium* and STI syndromes, less data exist to inform *M.genitalium* testing practices in women presenting with common genitourinary symptoms. While Bjartling *et al.* assessed a range of symptoms they found *M.genitalium* to be associated with the symptom of post-coital bleeding only (OR 2.1, 95%CI: 1.2-3.7)(Bjartling *et al.* 2012). Other research has reported associations between *M.genitalium* and abnormal vaginal discharge (Vandepitte *et al.* 2012; Walker *et al.* 2011) and dysuria (Vandepitte *et al.* 2012), but some studies conducted amongst STI clinic attendees in Sweden and America found no association between *M.genitalium* and genital symptoms in women (Anagrius *et al.* 2005; Mobley *et al.* 2012).

Women are disproportionately affected by the adverse consequences of STIs (Eng *et al.* 1997; Anderson 1995), however STI testing is associated with significant costs to services, and so it is important to have robust evidence that underpins recommendations for *M.genitalium* testing in women. We undertook a cross-sectional study of symptomatic and asymptomatic women attending Melbourne Sexual Health Centre to determine the prevalence of *M.genitalium* and macrolide-resistant *M.genitalium* in women, the prevalence of co-infections, and the association of *M.genitalium* with common genital symptoms and signs, to inform indications for testing and clinical practice.

5.4 Methods

This cross-sectional study was conducted amongst women attending Melbourne Sexual Health Centre, the largest public sexual health service in Victoria, Australia, between April 2017 and April 2019, with >50,000 consults per annum. Melbourne Sexual Health Centre provides a walk-in service, where on arrival clients are triaged as asymptomatic or symptomatic; if triaged as asymptomatic they are screened for STIs by a nurse, and if symptomatic they are seen by a clinician. In this study, women identified as asymptomatic at triage were screened for eligibility and recruited by a research nurse, and women triaged as symptomatic were screened for eligibility and recruited by select clinicians who were experienced in study recruitment. Women were eligible if they were sexually active, aged ≥18 years and were presenting with common genitourinary symptoms or presenting for routine STI screening. Women were ineligible if they were unable to consent to the study for reasons of language or mental state; if they were current sex workers; if they were presenting for *M.genitalium* test of cure or as an *M.genitalium* contact; or if they were aged under 18 years (Figure 19). Women with moderate or severe PID were not recruited in order to expedite their clinical care.

All participants completed a questionnaire which captured whether they had experienced any of the following genital symptoms in the week prior to presentation: abdominal or pelvic pain, dyspareunia, abnormal vaginal discharge, vaginal odour, post-coital bleeding, intermenstrual bleeding, vaginal itch, dysuria, urinary frequency or urgency, and/or fevers or sweats (Appendix C1). Participants also answered questions about prior sexual practices (Appendix C1).

Asymptomatic participants were not examined but provided a first pass urine for *C.trachomatis* and *N.gonorrhoeae* screening (in keeping with standard clinical practice), and were given instructions to self-collect a vaginal swab for *M.genitalium* screening (Figure 19). Symptomatic participants had a clinician collected cervicovaginal swabs for *M.genitalium*, *C.trachomatis* and *N.gonorrhoeae* testing (Figure 19). Clinicians completed a standardised checklist recording the presence or absence of each of the following clinical signs: abnormal vaginal discharge, abnormal vaginal odour, vulval redness or vulvitis, cervicitis (defined as mucopurulent cervicitis and/or cervical friability), cervical contact bleeding, and cervical or adnexal motion tenderness. Speculum and bimanual examination was performed in keeping with clinical practice at Melbourne Sexual Health Centre. Speculum examination is undertaken in women with vaginal discharge, abdominal and/or pelvic pain, whereas bimanual examination is generally restricted to women with abdominal or pelvic pain and those found to have cervicitis on examination. Asymptomatic women were not examined, also in keeping with clinic protocol.

All participants had vaginal pH recorded, and vaginal smear prepared for Gram stain to assess for bacterial vaginosis and vulvovaginal candidiasis. Wet preparation and culture for *Trichomonas vaginalis* (*T.vaginalis*) was performed in women with vaginal discharge and/or itch only, as *T.vaginalis* is extremely uncommon at Melbourne Sexual Health Centre and present in <1% of attendees (Marrone *et al.* 2008).

Detection of *M.genitalium* and macrolide-resistance mutations was performed using the ResistancePlus *M.genitalium* test (SpeeDx Pty Ltd., Sydney, Australia)(Pitt *et al.* 2018; Tabrizi *et al.* 2017). Samples were tested for *C.trachomatis* and *N.gonorrhoeae* by transcription mediated amplification (Aptima Combo 2, Hologic, Massachusetts, USA). *T.vaginalis* was detected using a wet preparation that was examined within 5 minutes of collection at the onsite Melbourne Sexual Health Centre laboratory, and culture. Bacterial vaginosis was diagnosed using both Amsel criteria and Nugent score (bacterial vaginosis defined as ≥3 Amsel criteria and NS=4-10). Vulvovaginal candidiasis was diagnosed based on the presence of typical clinical features (thick white or curdy candidal discharge and/or vulvovaginal erythema) and/or presence of visible pseudohyphae and/or budding yeasts on microscopy. Vaginal polymorphonuclear cell counts (PMNL) on Gram stain were recorded as either <5 or ≥5 vaginal PMNL/high power field (hpf).

5.4.1 Sample size and statistical methods

Sample size calculations were based on a study population of 1350 women, in which we assumed *M.genitalium* positivity would be 8% among 250 women with a specific symptom, and 4% among 1100 women without that specific symptom [estimates based on a prior Australian study (Walker *et al.* 2011)]; this would yield 80% power (α=0.05) to detect an OR of ≥2.3 for the symptom of interest. The proportion of women with each infection (*M.genitalium*, *C.trachomatis*, *N.gonorrhoeae*, *T.vaginalis*, bacterial vaginosis and vulvovaginal candidiasis) and with genital coinfections was determined with 95% binomial CIs. Firstly, we compared demographic and behavioural characteristics between asymptomatic and symptomatic women using logistic regression. Next, we compared the proportion of women with each individual infection by asymptomatic or symptomatic status using logistic regression, adjusting for number of male sexual partners (MSPs) in the prior 12 months, as a significant risk factor for STI acquisition. Logistic regression was then used to investigate the association between *M.genitalium* and each other genital infection, adjusting for MSPs in the prior 12 months.

Using logistic regression, we determined the association between demographic and behavioral factors, and clinical symptoms and signs and i) *M.genitalium* and ii) *C.trachomatis*, compared to women without *M.genitalium* or *C.trachomatis*. As *M.genitalium* and *C.trachomatis* have

overlapping genital symptoms and signs, and can be associated with cervicitis and/or PID, women with *C.trachomatis* were excluded from analyses of *M.genitalium*, and women with *M.genitalium* were excluded from analyses of *C.trachomatis*. All analyses were then adjusted for number of MSPs, vulvovaginal candidiasis, *N.gonorrhoeae* and bacterial vaginosis so that we could determine the independent association of *M.genitalium* (or *C.trachomatis*) with each characteristic. Analyses were not adjusted for *T.vaginalis*, as *T.vaginalis* was not assessed in all women. Additionally, we did not adjust for bacterial vaginosis in associations between *M.genitalium* / *C.trachomatis* and individual Amsel criteria (i.e. vaginal discharge, abnormal vaginal odour and vaginal pH) as these are used in the diagnosis of bacterial vaginosis (i.e. correlated with bacterial vaginosis). We also tested for interaction terms between *M.genitalium* (and *C.trachomatis*) and genital infections, and conducted stratified analyses where appropriate. Variables were considered significant if the p-value was <0.05. Statistical analyses were performed using Stata/IC (Version 14, StataCorp LP, College Station, USA). This project was approved by the Alfred Hospital Ethics Committee (project 100/17) and all participants provided written informed consent (Appendix C2).

5.5 Results

From April 2017 to April 2019, 16,956 individual women attended Melbourne Sexual Health Centre with a total of 36,891 consultations. Of these, 16.3% were sex workers and ineligible. As a public sexual health clinic, Melbourne Sexual Health Centre has a high proportion of non-English speaking clients who were not approached for the study, with the exact number unknown. A total of 1,355 women were recruited to the study by select clinicians and a research nurse. Thirty-seven women were excluded, 25 women disclosed post-recruitment that they were sex workers and 12 women were inadvertently recruited twice; 1,318 women were included in final analyses.

Of the 1,318 women analysed, 1,120 were symptomatic (reported at least one genital symptom in the week prior to presentation) and 198 were asymptomatic (reported no genital symptoms in the prior week). The most frequently reported symptoms were abnormal vaginal discharge (34%), abnormal vaginal odour (24%) and vulvo-vaginal itch (21%). Dyspareunia (10%), post-coital bleeding (8%), and fever (3%) were less frequently reported. Compared to asymptomatic women, symptomatic women were more likely to report inconsistent condom use in the prior 12-months (OR=1.79, 95%CI:1.07–3.00, p=0.026) and an STI in the past 6-months (OR=2.42, 95%CI:1.35–4.36, p=0.003; Table 18).

5.5.1 Prevalence of *Mycoplasma genitalium* and other genital infections

Of the 1,318 women, 15 (1%) had an invalid test for *M.genitalium*. Of 1,303 remaining women, 83 (6%, 95%CI:5–8) had *M.genitalium* detected, with no significant difference in the proportion with *M.genitalium* between completely asymptomatic women (5%, 95%CI:2–9) and women with one or more recent symptoms (7%, 95%CI:5–8, Table 18). Macrolide-resistance was detected in 39/82 *M.genitalium* positive samples (48%, 95%CI:36–59), and was not assessable in one sample. There was no difference in the proportion with macrolide-resistance between asymptomatic women and symptomatic women (40% versus 49%, p=0.741). One hundred women had *C.trachomatis* (8%, 95%CI:6–9), 12 had *N.gonorrhoeae* (1%, 95%CI:0–2), 379 had bacterial vaginosis (30%, 95%CI:28–33) and 314 had vulvovaginal candidiasis (24%, 95%CI:22–27). Only 6 of 684 participants tested by culture and wet preparation were positive for *T.vaginalis*. Bacterial vaginosis and vulvovaginal candidiasis were the only infections detected more frequently in symptomatic women compared to asymptomatic women (33% versus 17%, p<0.001, and 26% versus 15%, p=0.001, respectively), which is a reflection of how common the symptoms of vaginal discharge, odour and itch were in female STI clinic attendees. All women with *N.gonorrhoeae* were symptomatic.

5.5.2 *Mycoplasma genitalium* and genital co-infections

Of the 83 women with *M.genitalium*, 8 (10%, 95%CI: 4–18) were co-infected with *C.trachomatis*, 1 (1%, 95%CI:0–7) with *N.gonorrhoeae*, 29 (36%, 95%CI: 26–48) had concurrent bacterial vaginosis, 21 (26%, 95%CI:17–36) had concurrent vulvovaginal candidiasis, and 1 (1.2%, 95%CI: 0–7) was co-infected with *T.vaginalis*. *M.genitalium* was not significantly associated with presence or absence of any genital infection (Table 19).

5.5.3 Associations between demographic and behavioural characteristics and *Mycoplasma genitalium*

We investigated the association between demographic and behavioral characteristics and *M.genitalium* infection compared to women without *M.genitalium*. *M.genitalium* was not associated with specific demographic or behavioral characteristics following adjustment for number of MSPs and genital co-infections (Table 20). Similarly, *C.trachomatis* positivity was not associated with any demographic or behavioral characteristics in adjusted analyses (Table 21).

5.5.4 Association between self-reported symptoms and *Mycoplasma genitalium*

We investigated the association between self-reported symptoms in the week prior to recruitment and *M.genitalium*. *M.genitalium* was negatively associated with self-reported vaginal odour (AOR=0.48, 95%CI: 0.24–0.96, p=0.037), but was not associated with any other symptoms (Table 20). In contrast, *C.trachomatis* was associated with self-reported vaginal discharge (AOR=2.12, 95%CI: 1.32–3.42, p=0.002; Table 21). Additionally, in stratified analyses, *C.trachomatis* was associated with dyspareunia in women with vulvovaginal candidiasis (AOR=8.66, 95%CI: 1.90–39.54, p=0.005), but this association was not found in women without vulvovaginal candidiasis. Importantly very few women were co-infected with *C.trachomatis* and vulvovaginal candidiasis (n=11), which may have influenced this finding. There were no significant differences in symptoms between women with *M.genitalium* and *C.trachomatis*, although the small number of women with each infection is likely to have impacted this comparison (Table 22).

5.5.5 Association between signs on examination and *Mycoplasma genitalium*

We next investigated the association between clinical signs and *M.genitalium*. We tested for interaction terms between *M.genitalium* and genital infections and the only significant interaction was between *M.genitalium* and bacterial vaginosis for cervicitis (p=0.020). To account for potential confounding by bacterial vaginosis on the relationship between *M.genitalium* and cervicitis, data were then stratified by bacterial vaginosis status, and the association between *M.genitalium* and cervicitis was investigated within each stratum. In women without bacterial vaginosis, *M.genitalium* was strongly associated with cervicitis (AOR=4.38, 95%CI: 1.69–11.33, p=0.002, Table 23), but

this association was not found in women with bacterial vaginosis. *M.genitalium* was not associated with any other clinical signs, including vaginal PMNL count; although all women with *M.genitalium* -cervicitis had ≥5 PMNL/hpf detected (Table 23).

In order to determine if there were key differences in the clinical presentation between *C.trachomatis* and *M.genitalium* in our clinic population, we then assessed the association between clinical signs and *C.trachomatis*. *C.trachomatis* was associated with vaginal discharge (AOR=2.13, 95%CI:1.19–3.82, p=0.011), mucopurulent cervicitis (AOR=2.85, 95%CI: 1.49–5.44, p=0.002), and ≥5 PMNL/hpf on microscopy of vaginal secretions (AOR=2.50, 95%CI: 1.49–4.20, p=0.001; Table 24). There were no significant differences in clinical signs between women with *M.genitalium* and *C.trachomatis* (Table 22).

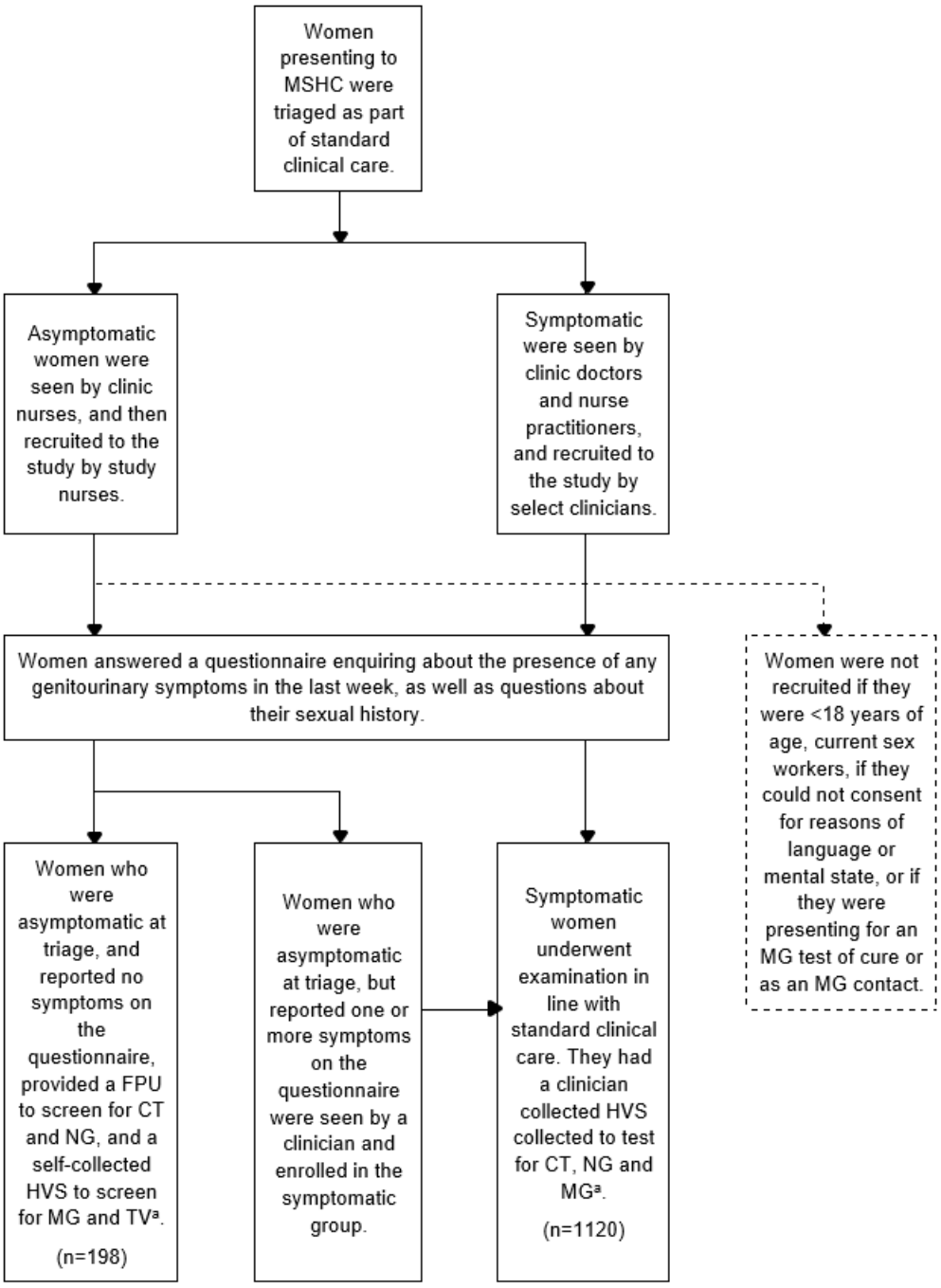

Figure 19. Flow diagram detailing the enrolment of participants in the study.

Notes:

Abbreviations: MSHC= Melbourne Sexual Health Centre; FPU= first pass urine; HVS= high vaginal swab; CT= *Chlamydia trachomatis*; NG=*Neisseria gonorrhoeae*; MG= *Mycoplasma genitalium*, TV=*Trichomonas vaginalis*. [a]Bacterial vaginosis and vulvovaginal candidiasis were also investigated. Vaginal smears for Gram stain and wet preparation were prepared for all participants, and vaginal pH was recorded for all participants.

Table 18. Demographic and epidemiological characteristics and prevalence of common genital infections in asymptomatic and symptomatic women[a]

	Asymptomatic women N=198 median (IQR) or n (%, 95%CI)		Symptomatic women[b] N=1120 median (IQR) or n (%, 95%CI)		Odds Ratio (95% CI)		p-value[c]
Median age	25	(22–29)	26	(23–29)	1.01	(0.98–1.04)	0.389
Median number of male partners in past 12m	4	(2–6)	4	(2–6)	1.01	(0.97–1.04)	0.638
Condom use in past 12m							
Always	21	(11, 7–16)	69	(6, 5–8)	1		
Not Always	175	(89, 84–93)	1,032	(94, 92–95)	1.79	(1.07–3.00)	**0.026**
STI in the past 6m[d]							
No	184	(93, 89–96)	934	(85, 83–87)	1		
Yes	13	(7, 4–11)	160	(15, 13–17)	2.42	(1.35–4.36)	**0.003**

	Asymptomatic women N=198 n (%, 95%CI)		Symptomatic women[b] N=1120 n (%, 95%CI)		Adjusted Odds Ratio[e] (95% CI)		p-value[c]
Mycoplasma genitalium							
Negative	185	(95, 91–98)	1035	(93, 92–95)	1		
Positive	10	(5, 2–9)	73	(7, 5–8)	1.26	(0.64–2.49)	0.506
Unassessable	3		12				
Chlamydia trachomatis							
Negative	183	(92, 88–96)	1025	(92, 91–94)	1		
Positive	15	(8, 4–12)	85	(8, 6–9)	0.99	(0.56–1.76)	0.977
Unassessable/ not tested	0		10				
Neisseria gonorrhoeae							
Negative	198	(100, 98–100)[f]	1098	(99, 99–100)			
Positive	0	(0, 0–2)[f]	12	(1, 1–2)	omitted		0.232[g]
Unassessable/ not tested	0		10				
Bacterial vaginosis[h]							
Negative	165	(83, 77–88)	713	(67, 64–70)	1		

Positive	33	(17, 12–23)	346	(33, 30–36)	2.40	(1.61–3.57)	**<0.001**
Not assessed	0		61				
Vulvovaginal candidiasis[i]							
Negative	164	(85, 80–90)	818	(74, 71–77)	1		
Positive	28	(15, 10–20)	286	(26, 23–29)	2.04	(1.34–3.12)	**0.001**
Not assessed	6		16				

Abbreviations: n=number, CI=confidence interval

Notes: [a]*T.vaginalis* is extremely uncommon at Melbourne Sexual Health Centre and present in <1% of attendees. Of the 684 participants who were tested by culture and wet prep, only 6 were positive for *T.vaginalis*. [b]Women were classified as 'Symptomatic' if they reported the presence of one or more of the following genital symptoms: abdominal pain, dyspareunia, vaginal discharge, abnormal odour, post-coital bleeding, intermenstrual spotting, vaginal itch, dysuria, urinary frequency, fevers. [c]p-value calculated using logistic regression and bold indicates significant findings. [d]STI in the past six months referred to bacterial STI only, however some women may have misinterpreted this question and answered with regards to warts or other nonbacterial STI. [e]Adjusted for number of male sexual partners in the past twelve months (continuous variable). [f]one-sided, 97.5% confidence interval. [g]p-value calculated using Fishers exact test, not adjusted for partner number. [h]Bacterial vaginosis diagnosis was defined as Nugent Score = 4-10 and 3-4 Amsel criteria OR Nugent Score=4-10 and presence of clue cells if client was either asymptomatic (i.e. Amsel criteria not assessed) or other factors (i.e. blood/menses) prevented clinical examination of Amsel Criteria. [i]Vulvovaginal candidiasis was diagnosed microscopically or clinically by a doctor.

Table 19 . Genital coinfections with *Mycoplasma genitalium*[ab]

	***Mycoplasma genitalium* negative n= 1220 (%, 95% CI)**		***Mycoplasma genitalium* positive n=83 (%, 95% CI)**		**Adjusted Odds Ratio (95% CI)[c]**		**p-value[d]**
***Chlamydia trachomatis*[e]**							
Negative	1118	(92, 91–94)	75	(90, 82–96)	1		
Positive	92	(8, 6–9)	8	(10, 4–18)	1.28	(0.60–2.74)	0.525
***Neisseria gonorrhoeae*[f]**							
Negative	1199	(99, 98–100)	82	(99, 93–100)	1		
Positive	11	(1, 0–2)	1	(1, 0–7)	1.27	(0.16–10.01)	0.818
Bacterial vaginosis[g]							
Negative	813	(70, 67–73)	51	(64, 52–74)	1		
Positive	349	(30, 27–33)	29	(36, 26–48)	1.29	(0.80–2.07)	0.295
Vulvovaginal candidiasis[h]							
Negative	918	(77, 74–79)	61	(74, 64–83)	1		
Positive	281	(23, 21–26)	21	(26, 17–36)	1.12	(0.67–1.88)	0.658

Abbreviations: n=number, CI=confidence interval

Notes: [a]Women with an unassessable *M.genitalium* result (n=15) were excluded from the analysis. [b] *T.vaginalis* is extremely uncommon at Melbourne Sexual Health Centre and present in <1% of attendees. 41 women with *M.genitalium* were assessed for *T.vaginalis* and only 1 (2%) was positive for *T.vaginalis*.

[c]Adjusted for number of male sexual partners in the last three months as a risk factor. [d]p-value calculated using logistic regression. [e]*C.trachomatis* result was unassessable in 9 women and not tested for in 1 woman. [f]*N.gonorrhoeae* result was unassessable in 8 women and not tested for in 2 women. [g]Bacterial vaginosis was not assessed in 61 women. [h]Vulvovaginal candidiasis was not assessed in 22 women.

Table 20. Associations between Mycoplasma genitalium and demographics, past sexual practices and self-reported symptoms in the prior week[a]

	Mycoplasma genitalium negative n=1128 median (IQR) or n (%, 95% CI)	*Mycoplasma genitalium* positive n=75 median (IQR) or n (%, 95% CI)	Adjusted Odds Ratio (95% CI)[b]	p-value[c]
Median age	26 (23-29)	26 (23-29)	0.96 (0.92-1.01)	0.136
Median number of male partners in past 12m	4 (2-6)	4 (3-7)	1.03 (1.00-1.07)	0.053
Condom use in past 12m[d]				
Always	82 (7, 6-9)	4 (5, 1-13)	1	
Not Always	1027 (93, 91-94)	70 (95, 87-99)	1.17 (0.41-3.31)	0.772
STI in the past 6m[de]				
No	959 (87, 85-89)	62 (85, 75-92)	1	
Yes	147 (13, 11–15)	11 (15, 8–25)	1.10 (0.55-2.22)	0.780
Self-reported symptoms (in the prior week to recruitment)				
Abdominal Pain				
No	962 (86, 83-88)	64 (86, 77-93)	1	
Yes	161 (14, 12-17)	10 (14, 7-23)	1.08 (0.54-2.17)	0.827
Missing	5	1		
Dyspareunia				
No	1010 (90, 89-92)	66 (90, 81-96)	1	
Yes	107 (10, 8-11)	7 (10, 4-19)	1.16 (0.51-2.61)	0.724
Missing	11	2		
Vaginal discharge				
No	752 (67, 64-70)	48 (65, 53-76)	1	
Yes	367 (33, 30-36)	26 (35, 24-47)	0.90 (0.53-1.54)	0.703
Missing	9	1		
Abnormal vaginal odour[f]				
No	850 (76, 73-79)	60 (81, 70-89)	1	
Yes	268 (24, 21-27)	14 (19, 11-30)	0.48 (0.24-0.96)	0.037
Missing	10	1		

Vaginal itch							
No	888	(79, 77-81)	55	(74, 63-84)	1		
Yes	234	(21, 19-23)	19	(26, 16-37)	1.16	(0.62-2.14)	0.644
Missing	6		1				
Post-coital bleeding							
No	1025	(92, 90-93)	64	(89, 79-95)	1		
Yes	92	(8, 7-10)	8	(11, 5-21)	1.32	(0.58-2.99)	0.511
Missing	11		3				
Intermenstrual bleeding							
No	1009	(90, 88-92)	65	(88, 78-94)	1		
Yes	110	(10, 8-12)	9	(12, 6-22)	1.28	(0.61-2.68)	0.512
Missing	9		1				
Dysuria							
No	973	(87, 85-89)	64	(85, 75-92)	1		
Yes	148	(13, 11-15)	11	(15, 8-25)	1.12	(0.56-2.25)	0.745
Missing	7		0				
Urinary frequency							
No	930	(83, 81-85)	62	(83, 72-90)	1		
Yes	191	(17, 15-19)	13	(17, 10-28)	0.85	(0.43-1.71)	0.654
Missing	7		0				

Abbreviations: n=number, CI=confidence interval, STI=sexually transmitted infection; m=month

Notes: [a]Women with an unassessable *M.genitalium* result (n=15) and/or *C.trachomatis* were excluded from the analysis (n=100; includes 8 women co-infected with *M.genitalium* and *C.trachomatis*). [b]All analyses were adjusted for number of male sexual partners, vulvovaginal candidiasis, *N.gonorrhoeae* and concurrent bacterial vaginosis. [c]p-value calculated using logistic regression. [d]Data missing for up to 3% of participants. [e]STI in the past six months referred to bacterial STI only, however some women may have misinterpreted this question and answered with regards to warts or other nonbacterial STI, and therefore this should be interpreted with caution. [f]Abnormal vaginal odour refers to any self-reported odour, not specifically a fishy odour

Table 21. Associations between *Chlamydia trachomatis* and demographics, past sexual practices and self-reported symptoms in the prior week[a]

	Chlamydia trachomatis negative N=1133 median (range) or n (%, 95% CI)	*Chlamydia trachomatis* positive N=92 [median (range) or n (%, 95% CI)]	Adjusted Odds Ratio (95% CI)[b]	p-value[c]
Median age	26 (23-29)	24 (22-29)	0.97 (0.92–1.01)	0.123
Median number of male partners in past 12m	4 (2-6)	4 (3-6)	1.02 (0.98–1.06)	0.341
Condom use in past 12m[d]				
Always	82 (7, 6-9)	3 (3, 1-9)	1	
Not Always	1032 (93, 91-94)	88 (97, 91-99)	2.90 (0.69–12.09)	0.144
STI in the past 6m[de]				
No	966 (87, 85-89)	77 (87, 78-93)	1	
Yes	146 (13, 11-15)	12 (13, 7-22)	0.65 (0.29-1.45)	0.295
Self-reported symptoms (in the week prior to recruitment)				
Abdominal Pain				
No	967 (86, 84-88)	79 (86, 77-92)	1	
Yes	161 (14, 12-16)	13 (14, 8-23)	0.86 (0.44-1.66)	0.647
Missing	5	0		
Dyspareunia				
Women with vulvovaginal candidiasis				
No	256 (93, 89-96)	8 (73, 39-94)	1	
Yes	20 (7, 4-11)	3 (27, 6-61)	8.66 (1.90-39.54)	**0.005**
Women without vulvovaginal candidiasis				
No	741 (90, 87-91)	71 (93, 85-98)	1	
Yes	87 (10, 9-13)	5 (7, 2-15)	0.48 (0.17-1.35)	0.164
Vaginal discharge				
No	755 (67, 64-70)	48 (53, 43-64)	1	
Yes	369 (33, 30-36)	42 (47, 36-57)	2.12 (1.32-3.42)	**0.002**
Missing	9	2		
Abnormal vaginal odour[f]				

No	859	(76, 74-79)	65	(71, 61-80)	1		
Yes	264	(24, 21-26)	26	(29, 20-39)	1.36	(0.80-2.32)	0.257
Missing	10		1				
Vaginal itch							
No	886	(79, 76-81)	77	(85, 76-91)	1		
Yes	241	(21, 19-24)	14	(15, 9-24)	0.74	(0.37-1.48)	0.399
Missing	6		1				
Post-coital bleeding							
No	1033	(92, 90-94)	81	(89, 81-95)	1		
Yes	89	(8, 6-10)	10	(11, 5-19)	1.22	(0.56-2.62)	0.620
Missing	11		1				
Intermenstrual bleeding							
No	1013	(90, 88-92)	82	(92, 84-97)	1		
Yes	111	(10, 8-12)	7	(8, 3-16)	0.78	(0.35-1.77)	0.557
Missing	9		3				
Dysuria							
No	978	(87, 85-89)	83	(92, 85-97)	1		
Yes	148	(13, 11-15)	7	(8, 3-15)	0.55	(0.23-1.30)	0.173
Missing	7		2				
Urinary frequency							
No	935	(83, 81-85)	71	(79, 69-87)	1		
Yes	191	(17, 15-19)	19	(21, 13-31)	1.22	(0.68-2.18)	0.508
Missing	7		2				

Abbreviations: n=number, CI=confidence interval, STI=sexually transmitted infection; m=month

Notes: [a]Women with an unassessable *C.trachomatis* result (n=10) or with *M.genitalium* (n= 83; includes 8 women co-infected with *M.genitalium* and *C.trachomatis*) were excluded from the analysis. [b]All analyses were adjusted for number of male partners, vulvovaginal candidiasis, *N. gonorrhoea* and concurrent bacterial vaginosis. [c]p-value calculated using logistic regression and bold indicates significant findings p<0.05. [d]Data missing for up to 3% of participants. [e]STI in the past six months referred to bacterial STI only, however some women may have misinterpreted this question and answered with regards to warts or other nonbacterial STI, and therefore this should be interpreted with caution. [g] We tested for interaction terms between *C.trachomatis* and genital co-infections and the only significant interaction was between *C.trachomatis* and vulvovaginal candidiasis for dyspareunia (p=0.016). Therefore, the association between dyspareunia and *C.trachomatis* could not be adjusted for vulvovaginal candidiasis. To account for potential confounding by vulvovaginal candidiasis on the relationship between *C.trachomatis* and dyspareunia, data were then stratified by vulvovaginal candidiasis status, and the association between *C.trachomatis* and dyspareunia was investigated within each stratum. [f]Abnormal vaginal odour refers to any self-reported odour, not specifically a fishy odour

Table 22. Self-reported symptoms and clinical signs identified in women with *Mycoplasma genitalium* compared to women with *Chlamydia trachomatis*[ab]

Self-reported symptoms	*Mycoplasma genitalium* positive, n=75 (%, 95% CI)		*Chlamydia trachomatis* positive, n=92 (%, 95% CI)		p-value[c]
Cohort					
Asymptomatic	8	(11, 5-20)	13	(14, 8-23)	
Symptomatic	67	(89, 80-95)	79	(86, 77-92)	0.502
Abdominal Pain					
No	64	(86, 77-93)	79	(86, 77-92)	
Yes	10	(14, 7-23)	13	(14, 8-23)	0.909
Missing	1		0		
Dyspareunia					
No	66	(90, 81-96)	81	(90, 82-95)	
Yes	7	(10, 4-19)	9	(10, 5-18)	0.930
Missing	2		2		
Vaginal discharge					
No	48	(65, 53-76)	48	(53, 43-64)	
Yes	26	(35, 24-47)	42	(47, 36-57)	0.136
Missing	1		2		
Abnormal odour[c]					
No	60	(81, 70-89)	65	(71, 61-80)	
Yes	14	(19, 11-30)	26	(29, 20-39)	0.150
Missing	1		1		
Vaginal itch					
No	55	(74, 63-84)	77	(85, 76-91)	
Yes	19	(26, 16-37)	14	(15, 9-24)	0.100
Missing	1		1		
Post-coital bleeding					

No	64	(89, 79-95)	81	(89, 81-95)	
Yes	8	(11, 5-21)	10	(11, 5-19)	0.980
Missing	3		1		
Intermenstrual bleeding					
No	65	(88, 78-94)	82	(92, 84-97)	
Yes	9	(12, 6-22)	7	(8, 3-15)	0.359
Missing	1		3		
Dysuria					
No	64	(85, 75-92)	83	(92, 85-97)	
Yes	11	(15, 8-25)	7	(8, 3-15)	0.158
Missing	0		1		
Urinary frequency					
No	62	(83, 72-90)	71	(79, 69-88)	
Yes	13	(17, 10-28)	19	(21, 13-31)	0.541
Missing	0		2		
Fever					
No	71	(96, 89-99)	87	(97, 91-99)	
Yes	3	(4, 1-11)	3	(3, 1-9)	0.807
Missing	1		2		

Clinical signs on examination[b]

	***Mycoplasma genitalium* positive, n=67 (%, 95% CI)**		***Chlamydia trachomatis* positive, n=79 (%, 95% CI)**		**p-value[c]**
Vaginal discharge					
No	15	(25, 14-37)	17	(32, 18-48)	
Yes	46	(75, 63-86)	57	(68, 52-82)	0.826
Not assessed/missing	6		5		
Abnormal odor[d]					
No	44	(72, 59-83)	50	(68, 56-78)	
Yes	17	(28, 14-41)	24	(32, 22-44)	0.566
Not assessed/missing	6		5		

Vulval redness					
No	46	(75, 63-86)	61	(82, 72-90)	
Yes	15	(25, 14-37)	13	(18, 10-28)	0.317
Not assessed/missing	6		5		
Mucopurulent cervicitis					
No	37	(82, 68-92)	46	(73, 60-83)	
Yes	8	(18, 8-32)	17	(27, 17-40)	0.263
Not assessed/missing	22		16		
Cervical or adnexal motion tenderness					
No	31	(86, 71-95)	37	(76, 61-87)	
Yes	5	(14, 5-29)	12	(24, 13-39)	0.227
Not assessed/missing	31		30		
Cervical contact bleeding					
No	36	(84, 69-93)	44	(75, 62-85)	
Yes	7	(16, 7-31)	15	(25, 15-38)	0.267
Not assessed/missing	24		20		
Vaginal pH					
≤4.5	37	(56, 43-68)	38	(51, 39-63)	
>4.5	29	(44, 32-57)	36	(49, 37-61)	0.577
Not assessed/missing					
High vaginal polymorph count					
<5	35	(54, 41-66)	31	(41, 30-53)	
≥5	30	(46, 34-59)	45	(59, 47-70)	0.121
Not assessed/missing	2		3		

Abbreviations: n=number, CI=confidence interval

Notes: [a] Analysis only includes women with *C.trachomatis* or *M.genitalium* infection. Women co-infected with *C.trachomatis* and *M.genitalium* have been excluded. [b]Asymptomatic women were not clinically assessed and have been excluded from the analysis. Clinical signs were elicited only in women with clinical indications for examination and in particular, cervical assessment and bimanual examination was undertaken in women with specific indications for a speculum and bimanual exam. [c]p-value calculated using Chi-square test. [d]Abnormal odour refers to any odour, not specifically a fishy odour.

Table 23. Associations between *Mycoplasma genitalium* and clinical signs among symptomatic women[ab]

	Total women n=1023	*Mycoplasma genitalium* negative n=956 (%, 95% CI)		*Mycoplasma genitalium* positive n=67 (%, 95% CI)		Adjusted Odds Ratio (95% CI)[c]		p-value[d]
Vaginal discharge								
No	322	307	(35, 32-38)	15	(25, 14-37)	1		
Yes	611	565	(65, 62-68)	46	(75, 63-86)	1.56	(0.84-2.87)	0.158
Not assessed/missing	90	84		6				
Abnormal odour[e]								
No	707	663	(76, 73-79)	44	(72, 59-83)	1		
Yes	228	211	(24, 21-27)	17	(28, 17-41)	1.22	(0.67-2.22)	0.517
Not assessed/missing	88	82		6				
Vulval redness								
No	679	633	(73, 70-76)	46	(75, 63-86)	1		
Yes	248	233	(27, 24-30)	15	(25, 14-37)	0.83	(0.41-1.66)	0.591
Not assessed/missing	96	90		6				
Mucopurulent cervicitis[f]								
Women with bacterial vaginosis								
No	208	188	(86, 81-90)	20	(95, 76-100)	1		
Yes	32	31	(14, 10-19)	1	(5, 0-24)	0.36	(0.05-2.85)	0.336
Women without bacterial vaginosis								
No	405	389	(91, 88-93)	16	(70, 47-87)	1		
Yes	46	39	(9, 7-12)	7	(30, 13-53)	4.38	(1.69-11.33)	0.002
Cervical or adnexal motion tenderness								
No	476	445	(78, 75-82)	31	(86, 71-95)	1		
Yes	127	122	(22, 18-25)	5	(14, 5-29)	0.46	(0.16-1.34)	0.155
Not assessed/missing	420	389		31				
Cervical contact bleeding								
No	591	555	(86, 83-88)	36	(84, 69-93)	1		
Yes	101	94	(14, 12-17)	7	(16, 7-31)	1.29	(0.55-3.02)	0.563

Not assessed/missing	331	307		24				
Vaginal pH								
≤4.5	594	557	(61, 58-64)	37	(56, 43-68)	1		
>4.5	381	352	(39, 36-42)	29	(44, 32-57)	1.29	(0.77-2.17)	0.334
Not assessed/missing	48	47		1				
High vaginal polymorph count								
<5	589	554	(60, 57-63)	35	(54, 41-66)	1		
≥5	400	370	(40, 37-43)	30	(46, 34-59)	1.33	(0.77-2.29)	0.307
Not assessed/missing	34	34		2				

Abbreviations: n=number, CI=confidence interval

Notes: [a]Women with an unassessable *M.genitalium* result (n=15), or *C.trachomatis* were excluded from the analysis (n=100; includes 8 co-infected women). In addition, asymptomatic women were not clinically assessed and have been excluded from the analysis (n=180). [b] Clinical signs were elicited only in women with clinical indications for examination and in particular, cervical assessment and bimanual examination was undertaken in women with specific indications for a speculum and bimanual exam. [c]All analyses were adjusted for number of male partners, vulvovaginal candidiasis, *N.gonorrhoeae* and concurrent bacterial vaginosis, with the exception that we did not adjust for bacterial vaginosis in models examining associations with individual Amsel criteria (i.e. vaginal discharge, abnormal vaginal odour and vaginal pH). [d] p-value calculated using logistic regression and bold indicates significant findings $p<0.05$. [e] Abnormal vaginal odour refers to any odour, not specifically a fishy odour. [f] We tested for interaction terms between *M.genitalium* and genital co-infections and the only significant interaction was between *M.genitalium* and bacterial vaginosis for cervicitis (p=0.020). Therefore, the association between cervicitis and *M.genitalium* could not be adjusted for bacterial vaginosis. To account for potential confounding by bacterial vaginosis on the relationship between *M.genitalium* and cervicitis, data were then stratified by bacterial vaginosis status, and the association between *M.genitalium* and cervicitis was investigated within each stratum.

Table 24. Associations between *Chlamydia trachomatis* and clinical signs among symptomatic women[ab]

	Total women n=1037	*Chlamydia trachomatis* negative n=958 (%, 95% CI)		*Chlamydia trachomatis* positive n=79 (%, 95% CI)		Adjusted odds ratio (95% CI)[c]		p-value[d]
Vaginal discharge								
No	325	308	(35, 32-39)	17	(23, 14-34)	1		
Yes	623	566	(65, 61-68)	57	(77, 66-86)	2.13	(1.19-3.82)	**0.011**
Not assessed/missing	89	84		5				
Abnormal odour[e]								
No	717	667	(76, 73-79)	50	(68, 56-78)	1		
Yes	233	209	(24, 21-27)	24	(32, 22-44)	1.42	(0.84-2.41)	0.193
Not assessed/missing	87	82		5				
Vulval redness								
No	690	629	(72, 69-75)	61	(82, 72-90)	1		
Yes	252	239	(28, 25-31)	13	(18, 10-28)	0.82	(0.40-1.67)	0.589
Not assessed/missing	95	90		5				
Mucopurulent cervicitis								
No	657	611	(89, 86-91)	46	(73, 60-83)	1		
Yes	93	76	(11, 9-14)	17	(27, 17-40)	2.85	(1.49-5.44)	**0.002**
Not assessed/missing	287	271		16				
Cervical or adnexal motion tenderness								
No	482	445	(78, 75-82)	37	(76, 61-87)	1		
Yes	135	123	(22, 18-25)	12	(24, 13-39)	0.99	(0.48-2.07)	0.986
Not assessed/missing	420	390		30				
Cervical contact bleeding								
No	600	556	(86, 83-88)	44	(75, 62-85)	1		
Yes	107	92	(14, 12-17)	15	(25, 15-38)	1.93	(0.98-3.78)	0.056
Not assessed/missing	330	310		20				
Vaginal pH								
≤4.5	602	564	(62, 59-65)	38	(51, 39-63)	1		
>4.5	384	348	(38, 25-41)	36	(49, 37-61)	1.42	(0.87-2.33)	0.161
Not assessed/missing								
High vaginal polymorph count								

<5	587	556	(60, 57-63)	31	(41, 30-53)	1		
≥5	414	369	(40, 37-43)	45	(59, 47-70)	2.50	(1.49-4.20)	**0.001**
Not assessed/missing	36	33		3				

Abbreviations: n=number, CI=confidence interval

Notes: [a]Women with an unassessable *C.trachomatis* result (n=10) or with *M.genitalium* (n= 83; including 8 women coinfected with *C.trachomatis* and *M.genitalium*) were excluded from the analysis. In addition, asymptomatic women (n=188) were not clinically assessed and have been excluded from the analysis.

[b] Clinical signs were elicited only in women with clinical indications for examination and in particular, cervical assessment and bimanual examination was undertaken in women with specific indications for a speculum and bimanual exam. [c] All analyses were adjusted number of male sexual partners, vulvovaginal candidiasis, *N. gonorrhoea* and concurrent bacterial vaginosis, with the exception that we did not adjust for bacterial vaginosis in models examining associations with individual Amsel criteria (i.e. vaginal discharge, abnormal vaginal odour and vaginal pH). [d]p-value calculated using logistic regression and bold indicates significant findings $p<0.05$. [e]Abnormal vaginal odor refers to any clinician-recorded odour, not specifically a fishy odour.

5.6 Discussion

M.genitalium was detected in 6% of women attending a large public sexual health center in Melbourne, Australia. *M.genitalium* was not associated with common genital symptoms, but was significantly associated with cervicitis (BASHH 2018; ASHA 2018). Specific symptoms were not helpful in informing additional indications for *M.genitalium* testing at our service. Importantly, 1 in 2 *M.genitalium* infections in women were macrolide-resistant, highlighting the value of resistance-testing and individualising therapy where possible.

M.genitalium was common in women attending our STI service (6%; 95%CI:5–8) compared to a previous study of 1116 women attending Australian primary health care services (2%; 95%CI 1-3) (Walker *et al.* 2011), which aligns with a recent meta-analysis reporting *M.genitalium* prevalence in the general population to be 1.3% (95%CI: 1.0–1.8) in developed nations (Baumann *et al.* 2018). In our study, *C.trachomatis* was detected in 8% (95%CI:6–9) of women, compared to 5% (95%CI 3-7%) in women attending primary care facilities in the previous Australian study (Walker *et al.* 2011). The high prevalence of *M.genitalium* and *C.trachomatis* in our study compared to the general population highlights the high-risk nature of our clinic population (Yeung *et al.* 2014).

Our study aligns with that of Bjartling *et al.* in that women with *M.genitalium* had a 4-fold increased odds of cervicitis after adjusting for genital coinfections (Bjartling *et al.* 2012). Both estimates are higher, but in the range of two prior meta-analyses, which found that women with *M.genitalium* had a 2-fold increased odds of cervicitis [OR=1.7;95%CI:1.35–2.04 (Lis *et al.* 2015) and OR=2.2;95% CI:1.6–2.9 (Taylor-Robinson *et al.* 2011)]. This association was only found in women without bacterial vaginosis, potentially because the pathogenesis of cervicitis in women co-infected with bacterial vaginosis and *M.genitalium* may be influenced/confounded by the presence of bacterial vaginosis-associated organisms; an association that has previously been observed (Marrazzo *et al.* 2006). However, lack of consistency in the criteria used for the diagnosis of cervicitis internationally is likely to have impacted on the comparability of estimates between countries (McGowin *et al.* 2011; Falk 2010). The CDC uses two major diagnostic signs to diagnose cervicitis: 1) mucopurulent endocervical exudate on examination, and/or 2) inducible endocervical bleeding when swabbing the cervical os (CDC 2015). While studies of asymptomatic cervicitis often rely on the presence of high vaginal or cervical PMNLs only, yet the criteria of increased PMNLs has not been standardised and is known to be less reliable (CDC 2015; Falk 2010). Our study did not find *M.genitalium* to be associated with elevated vaginal PMNL count, although all women with *M.genitalium*-cervicitis had an elevated PMNL count in vaginal secretions. A review of *M.genitalium* and cervicitis determined that a high vaginal PMNL count (>30 PMNL/hpf) was

not a specific sign of *M.genitalium*-cervicitis and may fail to detect less severe inflammation (McGowin *et al.* 2011).

Our study did not find *M.genitalium* to be positively associated with any symptoms in women which was similar to Bjartling *et al.* who only found *M.genitalium* to be associated with post-coital bleeding (Bjartling *et al.* 2012). In both studies, chlamydia was commonly associated with genital symptoms and signs in women including vaginal discharge, mucopurulent cervicitis, and elevated vaginal PMNL count, in line with other research (Latimer *et al.* 2019; Holmes *et al.* 2007; Bjartling *et al.* 2012). Although associated with symptoms and signs in our study, like *M.genitalium*, *C.trachomatis* was as common in women and without genital symptoms. *C.trachomatis* is known to be predominately asymptomatic in women, despite its established association with a range of symptoms and clinical syndromes (Holmes *et al.* 2007). We did not find significant differences in symptoms or signs between women with *M.genitalium* or *C.trachomatis*, although this is likely to have been due to limited numbers for comparison. Interestingly, Falk *et al.* also found no difference in presentation between women with *M.genitalium* and *C.trachomatis* among 461 women attending an STI clinic (Falk *et al.* 2005). In contrast Bjartling *et al.* reported that vaginal discharge, abdominal pain, and dysuria were significantly more common amongst women with *C.trachomatis* compared to *M.genitalium* (Bjartling *et al.* 2012). These differences may have been due to the fact that our and Falk's studies were in STI clinic attendees whereas Bjartling included women presenting to an emergency service who are likely to have more acute symptoms. Overall these data suggest that *C.trachomatis* seems to have capacity to cause more inflammation and symptoms of greater severity than *M.genitalium*.

Although perhaps more indolent than *C.trachomatis*, *M.genitalium* is associated with the considerable challenge of increasing antimicrobial resistance, and more complex and costly treatment strategies. We found 1 in 2 *M.genitalium* infections in women were macrolide-resistant, consistent with prior research at Melbourne Sexual Health Centre (Read *et al.* 2019a). Recent Australian studies have reported that 50-60% of *M.genitalium*-infections in heterosexuals are macrolide-resistant (Read *et al.* 2019a), with resistance exceeding 80% in MSM (Bissessor *et al.* 2015; Tagg *et al.* 2013; Couldwell *et al.* 2018). Our data highlight the value of resistance-testing and individualising therapy where possible, as up to 50% of women in our service can currently avoid quinolone use and achieve 95% first line cure using a doxycycline-2.5g azithromycin regimen (Durukan *et al.* 2019).

This was a large cross-sectional study, which captured detailed information on sexual practices, symptoms and signs in women tested for all common STIs and vaginal infections. However, as

recruitment occurred at a single sexual health clinic and non-English speaking women were excluded, prevalence estimates are not generalisable to the community. We were unable to approach all women attending the clinic as only select doctors recruited symptomatic women, and women with marked PID were not recruited in order to expedite clinical care. This study therefore did not assess the association between *M.genitalium* and PID, and is likely to have biased recruitment towards women with milder symptoms. Examination was performed in keeping with clinical indications and practice at our service, resulting in one third of the women having no information on cervicitis, which may have impacted on our findings. Doctors at our service systematically take patients in the order that they arrive to the walk in service and therefore there was no other bias related to medical staff. Vaginal symptoms (e.g. discharge and odour) were the most common symptoms reported in this study as they are the most likely reason for presentation to STI services. However, these symptoms are less likely to be associated with cervical STIs, which may have impacted on our ability to assess associations between other relevant symptoms and signs. As a consequence, our findings are most relevant to women with mild to moderate genitourinary symptoms attending outpatient STI services and general practices. Asymptomatic women were tested for *C.trachomatis* and *N.gonorrhoeae* using first pass urine, compared to symptomatic women who received an endocervical swab, in accordance with standard clinical care at Melbourne Sexual Health Centre. While vaginal samples have generally been considered to be the optimal specimen for *C.trachomatis* and *N.gonorrhoeae*, the Aptima Combo 2 assay used in our study is highly sensitive at detecting very low copies numbers of each organism. The Aptima Combo 2 assay has been shown to have near identical performance in urine compared to vaginal samples (Gaydos *et al.* 2003; Taylor *et al.* 2011). Lastly, the relatively small numbers of women with *M.genitalium* and *C.trachomatis* meant we were underpowered to detect statistical differences between the two STIs on direct comparison.

Overall, *M.genitalium* was common in women attending a high output urban STI service, and half of *M.genitalium* infections were macrolide-resistant. *M.genitalium* was not associated with specific genital symptoms, but was strongly associated with clinical signs of cervicitis. These data support an association between *M.genitalium* and cervicitis in women, particularly in the absence of other genital infections, and do not support routine testing for *M.genitalium* in women with common genital symptoms. These data are useful for clinicians in making decisions about indications for *M.genitalium* testing for women attending their services and help inform clinical practice and guidelines.

6. The Clinical Features and Response to Moxifloxacin of *Mycoplasma genitalium*-associated Pelvic Inflammatory Disease.

6.1 Background

M.genitalium is an established cause of PID (Lis *et al.* 2015). As *M.genitalium* testing is not routine in many clinical settings there are limited published data regarding the clinical characteristics of *M.genitalium*-PID.

Inadequately treated PID can result in chronic pelvic pain, ectopic pregnancy and infertility (Ness *et al.* 2002). Current international guidelines for the treatment of PID predominantly recommend antimicrobials such as tetracyclines, beta-lactams and nitro-imidazoles, and do not contain antimicrobials that are highly effective for treating *M.genitalium* (CDC 2015; Ross *et al.* 2011). When treated in accordance with CDC PID treatment guidelines with cefoxitin and doxycycline, as many as 41% of women tested positive for *M.genitalium* 30 days after treatment (Haggerty *et al.* 2008).

Moxifloxacin has been highly effective in the treatment of macrolide-resistant *M.genitalium* (Bradshaw *et al.* 2006a), is active against *C.trachomatis* and *N.gonorrhoeae* (Boothby *et al.* 2010; Ross *et al.* 2006). Moxifloxacin was reported to be as effective as ofloxacin and metronidazole for the treatment of PID in a RCT (Ross *et al.* 2006). While only three patients in this trial had *M.genitalium*-PID, all were microbiologically cured (Ross *et al.* 2006).

Further evidence is required as to the efficacy of moxifloxacin in the treatment of *M.genitalium*-PID. An effective agent is required for the treatment of *M.genitalium*-PID, as early effective treatment can avoid the serious long term sequalae associated with PID. Further data are also needed on the specific clinical characteristics of *M.genitalium*-PID, to aid prompt testing and diagnosis.

The study included in this chapter had the following aims:

1) To describe the clinical characteristics of *M.genitalium*-PID and to determine how they differ from those associated with *C.trachomatis*-PID.
2) To determine the proportion of women:
    a. microbiologically cured of *M.genitalium*-PID following moxifloxacin (i.e. *M.genitalium* not detected by NAAT at test of cure).
    b. clinically cured of *M.genitalium*-PID following 14 days of moxifloxacin (i.e. asymptomatic following treatment).

This paper was published in *Sexually Transmitted Diseases*: Latimer, R.L., Read, T.R.H., Vodstrcil, L.A., et al. 'Clinical Features and Therapeutic Response in Women Meeting Criteria for Presumptive Treatment for Pelvic Inflammatory Disease Associated With Mycoplasma Genitalium.' *Sexually Transmitted Diseases*. 2019; 46(2):73-79

This study was presented as an oral presentation at the IUSTI Asia Pacific Sexual Health Congress, Auckland, New Zealand, November 1st-3rd 2018 (Oral Presentation #196); at the Sexual Health Society of Victoria's annual World AIDS Day & Post Conference event, Melbourne, December 4th 2018; and at the CCS Postgraduate Symposium, Melbourne, November 12th 2018.

The paper has been included as text in the thesis to allow for inclusion of what was supplementary material in the published study. No alterations have been made, aside from changes to abbreviations, and figure and table numbers, for thesis consistency. Please see Appendix D for the PDF of the published study.

6.2 Abstract

6.2.1 Background

There are limited published data describing clinical features and therapeutic response in women meeting the criteria for presumptive treatment of PID associated with *Mycoplasma genitalium*. *M.genitalium*-PID has been reported to respond poorly to standard PID treatment regimens and while moxifloxacin is recommended in several treatment guidelines, published data to support its use are scant.

6.2.2 Methods

We conducted a retrospective study of women at Melbourne Sexual Health Centre between 2006 and 2017, who met the CDC criteria for presumptive treatment of PID, and had *M.genitalium* detected as the sole pathogen. Clinical and laboratory characteristics of *M.genitalium*-PID were compared to cases of *C.trachomatis*-PID by multivariable analysis. Microbiological and clinical cure following moxifloxacin and standard PID treatment were determined for women with *M.genitalium*-PID who returned for test of cure between 14 and 120 days.

6.2.3 Results

Ninety-two patients with *M.genitalium*-PID were compared with 92 women with *C.trachomatis*-PID. *M.genitalium*-PID was associated with increased lower abdominal tenderness [AOR=2.29 (95%CI: 1.14-4.60)], but a lesser vaginal PMNL response compared to *C.trachomatis*-PID by multivariable analysis. Of the 92 women with *M.genitalium*-PID, 54/92 (59%) received moxifloxacin (10-14 days) and 37/54 had a test of cure between 14-120 days; 27/37 (73%) cases had a median of 7 days of a standard regimen containing doxycycline and metronidazole +/- azithromycin prior to moxifloxacin. Microbial cure following moxifloxacin was 95% (95%CI: 82-99) and did not differ from standard therapy (p= 0.948), however clinical cure was significantly higher following moxifloxacin [89% (95%CI: 75-97, p=0.004)] although adverse effects were more common.

6.2.4 Conclusions

Women meeting CDC criteria for presumptive treatment of *M.genitalium*-PID did not significantly differ to those with *C.trachomatis*-PID. Moxifloxacin was associated with higher rates of symptom resolution in women with PID, and although microbial cure was high, it did not differ between regimens.

6.3 Introduction

M.genitalium has been associated with cervicitis, PID, spontaneous abortion and pre-term delivery in a recent meta-analysis (Lis *et al.* 2015; Wiesenfeld *et al.* 2017). However, there are limited published data on the contribution of *M.genitalium* to PID. More data are needed to establish the attributable risk of *M.genitalium* for female genital tract infections (Wiesenfeld *et al.* 2017). International guidelines for treatment of PID vary, however predominantly recommend presumptive use of antimicrobials such as tetracyclines, beta-lactams and nitro-imidazoles, prior to detection of the causative organism. These regimens aim to treat *C.trachomatis*, *N.gonorrhoeae* and anaerobes, and do not contain highly effective antimicrobials against *M.genitalium* (CDC 2015; Ross *et al.* 2011). When PID was treated in accordance with US CDC guidelines with cefoxitin and doxycycline, 41% of women with PID remained *M.genitalium* positive after 30 days (Haggerty *et al.* 2008). Inadequately treated PID increases the risk of chronic pelvic pain, ectopic pregnancy and infertility (Ness *et al.* 2002).

M.genitalium has marked propensity to develop antimicrobial resistance, with macrolide resistance seen in 40-60% of infections in many countries (Gesink *et al.* 2016; Dumke *et al.* 2016). Moxifloxacin has been highly effective against macrolide-resistant *M.genitalium* (Bradshaw *et al.* 2006a), is active against *C.trachomatis* and *N.gonorrhoeae* (Boothby *et al.* 2010; Ross *et al.* 2006), and was as effective as ofloxacin and metronidazole for all cause PID in a RCT (Ross *et al.* 2006). Although only three patients in this trial had *M.genitalium*-PID, all were cured (Ross *et al.* 2006). The US and UK guidelines now recommend 14 days of moxifloxacin for *M.genitalium*-PID (Ross 2017), although efficacy data are limited (Ross *et al.* 2006), and a recent meta-analysis has shown a decline in cure to 89% (95%CI: 82-94%) since 2010 (Li *et al.* 2017).

With recent regulatory approval of *M.genitalium* assays in many countries, testing for *M.genitalium* in presumptive PID may become increasingly common. However, little has been published on clinical characteristics of *M.genitalium*-PID and whether they differ from those associated with *C.trachomatis*-PID. In our study, we compared women who met CDC criteria for presumptive treatment of PID with *M.genitalium* to women with *C.trachomatis*-PID to investigate whether *M.genitalium*-PID is associated with distinct clinical and laboratory characteristics. In addition, we report the clinical and microbiological outcomes following moxifloxacin and standard antimicrobial therapy.

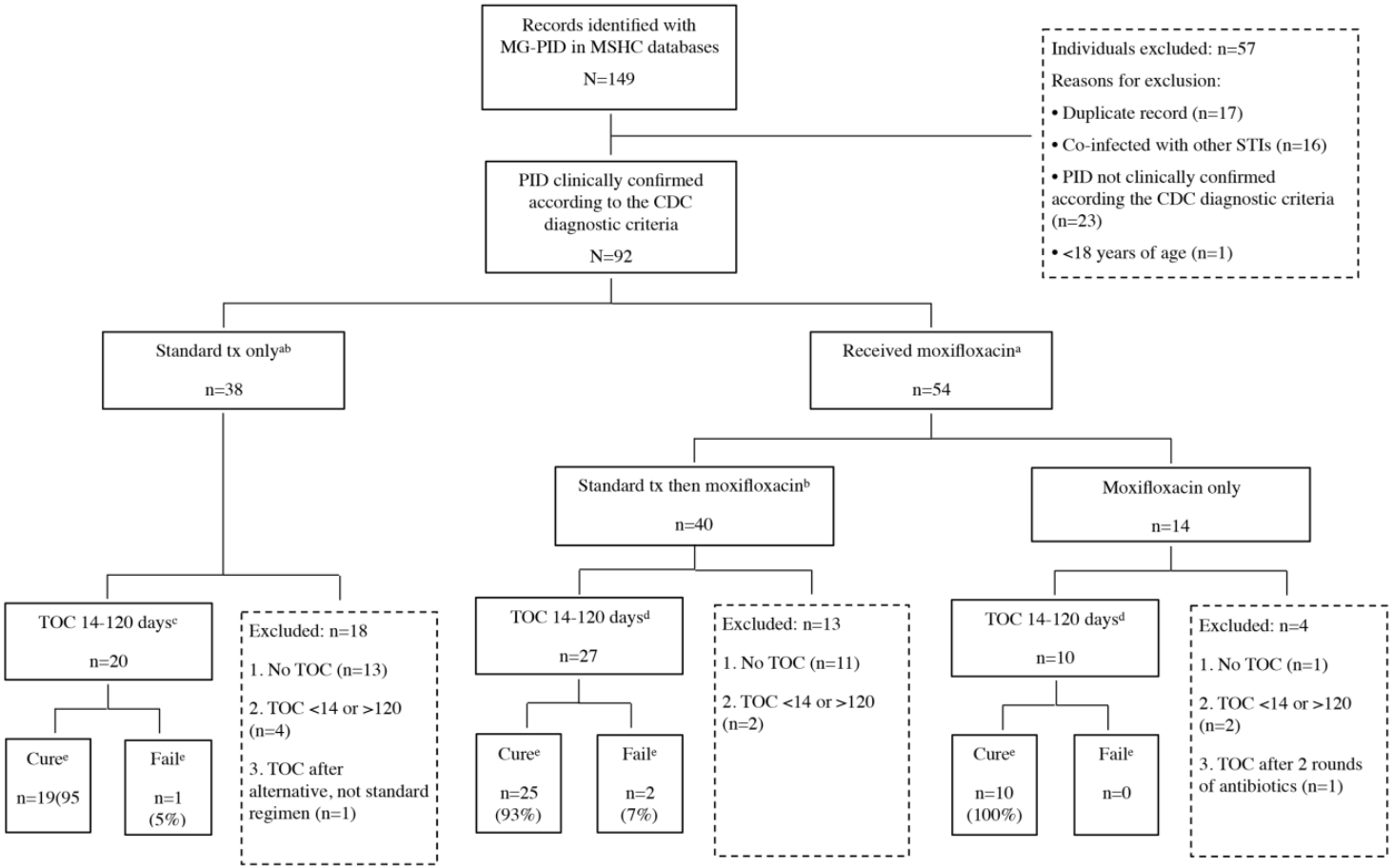

Figure 20. Selection of patients for analysis of effectiveness of moxifloxacin and standard treatment of *Mycoplasma genitalium* associated pelvic inflammatory disease

Abbreviations: MSHC=Melbourne Sexual Health Centre; MG= *Mycoplasma genitalium*; PID= pelvic inflammatory disease; n=number; STI=sexually transmitted infection; CDC=Centers for Disease Control and Prevention; tx= treatment; TOC= test of cure. [a]Patients were not randomised, selection of moxifloxacin versus standard therapy was at clinician discretion; [b]Standard therapy includes varied combinations of azithromycin 1g single dose, doxycycline 100 mg twice daily and metronidazole 400 mg twice daily for 14 days; [c]20 patients analysed for effectiveness of standard treatment; [d]27 patients who received standard therapy and moxifloxacin and 10 patients who received moxifloxacin only combined for analyses of effectiveness of moxifloxacin; [e]Cure refers to a negative test of cure, fail refers to a positive test of cure.

6.4 Materials and Methods

6.4.1 Study design and participants

We undertook a retrospective study of women with *M.genitalium* and presumptive PID between February 2006 and March 2017, at Melbourne Sexual Health Centre, Victoria, Australia. Women were included if they were ≥18 years, PID presumptively diagnosed using CDC criteria, and *M.genitalium* was the sole pathogen (i.e. negative for *C.trachomatis* and *N.gonorrhoeae*). Testing for *M.genitalium* in women with presumptive PID was at clinician discretion from 2006 to 2010, and from 2011 recommended in Melbourne Sexual Health Centre protocols. *M.genitalium*-PID cases were women with presumptive PID at presentation (87%), who underwent STI testing and were commenced on recommended therapy for PID (henceforth standard treatment). In a minority of cases (13%), women developed symptoms and signs following testing, and were diagnosed with presumptive PID at follow up.

We compared characteristics of *M.genitalium*-PID to those of *C.trachomatis*-PID (*C.trachomatis* sole pathogen), and treatment outcomes (clinical and microbial cure) following standard PID therapy and moxifloxacin. All cases diagnosed with *M.genitalium*-PID between February 2006-March 2017 were extracted from the clinic database. Cases of *C.trachomatis*-PID were selected over the same period from the clinic database.

6.4.2 Definitions and data collection

Presumptive PID was diagnosed using CDC criteria, which recommends treatment be initiated in sexually-active women at risk of STIs who are experiencing pelvic or lower abdominal pain and have one of the following: uterine, cervical motion or adnexal tenderness (CDC 2015). These criteria are designed to maximise sensitivity for PID diagnosis and err on the side of over rather than under-treatment of PID. Only approximately half of women meeting these criteria have been shown to have laparoscopic evidence of salpingitis or plasma cell endometritis (Jacobson *et al.* 1969; Sellors *et al.* 1991), although the positive predictive value for these criteria are higher in a STI clinic compared to a community setting (CDC 2015). All women included in the analysis were sexually-active and presented with abdominal pain or pelvic pain, and had either lower abdominal/pelvic tenderness or cervical/adnexal motion tenderness on examination. All reference to *M.genitalium*-PID and *C.trachomatis*-PID refers to presumptive PID diagnosis using CDC criteria. *M.genitalium* was detected on vaginal or cervical swab, or first pass urine, using an in-house PCR assay targeting the 16S rRNA gene of *M.genitalium* (Yoshida *et al.* 2002). *C.trachomatis* was detected using strand displacement amplification (Becton Dickinson, USA) before 2015, and Aptima transcription-mediated amplification assay from 2015 (Hologic, Marlborough, USA).

Bacterial vaginosis was diagnosed using Amsel's criteria (Money 2005). Trichomoniasis was diagnosed by wet preparation and culture. Vaginal and cervical PMNL counts on Gram stain were grouped in the following categories: vaginal PMNL/hpf: <1, 1-4 and ≥5 and cervical PMNL/hpf: <5, 5-8, >8).

6.4.3 Clinical characteristics

Epidemiological, clinical and laboratory data were extracted from paper-based and electronic records for women with *M.genitalium*-PID and *C.trachomatis*-PID. Clinical information recorded included: i) symptoms: lower abdominal pain, dyspareunia, vaginal discharge, fever, intermenstrual or post-coital bleeding; ii) signs: lower abdominal tenderness, cervical motion or adnexal tenderness, mucopurulent cervicitis, cervical-contact bleeding, abnormal vaginal discharge; iii) laboratory and microbiological results: vaginal and cervical PMNL count, detection of *C.trachomatis*, *N.gonorrhoeae*, *T.vaginalis*, and bacterial vaginosis; and iv) antimicrobial regimens prescribed including duration, adherence and adverse effects.

Patients were excluded if they did not fulfil presumptive PID criteria, were co-infected with another STI, or if symptoms were subsequently attributed to another condition.

6.4.4 Treatment outcomes

Women with presumptive PID were tested and treated with a standard regimen containing metronidazole 400 mg twice daily and doxycycline 100 mg twice daily for 14 days +/- azithromycin 1g (ASHA 2016). The *M.genitalium* test results were available 48 hours after testing. Beginning in 2011 all *M.genitalium* positive women were recalled and the standard regimen replaced with moxifloxacin 400mg once daily for 14 days, in accordance with Melbourne Sexual Health Centre guidelines. Before 2011, patients often received standard PID regimens only. Treatment outcomes in all women receiving moxifloxacin and standard regimens were analysed.

Microbiological cure was defined as a negative test of cure (TOC) between 14-120 days. Patients were excluded if: i) TOC was not performed or outside 14-120 days after treatment, ii) they received less than 7 days of moxifloxacin, iii) TOC was performed after more than 2 courses of antibiotics.

Clinical cure was defined as resolution of all PID related symptoms. Antibiotic adherence, partner treatment, re-infection risk, and microbiological and clinical outcomes were recorded, where available. Reinfection risk was defined as none (no sex), possible (sex with a new or treated partner), or probable (sex with a regular partner who had not been tested and/or treated).

The Alfred Hospital Research Ethics Committee approved this study (project number 304/15).

6.4.5 Statistical Analysis

Data were analysed using STATA (v14). Univariate and multivariable logistic regression analysis were performed to determine factors associated with *M.genitalium*-PID compared to *C.trachomatis*-PID. Variables were included in multivariable models if the P-value was 0.05 or less; if correlated, the variable most strongly associated with the outcome was used. Models were built in a forward-stepwise fashion, using the likelihood ratio test to determine the significance of the contribution of each variable to avoid over-fitting the model. Ninety-five percent CIs were calculated for proportions. As vaginal and cervical PMNL counts were correlated and vaginal PMNL count is a minor CDC criterion, only vaginal PMNL was included. Logistic regression was used to assess differences in outcomes by treatment group for those with *M.genitalium*-PID.

6.5 Results

During the study period (2006-2017), 149 records of women with *M.genitalium*-PID were identified, and *M.genitalium* accounted for 5.5% of PID cases at Melbourne Sexual Health Centre during this timeframe. Ninety-two women fulfilled the criteria for presumptive PID and had *M.genitalium* with no other pathogen detected; 57 women were excluded for reasons listed in Figure 20. Ninety-two *C.trachomatis*-PID cases were randomly selected, as described in materials and methods. Eight-seven percent of patients had PID diagnosed at their initial consultation, and 13% on return to clinic.

6.5.1 Demographic and Behavioural Characteristics

There was no significant difference between median ages of women with *M.genitalium*-PID and *C.trachomatis*-PID (25 years, IQR: 21-29, and 24 years, IQR: 21-28), respectively), Table25. In unadjusted analyses, compared to *C.trachomatis*-PID, women with *M.genitalium*-PID were more likely to be sex workers [OR=3.07 (95%CI: 1.33-7.08)], and to have fewer recent male partners (excludes sex work clients) [MSP>1 OR=0.54 (95%CI: 0.30-0.97)].

6.5.2 Clinical Characteristics

Univariate analyses showed women with *M.genitalium*-PID had similar clinical characteristics to women with *C.trachomatis*-PID, although were less likely to report post-coital bleeding [OR=0.42 (95%CI: 0.18-0.98)], Table25. On clinical examination, *M.genitalium*-PID was more likely to be associated with lower abdominal tenderness [OR=2.36 (95%CI: 1.29-4.28)].

6.5.3 Laboratory Characteristics

Women were tested for *M.genitalium* by cervical swab (64%), vaginal swab (20%) and first pass urine (16%). On unadjusted analyses women with *M.genitalium*-PID were less likely than women with *C.trachomatis*-PID to have elevated vaginal or cervical PMNL counts, Table25.

6.5.4 Associations with *Mycoplasma genitalium*-associated pelvic inflammatory disease compared to *Chlamydia trachomatis*- associated pelvic inflammatory disease by multivariable analyses

Women with *M.genitalium*-PID were more likely to have lower abdominal tenderness [AOR=2.29 (95%CI: 1.14-4.60)], but less likely to have a modest elevation in vaginal PMNL counts, compared to women with *C.trachomatis*-PID, Table26.

Table 25. ***Mycoplasma genitalium*** **presumptive pelvic inflammatory disease compared to** ***Chlamydia Trachomatis*** **presumptive pelvic inflammatory disease : an analysis of demographics, behavioural and clinical characteristics (N=184)**

	Chlamydia trachomatis pelvic inflammatory disease cases n=92 (%, 95% CI) or median (IQR)		Mycoplasma genitalium pelvic inflammatory disease cases n=92 (%, 95% CI) or median (IQR)		Unadjusted Odds Ratios (95%CI)		P-value	Adjusted[a] Odds Ratio (95%CI)		P-value
Age	24	(21-28)	25	(21-29)						
Number of MSP last 3months[bc]										
≤1	35	(38, 28-49)	49	(53, 43-64)	1.0			1.0		
>1	57	(62, 51-72)	43	(47, 36-57)	0.54	(0.30-0.97)	0.039	0.54	(0.27-1.06)	0.071
Consistency of condom usage[b]										
Not always	79	(94, 87-98)	72	(85, 75-92)	1.0					
Always	5	(6, 2-13)	13	(15, 8-25)	2.85	(0.97-8.40)	0.057			
Current sex worker										
No	83	(90, 82-95)	69	(75, 65-83)	1.0			1.0		
Yes	9	(10	23	(25, 17-35)	3.07	(1.33-7.08)	0.008	1.92	(0.75-4.97)	0.176
Sex within Australia only[b]										
No	36	(42, 32-54))	23	(28, 19-39)	1.0					
Yes	49	(58, 46-68)	59	(72, 61-81)	1.88	(0.99-3.60)	0.054			
Reported Symptoms										
Abdominal pain[d]										
No	23	(25, 17-35)	13	(14, 8-23)	1.0					
Yes	69	(75, 65-83)	79	(86, 77-92)	2.03	(0.95-4.30)	0.066			
Dyspareunia[d]										
No	43	(47, 36-57)	45	(49, 38-60)	1.0					
Yes	49	(53, 43-64)	47	(51, 40-62)	0.92	(0.51-1.63)	0.768			
Post-coital bleeding										
No	73	(79, 70-87)	83	(90, 82-95)	1.0			1.0		
Yes	19	(21, 13-30)	9	(10, 5-18)	0.42	(0.18-0.98)	0.044	0.40	(0.15-1.12)	0.082
Intermenstrual bleeding										
No	77	(84, 75-91)	71	(77, 67-85)	1.0					
Yes	15	(16, 9-25)	21	(23, 15-33)	1.52	(0.73-3.17)	0.267			

Dysuria										
No	64	(70, 59-79)	72	(78, 68-86)	1.0					
Yes	28	(30, 21-41)	20	(22, 14-32)	0.63	90.33-1.23	0.181			
Urinary frequency[e]										
No	72	(78, 68-86)	82	(89, 81-95)	1.0			1.0		
Yes	20	(22, 14-32)	10	(11, 5-19)	0.44	(0.19-1.00)	0.050	0.56	(0.22-1.46)	0.234
Vaginal discharge (symptom)										
No	36	(39, 29-50)	40	(43, 33-54)	1.0					
Yes	56	(61, 50-71)	52	(57, 46-67)	0.84	(0.46-1.50)	0.549			
Recorded Clinical Signs										
Lower abdominal tenderness[d]										
No	49	(53, 43-64)	30	(33, 23-43)	1.0			1.0		
Yes	43	(47, 36-57)	62	(67, 57-77)	2.36	(1.29-4.28)	0.005	2.29	(1.14-4.60)	0.020
Cervical or adnexal motion tenderness[d]										
No	15	(16, 9-25)	13	(14, 8-23)	1.0					
Yes	77	(84, 75-91)	79	(86, 77-92)	1.18	(0.53-2.65)	0.682			
Mucopurulent cervicitis										
No	57	(62, 51-72)	62	(67, 57-77)	1.0					
Yes	35	(38, 28-49)	30	(33, 23-43)	0.79	(0.43-1.44)	0.441			
Cervical contact bleeding										
No	78	(95, 76-91)	81	(88, 80-94)	1.0					
Yes	14	(15, 9-24)	11	(12, 6-20)	0.76	(0.32-1.77)	0.520			
Vaginal discharge (sign)										
No	42	(46, 37-56)	43	(46, 37-56)	1.0					
Yes	50	(54, 44-63)	49	(54, 44-63)	0.96	(0.54-1.71)	0.882			
Bacterial Vaginosis[b]										
Not detected	49	(53, 43-64)	51	(55, 45-66)	1.0					
Detected	33	(36, 26-47)	32	(35, 25-45)	0.93	(0.50-1.74)	0.824			
Vaginal PMNL count[bfg]										
< 1	23	(28, 19-39)	38	(46, 35-58)	1.0					
1-4	19	(23, 15-34)	12	(15, 8-24)	0.38	(0.16-0.93)	0.034	0.34	(0.13-0.89)	0.027
≥5	40	(49, 38-60)	32	(39, 28-50)	0.48	(0.24-0.97)	0.041	0.72	(0.34-1.54)	0.400
Cervical PMNL count[bfg]										
<5	5	(11, 4-25)	9	(24, 12-41)	1.0					
5-8	6	(14, 5-27)	14	(38, 22-55)	1.29	(0.30-5.54)	0.726			

>8	33	(75, 60-87)	14	(38, 22-55)	0.24 (0.24-0.15)	0.024

Abbreviations: n= number; PID = pelvic inflammatory disease; CI= confidence interval; IQR=interquartile range; MSP= male sexual partners; PMNL= polymorphonuclear lymphocyte

Notes: [a]Adjusted model includes: number of male sexual partners, current sex worker, post-coital bleeding, urinary frequency, lower abdominal tenderness, and vaginal PMNL count; [b]Denominator varied due to exclusion of patients with unrecorded data; [c]Male sexual partners does not include commercial partner numbers; [d]All women included in the analysis had presumptive PID based on CDC criteria: were sexually active and presented with pelvic pain, defined as either abdominal pain or deep dyspareunia (pelvic pain elicited during sexual intercourse) and had lower abdominal/pelvic tenderness or cervical motion/adnexal tenderness on examination; [e]Urinary frequency was also included in the multivariate analysis based on its contribution to the model using the likelihood ratio statistic; [f]Vaginal PMNL from high vaginal swab; [g] Vaginal and cervical PMNL counts were correlated and since vaginal PMNL count is a minor CDC criterion for PID, only vaginal PMNL were included in the multivariable model.

6.5.5 Treatment outcomes in *Mycoplasma genitalium* associated pelvic inflammatory disease

Of the 92 women with *M.genitalium*-PID, 54 (59%) received moxifloxacin and 38 (41%) a standard regimen only (Figure 20). Thirty-seven of 54 women who received moxifloxacin returned for TOC at 14 to 120 days (Figure 20). Of these women, 10 of 37 (27%) received moxifloxacin only and 27 of 37 (73%) started a standard regimen before being recalled for moxifloxacin. Pre-moxifloxacin, standard therapy in 23 of 27 women (85%) was azithromycin followed by doxycycline and metronidazole and in 4 of 27 women (15%) was doxycycline and metronidazole without azithromycin. Ceftriaxone, which has no effect on *M.genitalium*, was only dispensed presumptively for individuals considered at risk of *N.gonorrhoeae*. Median duration of standard therapy pre-moxifloxacin was 7 days (range 2-14 days). Median time to TOC was 32 days (IQR 24-41 days). A higher proportion of women receiving moxifloxacin returned for TOC at 14-120 days [37/54 (69%)] in contrast to standard treatment [20/38 (53%)] (Figure 20).

Of the 37 women with *M.genitalium*-PID who had moxifloxacin and a 14 to 120 day TOC, 35/37 [95% (95%CI: 82-99)] were microbiologically cured (Table26, Figure 20). There was no significant difference in microbiological cure between the moxifloxacin group and the standard treatment group (p=0.948). Of note, both of the moxifloxacin failures received standard PID treatment prior to moxifloxacin. Clinical cure, defined as resolution of all pelvic symptoms, was significantly higher in women treated with moxifloxacin [33/37, 89% (95%CI: 75-97)] compared to standard treatment [10/19, 53% (95%CI: 29-76), p=0.004], (Table26). Of the four patients in the moxifloxacin group with persistent symptoms, all had abdominal pain and two reported dyspareunia. Of the 9 patients in the standard treatment group with ongoing symptoms, seven had persistent abdominal pain, and three had persistent dyspareunia.

6.5.6 Adherence to antimicrobial therapy and re-infection risk

Adherence to therapy was documented in 32 of 37 (86%) patients who had moxifloxacin and returned for a TOC visit, with 27 of 32 [84% (95% CI 67-95)] reporting 100% adherence and 5 of 32 reporting <100% adherence (all took >7 days) (Table26). Of the two microbiological failures following moxifloxacin, one had sex with an untreated partner before TOC and was at high risk of reinfection; the other did not have reinfection risk or adherence documented. Adherence to standard treatment was documented in 16 of 20 patients returning for TOC with 12 of 16 [75% (95%CI 48-93)] reporting 100% adherence. There was no difference in adherence between the two groups (p=0.436).

6.5.7 Adverse Effects

Adverse effects were significantly more common amongst those who took moxifloxacin (p=0.026), with 15/37 (41%) women who received moxifloxacin reporting side effects (Table26). The most common side effects were nausea (7 of 15), diarrhoea (3 of 15) and candidiasis (2 of 15); one patient reported tendon pain. No serious adverse effects were reported but four of five patients who ceased moxifloxacin early experienced side effects. Of the 20 women who received standard treatment, only two (10%) reported any adverse effects, the nature of which was not recorded.

Table 26. Clinical and microbiological outcomes, adherence and adverse effects of moxifloxacin compared to standard treatment for *Mycoplasma genitalium* associated Pelvic Inflammatory Disease[ab] (N=57)

	Standard treatment[d] n=20 (%, 95% CI)		Moxifloxacin[c] n=37 (%, 95% CI)		p-value
Adherence[e]					
Incomplete	4	(25, 7-52)	5	(16, 5-33)	
Complete	12	(75, 48-93)	27	(84, 67-95)	0.436
Reinfection risk[ef]					
None	6	(55, 23-83)	12	(41, 24-61)	
Possible	3	(27, 6-61)	7	(24, 10-44)	0.856
Probable	2	(18, 2-52)	10	(34, 18-54)	0.320
Reported side effects					
No	18	(90, 68-99)	22	(59, 42-75)	
Yes	2	(10, 1-32)	15	(41, 25-58)	0.026
Microbiological outcome					
Fail	1	(5, 1-25)	2	(5, 1-18)	
Cure	19	(95, 75-100)	35	(95, 82-99)	0.948
Resolution of symptoms[e]					
No	9	(47, 24-71)	4	(11, 3-25)	
Yes	10	(53, 29-76)	33	(89, 75-97)	0.004

Abbreviations: PID = pelvic inflammatory disease; n= number; CI= confidence interval; TOC=test of cure

Notes: [a]Patients were excluded if no TOC was performed (n=25), the TOC occurred <14 day or >120 days after treatment (n=8), TOC occurred after an alternative, not standard regimen (n=1), or the TOC was performed after multiple rounds of antibiotics (n=2); [b]Patients were not randomised, selection of moxifloxacin versus standard therapy was at clinician discretion; [c]27 patients who had standard therapy and moxifloxacin and TOC 14-120 days; 10 patients who had moxifloxacin only and TOC 14-120 days; [d]Standard treatment group includes 22 patients who had a TOC at 14-120 days and includes varied combinations of azithromycin 1g single dose, doxycycline 100 mg twice daily and metronidazole 400 mg twice daily for 14 days; [e]Denominator varied due to exclusion of patients with unrecorded data; [f]Reinfection risk was defined as none (no sex), possible (sex with a new or treated partner), or probable (sex with a regular partner who has not yet been tested and/or treated).

6.6 Discussion

This study of women meeting the criteria for presumptive treatment of PID associated with *M.genitalium,* found a similar clinical presentation to that of *C.trachomatis*-PID. Among 37 evaluable women, moxifloxacin microbiologically cured 95% of *M.genitalium* infections but this did not differ to standard treatment. It is interesting that moxifloxacin was associated with significantly higher resolution of clinical symptoms (89%) compared to standard treatment (53%), although side effects were common with moxifloxacin.

There are limited published data examining the association between *M.genitalium* and PID. While cases included in this dataset fulfilled the CDC criteria for presumptive treatment for PID, past studies found that up to half of all cases of presumptive PID did not have histological endometritis or salpingitis (Jacobson *et al.* 1969). Mild abdominal pain has previously been reported in *M.genitalium*-associated acute endometritis (Cohen *et al.* 2002). Investigators in one study found *N.gonorrhoeae*-PID to be more severe than *M.genitalium*-PID, but found no difference in the clinical presentation of *C.trachomatis*-PID and *M.genitalium*-PID (Short *et al.* 2009). Although we found *M.genitalium*-PID was more likely to be associated with abdominal tenderness than *C.trachomatis*-PID, the significance of this finding is unclear. Universal testing for *M.genitalium* in PID at Melbourne Sexual Health Centre was not recommended until 2011, and although the case definition of presumptive PID required the presence of abdominal or pelvic pain and lower abdominal/pelvic tenderness or cervical/adnexal motion tenderness on examination, it is possible that only more severe cases were tested for *M.genitalium* prior to 2011. In contrast to the clinical findings, *M.genitalium*-PID was associated with modest reduction in cervico-vaginal PMNL response compared to *C.trachomatis*-PID, supporting the role of *C.trachomatis* as an established cause of PID and morbidity. An important limitation of this study is that outpatient sexual health services are likely to see milder PID. Studies in hospitals attended by women with more severe PID may have different findings. While records were reviewed by the same researcher to control for differences in interpretation of records, missing data and clinician variability in documentation are unavoidable limitations of a retrospective case review. Despite these limitations, this remains the largest study to date of *M.genitalium*-PID examining clinical features.

In this study both regimens achieved high levels of microbial cure, although clinical cure was significantly higher with moxifloxacin, and only 53% of women receiving standard regimens experienced complete resolution of PID symptoms. This raises the possibility of persistent low load infection, as this pattern occurs in *M.genitalium*-associated urethritis (Read *et al.* 2019b). However, a direct comparison of treatment outcomes should be interpreted with care due to substantial

differences between those who received standard treatment, and those given moxifloxacin. Firstly, treatment regimens were not randomised and were at clinician discretion, with temporal differences between the two treatment groups. Women receiving standard treatment only were predominantly treated earlier in the study (60% treated before 2013), while the majority of those in the moxifloxacin group were treated later in the study (85% after 2013). Melbourne Sexual Health Centre has experienced an extraordinary rise in macrolide-resistant *M.genitalium* and azithromycin-failure from <10% in 2006 to >40% in 2016 (Read *et al.* 2019b; Jensen *et al.* 2008; Bissessor *et al.* 2015). The group who received standard treatment only predominantly reflected the period when macrolide resistance was uncommon and this regimen may be less effective as high levels of macrolide resistance (>50%) are now seen in 2018 (Read *et al.* 2019b). These data suggest that regimens containing azithromycin may be effective in regions with low levels of macrolide resistance. It is possible that cure is also enhanced by the presence of doxycycline, which we have recently shown has a substantial effect on *M.genitalium* load and selection of macrolide-resistance (Read *et al.* 2019b). Neither treatement regimen contained ceftriaxone as this is the standard of care at MSHC as *N.gonorrhoeae* is rare in Australia. A previous study found that adding ceftriaxone to a doxycycline/metronidazole regimen overall improved outcomes (Piyadigamage *et al.* 2005). This may have been because PID is usually a polymicrobial condition and ceftriaxone provides broad spectrum cover (beyond just *N.gonorrhoeae*). It is possible its omission from the control group at MSHC resulted in lower clinical cure. Published data suggests that it is the inclusion of azithromycin rather than doxycycline or ceftriaxone that is likely to have affected cure, as when *M.genitalium*-PID was treated with cefoxitin and doxycycline, the microbiological cure rate was only 59% (Haggerty *et al.* 2008). Further data regarding the efficacy of doxycycline-azithromycin inclusive regimens, particularly with respect to clinical cure, would be of value considering the cost and lower tolerability of moxifloxacin.

Adverse effects were significantly more common in women treated with moxifloxacin than standard therapy. Although this may be directly attributable to moxifloxacin, 73% of women receiving moxifloxacin had also been exposed to a standard PID regimen. Gastrointestinal side effects are common with quinolones, however, rare serious adverse effects including arrhythmias, neuropathy and tendon rupture have also been reported. Risk of adverse effects needs to be balanced with risk of serious sequelae from delaying antimicrobial therapy in PID (Ness *et al.* 2002; Ross 2014; Hillis *et al.* 1993). This study was conducted at a time of rising macrolide resistance in Australia and during the emergence of quinolone resistance in the region. A recent meta-analysis reported the efficacy of moxifloxacin for *M.genitalium* to be declining, from 100% (95%CI: 99–100) in studies prior to 2010, to 89% (95%CI: 82–94) since (Li *et al.* 2017). Due to the small numbers of treatment

failures in this study, temporal trends in efficacy could not be assessed. Of note, a significant proportion of women treatment with moxifloxacin had exposure to standard PID regimens prior to moxifloxacin, which may have improved moxifloxacin cure. Although this may have impacted on the reported efficacy of moxifloxacin, this is also reflection of real world practice in the absence of point of care tests. Moxifloxacin, although costly and associated with more adverse effects, currently remains the only available therapeutic option in most clinical settings for patients with macrolide-resistant *M.genitalium*.

This study reports a case series of women with presumptive PID and *M.genitalium* detected as the sole pathogen. It did not find clinically meaningful differences between women with *M.genitalium*-PID and *C.trachomatis*-PID and does not provide prospective data to inform clinicians of the likelihood that *M.genitalium* will lead to PID. The CDC recommended standard PID regimen includes doxycycline but not azithromycin, and has been shown to have low microbiological and clinical cure for *M.genitalium*-PID (Haggerty *et al.* 2008). Macrolide resistance is becoming increasingly common worldwide and in the majority of high-income countries exceeds 40%, so azithromycin-based regimens can be expected to have declining efficacy. If *M.genitalium* is identified in a woman presenting with the clinical features of PID and no other pathogen is detected, then this study provides data showing moxifloxacin is highly effective in achieving microbiological cure of *M.genitalium*, but that 2 in 5 women will experience predominately mild adverse effects. The future will see point-of-care assays for *M.genitalium* that incorporate resistance markers, and this will assist researchers in determining the efficacy of specific regimens for *M.genitalium*-associated syndromes and clinicians in selecting appropriate antimicrobials to ensure high level clinical and microbial cure.

SECTION B

‘STEALTHING’

7. Introduction to ‘Stealthing’

‘Stealthing’ is a colloquial term for non-consensual condom removal, where consent for sexual intercourse is given on the proviso of using condoms. As condoms are a primary preventative barrier method for protection against STIs and pregnancy, ‘stealthing’ may lead to serious consequences.

‘Stealthing’ became a widespread topic of discussion in the media, following a 2017 legal publication by Alexandra Brodsky- ‘“Rape-Adjacent”: Imagining Legal Responses to Nonconsensual Condom Removal’, which included interviews with victims’, and describes the legal implications of ‘stealthing’ and legal avenues to address ‘stealthing’ as sexual assault (Brodsky 2017). In Australia the legal repercussions vary state to state. Under Victorian law, ‘stealthing’ could come under the classification of sexual assault, but ‘stealthing’ is not explicitly stated as a crime under common or statutory law. The first criminal charge in Australia for ‘stealthing’ as an act of sexual assualt was filed in Melboure, Victoria in 2018, and is awaiting trial as of June 2021.

There are no other papers in any literature directly examining nonconsensual condom removal or the rate at which it happens, although the National Sexual Assault Hotline in America does report receiving calls related to this practice (Nedelman 2017). Clinicians at Melbourne Sexual Health Centre have reported patients presenting for STI testing, following non-consensual condom removal (personal communication with Prof. Catriona Bradshaw and Dr. Vincent Cornelisse).

In order to take create preventative measures to protect against ‘stealthing’, it is important to define who it is happening to, and the situational factors around the event. This knowledge gap led me to undertake a study to determine the percentage of patients who had experienced ‘stealthing’, and the risk factors associated with it (Chapter 9).

The following literature review will discuss the use of condoms and reasons for non-use, consent, and any existing literature which pertains to ‘stealthing’. ‘Stealthing’ is a novel area of sexual health research with many gaps and as outlined above, this PhD will seek to address some of these.

Figure 21. Picture by artist Cheeky Palm, commissioned by YWCA Australia, based on the study in Chapter 9

Artists permission given for picture inclusion in thesis.

8. Literature Review B- ‘Stealthing’

8.1 The Male Condom

8.1.1 The purpose of condoms

Condoms are a form of barrier protection used during sexual intercourse to protect against STIs and pregnancy (Roper *et al.* 1993). It is well documented that condoms are highly effective, however efficacy is dependent on correct use (Roper *et al.* 1993). According to the CDC condom use data, between 2011–2015, 23.84% of women and 33.7% of men used a condom during their last sexual intercourse in the past 12 months (Copen 2017), and four hundred and fifty million condoms are sold every year in the US alone (Planned Parenthood 2020). Prevalence of condom use is influenced by demographics such as age, ethnicity, education and relationship status (Copen 2017; Reece *et al.* 2010).

Condoms are a primary preventative method designed to protect against the spread of STIs, providing partial protection against all STIs (Holmes *et al.* 2004). The US CDC have reported that in the US there are nearly 20 million cases of STIs yearly, half of which occur in patients below the age of 25 (Owusu-Edusei *et al.* 2013). These infections pose a significant public health epidemic and place a large burden on limited healthcare resources, with the estimated direct medical cost of selected STIs in the US in 2008 alone being approximately $15.6 billion (Owusu-Edusei *et al.* 2013). The risk of HIV transmission has been shown to be reduced by between 80% and 95% with consistent condom use (Weller *et al.* 2002; Pinkerton *et al.* 1997). Exact rates of STI prevention are difficult to ascertain due to methodological difficulties, but sufficient evidence exists to conclude that condoms are partially efficacious in protecting against STIs other than HIV. A review by leading STI researcher King Holmes in 2004, found that condoms significantly reduce acquisition of syphilis, *C.trachomatis*, *N.gonorrhoeae*, HSV-2, and possibly trichomoniasis as well (Holmes *et al.* 2004).

Condoms are also an effective form of birth control, and birth control is the primary purpose of use for many users (Cassell *et al.* 2006). In a nationwide population study in the US among those using condoms for contraceptive indications, 59.9% of women and 56.4% of men use only condoms; 25.0% of women and 33.2% of men use condoms and adjunctive hormonal contraception; and 15.1% of women and 10.5% of men used condoms and adjunctive non-hormonal contraceptive methods (Copen 2017). When condoms are used for correctly, they are 98% effective as birth control (Hatcher *et al.* 2007; Trussell 2011). However condoms are often used improperly, and with typical use condoms are 82% effective in protecting against pregnancy (Trussell 2011).

8.1.2 Demographics and situational factors associated with condom use

There have been two large population surveys conducted in the US and UK examining condom use, which have found prevalence of condom use to be affected by demographics such as age, ethnicity, education and relationship status (Copen 2017; Cassell *et al.* 2006). Condom use is also affected by situational and physiological factors. Inconsistent condom use is associated with an increased odds of imperfect condom use (Hatherall *et al.* 2007).

The United States 2011–2015 National Survey of Family Growth (NSFG), and the combined 1990+2000 British National Surveys of Sexual Attitudes and Lifestyles (Natsal), are large nationwide population surveys of over 20,000 people, which contain questions around condom use (Copen 2017; Cassell *et al.* 2006). These surveys found that younger men are more likely to use condoms, with condom use decreasing with older age. Amongst 16-24-year old's in the UK, 51.6% used a condom at their last sexual intercourse, while in the US, 53.5% of 15-19-year-old men used condoms every time they had had sexual intercourse in the past year. Older men used condoms significantly less, with 18% of 35-44 year old's in the UK using a condom at their last sexual encounter, while in the US, 9.4% of men in this age group used a condom every time they had sex in the past year (Copen 2017; Cassell *et al.* 2006). In the US 70.0% of men over the age of 35 had not used a condom in the last 12 months, compared to 6.9% of those under the age of 20 (Copen 2017). Although older men use condoms infrequently, studies have shown that they are more likely to use them correctly (Warner *et al.* 2008; Hernández-Romieu *et al.* 2014; Shlay *et al.* 2004).

Education status impacted on condom use in the US, but was not examined in the British surveys. Men with higher levels of educational attainment were more likely to use condoms every time, compared to those who had a lower level of education attainment (Copen 2017). Interestingly level of education did not impact on women's use of condoms in this study (Copen 2017).

Ethnicity impacted on condom use in both studies, with those self-classified as white reporting significantly lower rates of condom usage at both last sexual intercourse or ever, in both studies (Copen 2017; Cassell *et al.* 2006). In the US, those who were self-classified as black or African American had the highest rates of condom use, followed by Hispanic or Latino, followed by those who are white. In the UK, those who classified themselves as Black African had the highest rates of use, followed by Indian, Black Caribbean, Pakistani, followed by those who are white. Interestingly, one study of 1973 consistent condom users in Denver, USA found that those who were white had a significantly lower error rate when using condoms than blacks or Hispanics, with the highest error rate found amongst black MSM (Shlay *et al.* 2004).

Relationship status greatly affects condom use. In the CDC population study, men were more likely to have reported using a condom "every time" they had intercourse with a casual partner compared to a regular partner, and more likely to use condoms if they had two or more casual partners compared to one partner only (Copen 2017). Similarly in the British study, those starting relationships stated that they were significantly more likely to have used condoms during their last sexual intercourse, compared to those in relationships of five years or longer (Cassell *et al.* 2006). Rates of condom use at last sex fell and plateaued around six months in to a relationship (Cassell *et al.* 2006). The limitation of both of these studies is that condom-use is self-reported, and therefore may be over or under estimated.

Other factors known to be associated with condom use were not explored in either of these population studies. Situational factors, such as either partner partaking in alcohol or drugs, have consistently been shown to be associated with decreased condom usage (Crosby *et al.* 2007; Abbey *et al.* 2005; Rietmeijer *et al.* 1998; Lachowsky *et al.* 2016; Race *et al.* 2017). Physiological factors such as erectile issues also play a role in use (Hensel *et al.* 2011; Graham *et al.* 2006). In a study of men 278 men presenting to a public STI clinic, 37.1% reported condom-associated erection loss (Graham *et al.* 2006). In this study men with erection issues were more likely to engage in unprotected sex ($p=0.04$) and more likely to have inconsistent condom use ($p=0.014$). A larger study of 1875 men in the USA had similar findings, with erection issues such as difficulty maintaining an erection, less rigidity, or less than typical length associated with greater odds of incomplete condom use (Hensel *et al.* 2011). An online study of 761 heterosexual men from the UK found that men who have difficulty reaching an orgasm were significantly more likely to remove condoms before sex is over (AOR=2.08) (Graham *et al.* 2011), indicating sexual arousal may be an important factor in condom removal.

In studies of condom use during penetrative vaginal intercourse, it has been found that condoms are often used incorrectly with errors related to function, human error and use errors. Functional errors include condom breakage or slippage. Rates of condom breaking or slipping vary; in the population study conducted in the US, 6.5% of women stated the condom broke or fell off during their last sexual intercourse (Copen 2017). Human errors include turning a condom inside out or reusing a condom (Warner *et al.* 1998; Shlay *et al.* 2004; Grimley *et al.* 2005), not securing a condom at withdrawal (Grimley *et al.* 2005; Bortot *et al.* 2006), or failing to leave space at the tip of the condom (Grimley *et al.* 2005; Bortot *et al.* 2006). Incomplete use errors occur frequently during sexual intercourse, with penetration before applying condoms occurring in 8-12% of intercourse involving condoms (Hensel *et al.* 2011; Warner *et al.* 1998; Shlay *et al.* 2004), removing condoms before completion of intercourse occurring in 3-9% of interactions (Hensel *et al.* 2011; Hatherall *et*

al. 2007; Warner *et al.* 1998; Shlay *et al.* 2004), and both types of incomplete use occurring in 2.0% of sexual events (Hensel *et al.* 2011). The amount of experience men have had in using condoms has been significantly associated with all types of error, with those less experienced misusing condoms more frequently (Shlay *et al.* 2004; Crosby *et al.* 2008a; Crosby *et al.* 2008b).

Reasons for condom use and condom non-use are complex and diverse, and not usually attributable to a single demographic or situational factor. While condom use is quite similar between heterosexuals and MSM, these are two distinctly different populations, and the following sections will discuss the literature uniquely specific to these two groups.

8.1.3 Condom use amongst heterosexuals

Data from the NATSAL studies in the UK indicate that condoms are commonly used amongst heterosexuals, with 29.3% of heterosexual men reporting that they used a condom during their last sexual intercourse (Cassell *et al.* 2006). The primary purpose of condom use among men in this group was to prevent pregnancy, with 69.6% of male respondents stating this was the only purpose, and 18.2% stating they used condoms for the dual purpose of protecting against pregnancy and STIs (Cassell *et al.* 2006).

In men having heterosexual intercourse, condom use errors and problems are largely similar between vaginal and anal sex, however they are significantly more likely to remove a condom during anal sex rather than vaginal sex ($p<0.001$) (Topping *et al.* 2011).

Amaro, a leading theorist on issues of gender and power, has stated condom use is distinctly different men than it is for women: “for men, the behaviour is wearing the condom; for women, the behaviour is persuading the male partner to wear a condom or, in some cases, deciding not to have sex when the male partner refuses to wear a condom” (Amaro 1995). Feminist scholars have opined that it is impossible to discuss condom use in heterosexual relationships without acknowledging issues of power, namely that sex typically occurs in contexts in which men have greater power than women (Amaro 1995). These theories are supported by an American study of 219 American college students which found that women play a more active role in the negotiation of condom use, while men play a more reactive role (Carter *et al.* 1999).

Female involvement in the decision to use condoms can influence the rate of condom use errors. In an online Canadian study of 2000 people, men who have decided to use condoms unilaterally, without the input of the female partner, were more likely to report early removal of condoms and slippage during withdrawal compared with those who made the decision to use condoms with female input (Crosby *et al.* 2008a). The authors concluded that female involvement in condom use

decisions provides a protective effect for condom use errors. Concerningly in a study of incarcerated female teens, 34.3% reported not discussing condom use prior to sex, while 48.5% wanted to use a condom but did not have one available. Of those who used condoms, 26.9% removed them before sex was over. Condom errors were more likely to occur if either of the teens was high on alcohol or drugs, however condom errors occurred more frequently when it was the female partner who was drunk compared to the male partner (Crosby *et al.* 2007). This study again highlights the importance of female involvement and awareness in a male's decision to use a condom during heterosexual sex.

8.1.4 Condom use amongst men who have sex with men

Data from the NATSAL studies in the UK indicate that condoms are commonly used amongst MSM, with 35.8% of MSM using a condom at their last sexual intercourse (Cassell *et al.* 2006). The primary purpose of condom use in this group is to prevent STI and HIV transmission and acquisition. According to the British NATSAL studies, MSM are more likely than heterosexuals to report consistent condom use (Shlay *et al.* 2004; Cassell *et al.* 2006). However these studies were conducted prior to the introduction of PrEP, with research indicating a significant decrease in consistent condom use amongst MSM since the introduction of PrEP (Holt *et al.* 2018; Golub *et al.* 2010).

Group sex is a particularly risky situation amongst MSM, with increased risk of STI (van den Boom *et al.* 2016). Amongst 393 MSM in Washington D.C., 27.2 % reported engaging in group sex in the prior year, with 33.0% reporting no condom use with their sex partners (Phillips *et al.* 2014). Although group sex was common, cross-sectional research by van den Boom in Amsterdam (n=2045) found that condoms were more likely to be used in group sex situations compared to dyadic situations (van den Boom *et al.* 2016). This study was conducted prior to the introduction of PrEP, which may have influenced how condoms are used in group sex as well as dyadic sex. An online survey of men in Paris (n=444) found those reporting condomless group sex were much more likely to taking PrEP than men with no group sex experience (41.5% vs 7.7%, $p < .001$) (Callander *et al.* 2019). PrEP and the HIV epidemic has had a significant impact on the way the MSM population utilizes condoms, and new research on condom use amongst this group is needed since the relatively recent introduction of PrEP.

There is limited literature on the power dynamics of condom use amongst MSM, compared to the literature on heterosexuals. While there is limited literature, we can acknowledge similarly there is still may be a power dynamic at play, with one partner wearing the condom, and the other

negotiating condom use, however the power dynamic may not be influenced by traditional gender stereotypes or perceptions.

Psychological research has predominately focused on the impact of stigma on sexual health and risk-taking behaviours (Hubach *et al.* 2015; Coker *et al.* 2010; Ramirez-Valles *et al.* 2010; Starks *et al.* 2013; Hamilton *et al.* 2009). Stigma against MSM has been associated with increased substance use (Coker *et al.* 2010; Race *et al.* 2017), and substance use has consistently been associated with less consistent condom use (Rietmeijer *et al.* 1998; Lachowsky *et al.* 2016; Race *et al.* 2017). Stigma has also been associated with psychological characteristics which influence sexual risk-taking behaviours, including anxiety, depression, and social isolation (Hubach *et al.* 2015; Frost *et al.* 2007; Hart *et al.* 2005). A study of 100 HIV positive men in rural USA found that HIV-stigma was correlated with loneliness (r =0.619, $p<0.01$), and loneliness was associated with lack of condom use ($p<0.05$) (Hubach *et al.* 2015). Another study examining psychological factors that influence condom use amongst MSM interviewed 100 men in the USA, and found social anxiety was associated with increased probability of unprotected insertive anal intercourse in the past six months (Hart *et al.* 2005). The authors concluded that the findings highlighted the importance of examining the decision to use a condom as a shared activity with either perceived or actual social implications, and not as a unilateral decision (Hart *et al.* 2005).

A study of 245 MSM in New York, USA found that stigma is also significantly associated with a perception that condoms reduce intimacy during sexual intercourse (Starks *et al.* 2013). Studies have suggested the perception of intimacy is critical in condom use, particularly amongst MSM, with qualitative research finding the use of condoms communicates mistrust of a partner (Blechner 2002; Shernoff 2005; Smith *et al.* 2009). A survey of 318 MSM in New York found that intimacy interference attitudes were a significant predictor for condom non-use.

8.1.5 Consequences of condom removal

While the reasons people choose not to use condoms are many and varied, sexual intercourse with partial condom use can carry the same risks as sex with no condom use, as efficacy is dependent on correct use (Roper *et al.* 1993). Delayed application of condoms for receptive anal sex are risk factors for the transmission of HIV infection amongst MSM (OR=5.8, p=0.01) (Calzavara 2003), and condom use errors have been significantly associated with STI acquisition (Shlay *et al.* 2004). By removing a condom during sex, there is increased risk of pregnancy and STIs (Roper *et al.* 1993), and those who have not consented to the removal of a condom may experience emotional and psychological distress (Brodsky 2017).

8.2 Sexual Consent

8.2.1 Definition

Consent is defined as a free and voluntary agreement between participants, which requires both reason and deliberation ("ALRC Report 114" 2010; Lehman *et al.* 2008). A person who enjoys adequate mental ability and capacity to make an informed and intelligent choice has the capacity to provide consent ("ALRC Report 114" 2010; Lehman *et al.* 2008). Consent should be free from fraud, duress, persuasion, or any variation of these ("ALRC Report 114" 2010; Lehman *et al.* 2008). In the context of agreement due to apprehension or terror, the consent given is not real consent and is instead acquiescence (Lehman *et al.* 2008).

In a review of 'The Complexities of Sexual Consent', Muehlenhard concluded there are two main aspects that are important in defining sexual consent (Muehlenhard 1995/1996). The first is that consent requires knowledge. A person must be able to understand all aspects of the sexual act to occur, as well the social implications and meaning of the activities. The second aspect is that consent must be given freely, with no excessive influence or coercion.

All Australian states have a statutory definition of consent based on free agreement ("ALRC Report 114" 2010). In legal cases of sexual offences against adults, the prosecution must prove the complainant did not consent to the sexual conduct ("ALRC Report 114" 2010). Defendants will often assert that they believed the intercourse to be consensual, with juries left to conclude what is the reasonable or honest belief in each situation ("ALRC Report 114" 2010). A report by the National Council to Reduce Violence Against Women and their Children noted variations across Australia in terms of the conditions and circumstances that negate consent or the way 'honest belief' is dealt with ("ALRC Report 114" 2010; "Time for Action" 2009).

8.2.2 Communicating consent

In 1987, McCormick categorised the initiation of sexual activity initiation as through either direct or indirect means, and either verbal or non-verbal means (McCormick 1987) This premise has underpinned majority of sexual initiation and consent literature since then (Hickman *et al.* 1999; Byers *et al.* 1989; McCormick 1979). Direct means include words or actions that are unambiguous and cannot be misconstrued, such as directly asking a partner if they would like to have sex. Indirect means include words or actions that do not explicitly imply a desire to engage in sexual activity, such as kissing a partner. The majority of studies on sexual initiation were conducted in the 1980s, and concluded that indirect strategies were more commonly employed (Greer *et al.* 1994; McCormick 1979), with non-verbal indirect means preferred by both the initiator and the receiver (Greer *et al.* 1994; Hickman *et al.* 1999; Mitchell *et al.* 1998). As relationships increase in length,

verbal methods increase in use, with both indirect and direct means acceptable to both parties (Humphreys *et al.* 2007). The literature concludes that both women and men prefer indirect communication as it enables sexual initiation while avoiding explicit rejection.

Sexual consent follows similar patterns to sexual initiation, with consent commonly communicated through non-verbal means (Hickman *et al.* 1999; Humphreys 2007). In heterosexual relations, women are more likely to use indirect verbal methods of consent, such as asking for a condom, while men are more likely to use indirect nonverbal methods of consent, such as responding with kissing or touching (Hickman *et al.* 1999). The most common form of non-verbal consent is not displaying resistance tactics (Hickman *et al.* 1999), with Hall et al. concluding in 1998 that most sexual activity occurs without overt consent being communicated (Hall 1998). Literature on communicating consent is limited, however there is particularly limited literature on the communication of consent in non-heterosexual relationships. Existing literature shows that those in same-sex relationships also predominately use non-verbal means to communicate consent, with no significant differences between MSM and WSW (Beres *et al.* 2004). Consent literature also predominately views consent as a static process, with few papers referring to consent as evolving process or evaluation of a partners behaviours throughout a sexual encounter (Beres *et al.* 2014; Beres 2010). Further research is required to understand consent as it changes throughout a sexual encounter.

8.2.3 Interpreting consent

The communication of sexual consent is an under-researched area. Existing research has been predominately conducted amongst heterosexual college students, with further research needed amongst groups which do not identify as heterosexual, such as MSM, women who have sex with women, and other non-cis identifying partners, as well as further research needed amongst a more age diverse population. Complicating consent literature is that it predominately ascribes to sexual script theory, with the man as the initiator and the woman as the receiver of sex. Sexual script theory was developed in 1973, and states that sexual behaviour is both instinctual and learned, and that sexual behaviours or 'scripts' are gendered (Gagnon *et al.* 1973).

Research has been inconclusive of the ease with which consent can be misinterpreted. A study by Humphreys *et al.* (2007) exposed 415 Canadian university students to a vignette of a couple engaging in intercourse following non-verbal initiation and passive consent techniques (Humphreys 2007). The study concluded that gender is a factor in the perception of whether or not consent was given in situations where researchers intended for consent to be ambiguous. Men were more likely than women to perceive the situations as consensual, acceptable and unambiguous, regardless of the

previous relationship between the couple described in the vignette (Humphreys 2007). An earlier study by Hickman & Muehlenhard, of 68 students from the University of Kansas, USA, asked participants to imagine themselves in scenarios in which sexual intercourse was initiated either verbally or nonverbally by either themselves or their date, along with a list of possible responses someone could make to such initiations. This study concluded women were more likely to use indirect verbal signals to communicate consent, while men used nonverbal signals, and this may lead to gender-based misunderstandings. However the study took care to emphasise there were far more similarities than differences in women's and men's self-reported use and interpretation of consent signals, and importantly both men and women reported direct refusal is an unambiguous signal, with miscommunication an unlikely explanation for rape (Hickman *et al.* 1999). One study surveying 366 participants about sexual influence techniques concluded sexually aggressive men are more likely to selectively ignore or reinterpret women's non-consent (Christopher *et al.* 1990).

An interesting conclusion from the study by Hickman & Muehlenhard was that a condom is a common way for women and men to communicate consent, and that consent for sexual intercourse is implied in the process of asking or applying, or the appearance of a condom (Hickman *et al.* 1999). A study analysing strategies used to influence condom use amongst 90 heterosexual couples stated that participants regularly use non-verbal signals such as putting a condom on, buying or getting condoms, or presenting a condom to their partner (Bird *et al.* 2001). There is no literature addressing the role of condoms in communicating consent outside of heterosexual relationships.

8.3 ‘Stealthing’ or Non-consensual Condom Removal

8.3.1 ‘Stealthing’ definition

Non-consensual condom removal refers to removal or non-use of a condom during consensual sexual intercourse, that had been consented to on the proviso of condom use. When this condom is removed, the terms of consent are violated. As mentioned above, this act is popularly referred to as ‘stealthing’(Brodsky 2017).

8.3.2 Literature on ‘stealthing’

Recently, ‘stealthing’ or non-consensual condom removal has been a rising topic of discussion, particularly prompted by the Julian Assange case in Sweden ("Assange v Swedish Prosecution Authority" 2011). There is very limited academic literature related specifically to ‘stealthing’, rather it has been alluded to in the context of being a unique phenomenon related to birth control sabotage amongst heterosexuals, and intentional HIV transmission amongst MSM. Personal correspondence with clinicians at Melbourne Sexual Health Centre informs that patients present following ‘stealthing’ episodes, of which majority do not appear related to these purposes (personal communication with Prof. Bradshaw and Dr. Vincent Cornelisse).

There is one paper discussing ‘stealthing’ amongst MSM as a unique phenomenon amongst those attempting to intentionally transmit HIV, also termed ‘gift givers’. The study recruited 332 men randomly online from among men who were using the Internet specifically to find other men to engage in unprotected sex with (Klein 2014a). This study was qualitative, so did not examine the prevalence of ‘stealthing’. It also considered ‘stealthing’ to be a practice in which an HIV-positive man intentionally tries to infect an HIV-negative man without the latter’s knowledge or consent, which could include omission of a condom, or by other means such as non-disclosure of HIV status.

In heterosexuals, the majority of studies that mention ‘stealthing’ are in relation to birth control sabotage and have been conducted amongst domestic violence victims (Bergmann *et al.* 2015; Miller *et al.* 2010). Amongst these women, condom negotiation has been significantly associated with physical abuse (Lang *et al.* 2007; Davila *et al.* 1999). Birth control sabotage has been significantly associated with unintended pregnancy (AOR 1.58, 95% CI 1.14–2.20) (Miller *et al.* 2010).

The only paper that directly pertains to ‘stealthing’ outside of birth control sabotage or HIV-transmission is a legal paper by Brodsky, which described victims experiences and acknowledges that this act can be outside of these parameters (Brodsky 2017). The paper focuses on the legal implications of ‘stealthing’ and legal avenues to address it (Brodsky 2017). This paper did not

explore prevalence of this practice, and largely focused on ‘stealthing’ amongst heterosexuals and ignores the experiences of MSM.

8.3.2 The legality of ‘stealthing’

Until recently, Western courts have largely been ignorant of non-consensual condom removal. The National Sexual Assault Hotline in America has reported receiving calls related to this practice (Nedelman 2017), as has a Victorian Police Sexual Crimes Squad spokesperson in Australia. The Victorian Police spokesperson stated that the “removal of a condom during sex without consent could be construed as rape though all the facts would need to be considered” (Lambert 2017). Various legal cases have now occurred around the world, with the most infamous the case against Julian Assange ("Assange v Swedish Prosecution Authority" 2011; Brodsky 2017). A case around non-consensual condom removal is currently awaiting trial for sexual assault in Victoria, and will be Australia’s first ‘stealthing’ case.

Brodsky, an American lawyer, examined ‘stealthing’ in the context of American law in her paper ‘“Rape-Adjacent”: Imaging legal response to non-consensual condom removal’, published in the Columbia Journal of Gender and Law (Brodsky 2017). This paper examined whether or not removing a condom violated the terms of consent to continued intercourse, and assumed that consent is the standard of distinguishing consensual sex from sexual violence. Brodsky argued consent was violated for in two ways. The first being that the victim had consented to touch by a condom, not touch by the skin of a penis (Brodsky 2017). She stated that according to law, one may consent to one form of sexual contact without providing future consent to all sexual contact, and thus consenting to touch with a condom was a separate prior act to touch with a penis, even if they occurred in the same sexual encounter. Brodsky’s second argument for viewing ‘stealthing’ as a violation of consent due to the fact that there are inherently different risks associated with sex with a condom and sex without a condom (Brodsky 2017). There have been legal cases in which a respondent was found to have committed battery when his sexual partner agreed to intercourse after he claimed to be infertile when he actually was not, and thus the risk of pregnancy was re-introduced ("Barbara A. Vs John G. " 1983). Brodsky argued that this is the same deception as ‘stealthing’(Brodsky 2017).

Brodsky could not determine how a ‘stealthing’ case would be prosecuted, whether it would be classified as a felony (such as rape) or a misdemeanour (such as sexual harassment) (Brodsky 2017). This hinged on the ambiguity of consent. As discussed in chapter 8.2.2, most consent during sexual intercourse is nonverbal. This could create difficulties for judges and juries in determining whether or not consent was violated- a ‘he said she said’ situation.

The law in Australia is unclear whether or not a case could be prosecuted, as removing a condom during sex is not expressly written as illegal in the law (statutory law), and there is no case precedence (common law). There is a law in Sweden which expressly states removing a condom without the consent of the partner is classified as rape. It was under this law that Julian Assange, founder of WikiLeaks, was charged for rape ("Assange v Swedish Prosecution Authority" 2011). The pending trial in Victoria, Australia is significant, and will provide legal clarification around the prosecution of a ‘stealthing’ case in Western societies.

8.4 Summary of literature review exploring *'Stealthing'*

'Stealthing' is a relatively new phenomenon in the eyes of the law and society. However, 'stealthing' has clearly been occurring amongst women and MSM, with literature documenting birth control sabotage and 'gift-giving' (Bergmann *et al.* 2015; Miller *et al.* 2010; Klein 2014a). Despite the minimal legal and academic recognition, there is abundant evidence that this practice is common, with extensive discussion on various online forums such as Reddit (Brodsky 2017), and numerous clinicians' personal anecdotes at Melbourne Sexual Health Centre, the largest sexual health clinic in Australia. While many academic papers discuss early removal of condoms (Hensel *et al.* 2011; Hatherall *et al.* 2007; Warner *et al.* 1998; Shlay *et al.* 2004), none of these studies discuss whether this removal was consensual. 'Stealthing' is likely to soon be classified as sexual assault in common law, due to pending criminal trials, as it violates the terms of consent for sexual intercourse. This violation may lead to serious consequences, with condom use errors significantly associated with STIs, HIV and pregnancy, and the additional mental burden of becoming a victim of sexual assault.

With current debate in the legal community as to the nature of sexual assault, the medical implications of condom removal, and widespread public interest, there is an increasing need to determine the prevalence of 'stealthing' and the risk factors associated with it. This section of my thesis seeks to expand the literature around the proportion of women and MSM who have experienced 'stealthing', and the situational factors that were associated with the incident.

8.5 Hypotheses and Aims of Section B

8.5.1 Hypotheses

1) ‘Stealthing’ will be a commonly experienced practice amongst attendees at Melbourne Sexual Health Centre.
2) ‘Stealthing’ will be associated with demographic and situational risk factors, similar to condom use.
3) ‘Stealthing’ is likely to result in serious consequences for a proportion of those who experience it.

8.5.2 Aims

1) To determine the proportion of attendees at Melbourne Sexual Health Centre who have experienced ‘stealthing’.
2) To determine the risk and situational factors associated with ‘stealthing’ incidents.

9. How often do patients report non-consensual condom removal when presenting to a sexual health clinic.

9.1 Background

'Stealthing' has been a widespread topic of conversation in the community and in the media, prompted by both the infamous Julian Assange case and by a paper by Alexandra Brodsky, which explored the legal implications of 'stealthing' ("Assange v Swedish Prosecution Authority" 2011; Brodsky 2017). 'Stealthing' has been mentioned in the medical literature as unique phenomenon, related to birth control sabotage or related to intentional HIV transmission (Klein 2014a). However, the anecdotal evidence collected by Brodsky shows that this is not the primary purpose for this act, and 'stealthing' is likely occurring commonly outside of these acts. Due to widespread public, legal and medical interest, there is an increasing need to determine the prevalence of 'stealthing' and the risk factors associated with it.

This chapter had the following aims:

1) What proportion of sexual health center patients report experiencing 'stealthing':
    a. among heterosexuals?
    b. among MSM?
2) What are the risk factors associated with 'stealthing'?

The findings of this study were published in *PLoS One*: Latimer RL, Vodstrcil LA, Fairley CK, Cornelisse VJ, Chow EPF, Read TRH, et al. (2018) Non-consensual condom removal, reported by patients at a sexual health clinic in Melbourne, Australia. *PLoS One* 13(12): e0209779. https://doi.org/10.1371/journal.pone.0209779

This study has been presented as a poster presentation at the IUSTI Asia Pacific Sexual Health Congress, Auckland, New Zealand, November 1st-3rd 2018 (Poster Presentation #18).

This study also generated a strong interest from the media, and I was invited to discuss the findings on 'Afternoons' with Richelle Hunt and Raf Epstein on ABC Radio Melbourne, June 4th 2019; on 'The Hook Up' with Nat Tencic on triple J Radio, November 4th 2018; and in the newspaper 'The Age' by Melissa Cunningham, 'One in three women victim to 'stealth' condom removal', June 3rd 2019.

The paper has been included as text in the thesis to allow for inclusion of what was supplementary material in the published study, and for the addition of appendices. No alterations have been made,

aside from changes to abbreviations, and figure and table numbers, for thesis consistency. Please see Appendix E for the PDF of the published study.

9.2 Abstract

9.2.1 Background

Non-consensual removal of condoms, colloquially referred to as ‘stealthing’, is the removal of a condom during sex by a sexual partner when consent has been given for sex with a condom only.

9.2.2 Methods

We conducted a cross-sectional survey to determine how commonly women and MSM attending Melbourne Sexual Health Centre had experienced stealthing, and analysed situational factors associated with the event. Responses were linked to demographic information extracted from patient files.

9.2.3 Results

1189 of 2883 women (41.2%), and 1063 of 3439 MSM (30.9%) attending the clinic during the study period completed the survey. Thirty-two percent of women (95% CI: 29-35%) and 19% of MSM (95% CI: 17%,22%) reported having ever experienced stealthing. Women who had been stealthed were more likely to be a current sex worker (AOR 2.87, 95% CI: 2.01-4.11, p <0.001). MSM who had experienced stealthing were more likely to report anxiety or depression (AOR 2.13, 95% CI: 1.25-3.60, p=0.005). Both female and male participants who had experienced stealthing were three times less likely to consider it to be sexual assault than participants who had not experienced it (OR 0.29, 95% CI: 0.22-0.4 and OR 0.31, 95% CI: 0.21-0.45 respectively).

9.2.4 Conclusions

A high proportion of women and MSM attending a sexual health service reported having experienced stealthing. While further investigation is needed into the prevalence of stealthing in the general community, clinicians should be aware of this practice and consider integrating this question into their sexual health consultation. Understanding situational factors would assist in the development of preventive strategies, particularly female sex workers and MSM.

9.3 Introduction

Non-consensual removal of condoms, colloquially referred to as 'stealthing' (Brodsky 2017) or 'stealth-breeding' (Brennan 2017), refers to the practice of a sexual partner covertly removing a condom, when consent has been given for condom protected sex only (Brodsky 2017). Condoms are used as a primary preventative method of protecting against STIs, HIV and pregnancy, being 80 to 98.6% effective (Holmes *et al.* 2004; Pinkerton *et al.* 1997; Weller *et al.* 2002). Stealthing may result in the transmission of STIs, HIV, or unintended pregnancy, and could have significant personal and public health implications.

Studies of undergraduate students have found consent for sexual intercourse to be mostly communicated through non-verbal means (Hickman *et al.* 1999; Humphreys 2007), with consent for sexual intercourse often implied in the process of asking for or applying a condom (Hickman *et al.* 1999). Brodsky has argued that condom removal without mutual agreement violates consent to sex (Brodsky 2017).

In young adult heterosexual relations, it is common for male partners to engage in condom resistance tactics (Davis *et al.* 2014). Several studies have identified stealthing as a method of birth control sabotage (Miller *et al.* 2010; Moore *et al.* 2010), as well as a means of intentional HIV transmission (Klein 2014b). Anecdotal research by Brodsky focusing on heterosexual and heteronormative relations, and theoretical research by Brennan focusing on condom-less sex between men, argue these are not the primary motivators for this act (Brodsky 2017; Brennan 2017).

In spite of public interest in stealthing, there are no scientific articles that investigate how common it is, who is most at risk, and the outcomes for those who report being stealthed. We aimed to investigate the proportion of sexual health centre patients reporting nonconsensual removal of condoms: 1) among heterosexual women and 2) among MSM, as well as associated risk factors. For the purpose of this study, 'stealthing' was defined as condom removal without consent, where consent to sex was conditional upon use of a condom.

9.4 Methods

9.4.1 Population and setting

This was a cross-sectional questionnaire-based study conducted amongst women and MSM attending the Melbourne Sexual Health Centre in Victoria, Australia, between the 22nd December 2017 and the 22nd February 2018. Melbourne Sexual Health Centre is the largest public sexual health service in Victoria, Australia. The centre provides around 50,000 consultations every year, 37% with women and 36% with MSM (Chow *et al.* 2014). Clinic attendees routinely complete a computer assisted self-interview about their sexual history prior to seeing a triage nurse.

9.4.2 Study measurement

Women and MSM presenting to Melbourne Sexual Health Centre, aged 18 or over, were invited to complete an electronic questionnaire containing questions about stealthing after completing the computer assisted self-interview (Appendix E2). Participants read a patient information and consent form which detailed the nature of the survey, and patients could only commence the questionnaire after ticking a box stating 'Yes- willing to help'. Due to the potential of the questionnaire to cause distress when recalling the stealthing event, the participant information included advertisement of free counselling services available at Melbourne Sexual Health Centre and elsewhere. The Alfred Hospital Ethics Committee approved the study (number 494/17).

Age, number of sexual partners, and HIV status were extracted electronically from routinely collected clinic records for respondents and non-respondents, de-identified for non-respondents, and linked to questionnaire responses for respondents (Figure 22).

The questionnaire asked whether the participant had ever had a condom removed during sex with or without permission and at what point the participant noticed. Participants could choose from a hierarchy of seven responses describing the circumstances. Multiple responses were allowed for those reporting multiple occurrences, and there was no time limit applied to the reported event. Participants were deemed not to have experienced stealthing if they responded either: 1) they had never had a condom removed during sex, 2) that a condom had been removed with permission, or 3) that a condom was removed without permission but they willingly continued sex. Participants were deemed to have experienced stealthing if they reported: 4) condom removal without permission and sex continued unwillingly, 5) condom removal without permission and sex was discontinued, 6) condom removal during sex but they did not realise until afterwards, or 7) the condom was never put on despite being requested. If a participant only selected options between 1 and 3 they were classified as never having been stealthed. If a participant selected any option

between 4-7, regardless of whether they had also selected options between 1 and 3, they were classified as ever having been stealthed (Figure 22).

Participants who reported stealthing were asked further questions about the specific event (Figure 22). Participants who had selected multiple options were asked about the incident with the highest assigned number. For instance if they reported several stealthing events with differing scenarios and selected both response 4 and 5, then specific questions were asked about "event 5" only – i.e. condom removal without permission and sex was discontinued. Questions included: when the incident occurred, how long they had known the partner, how they would describe the relationship, where they had met, whether either person had been using drugs or alcohol, whether the event was reported to the police, and what they perceived were the consequences of the condom removal. All respondents were asked whether they considered the removal of a condom without consent to be sexual assault.

9.4.3 Statistical analysis

All analyses were performed using Stata IC version 14. MSM who reported only insertive anal sex and no receptive anal sex while completing the computer assisted self-interview were excluded from the dataset prior to analysis of questionnaire responses, as experiencing stealthing was considered unlikely if the male was only the insertive partner. Risk factors for experiencing stealthing in women and MSM were not compared to each other as they are different populations. Univariable and multivariate analyses were performed to determine the differences in demographics between non-respondents and respondents, and the differences between those who had and had not experienced stealthing. Variables were included in multivariate models if the p-value was ≤ 0.1; if correlated, the variable most strongly associated with the outcome was used. Models were built in a backward-stepwise fashion, using the likelihood ratio test to determine the significance of the contribution of each variable. Ninety-five percent binomial CIs were calculated for all proportions. We assumed 100 patients would complete the survey each week and estimated 2% would report ever being stealthed. The 95%CIs around an estimated 2% prevalence of stealthing after six weeks (600 responses) would be 1.0-3.5%.

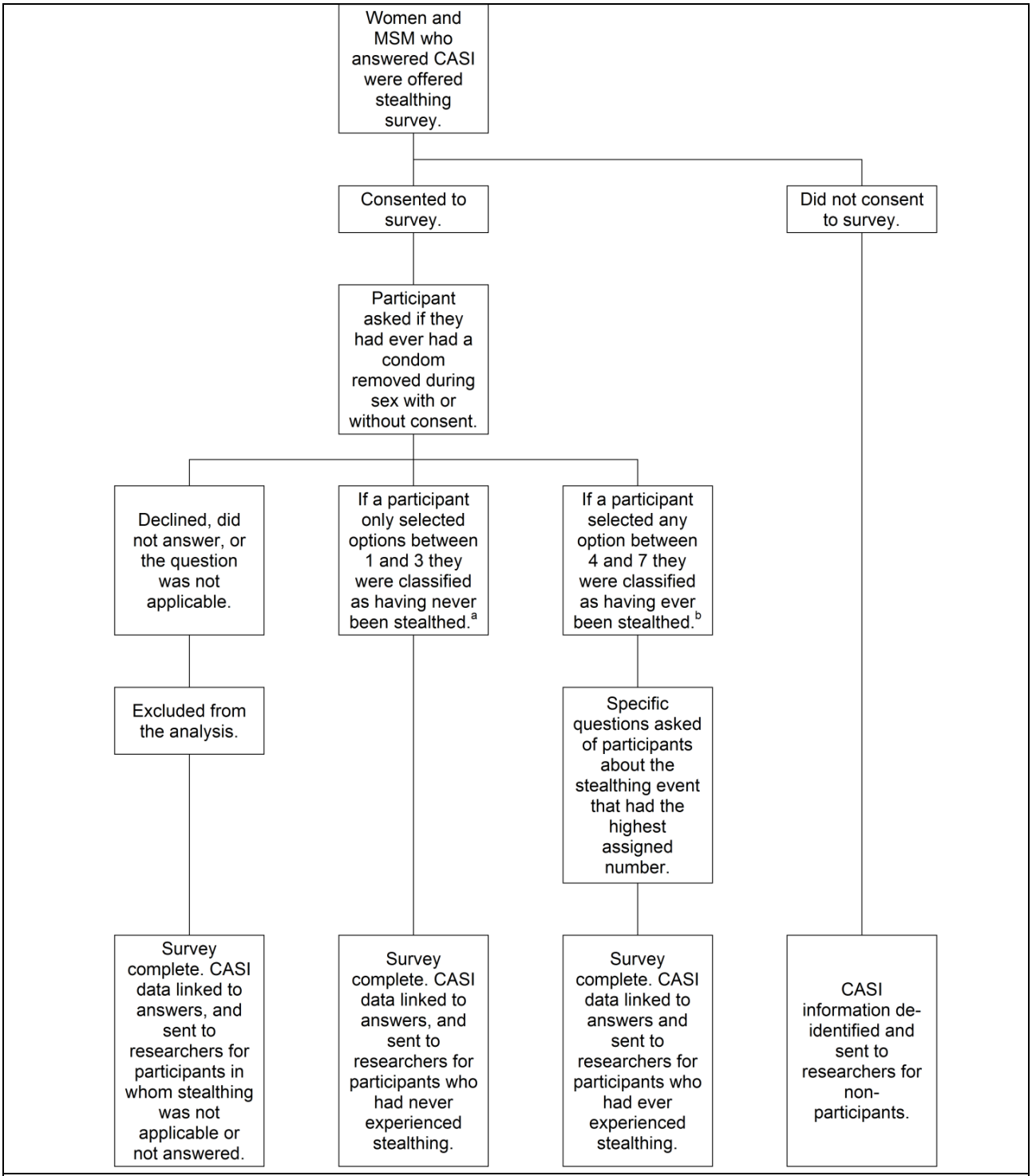

Figure 22. Possible pathways for patients offered the survey, and the classification for analysis of nonconsensual condom removal.

Abbreviations: MSM= men who have sex with men; CASI= computer assisted self-interviewing. [a]Participants were classified as never having experienced stealthing if they responded either: 1) they had never had a condom removed during sex, 2) that a condom had been removed with permission, or 3) that a condom was removed without permission but they willingly continued sex. [b]Participants were deemed to have experienced stealthing if they reported: 4) condom removal without permission and sex continued unwillingly, 5) condom removal without permission and sex was discontinued, 6) condom removal during sex but they did not realise until afterwards, or 7) the condom was never put on despite being requested.

9.5 Results

During the study period, 2883 women and 3439 MSM attended the clinic, of whom 1189 women (41%, 95%CI: 39-43%) and 1063 MSM (31%, 95%CI: 29-32%) completed the survey (classified as respondents).

Female respondents were more likely than non-respondents to have had sex overseas in the last twelve months (AOR 1.49, 95% CI: 1.26-1.77, p<0.001) and were less likely to be a current sex worker (AOR 0.78, 95% CI: 0.63-0.96, p=0.02) (Table27). Compared to MSM non-respondents, the men who responded were more likely to have had sex overseas in the last twelve months (AOR 1.70, 95% CI: 1.37-2.11, p<0.001), and were less likely to be HIV positive (AOR 0.60, 95% CI: 0.38-0.95, p=0.029) (Table27).

Of the 1189 women and 1063 MSM who consented to the survey and answered the first question: 60 (5%) women and 64 men (6%) declined to answer whether they had experienced stealthing, 45 (4%) women and 37 (3%) men deemed the question to be not applicable to them i.e. they never used condoms, or did not engage in penetrative sex with men and 90 (8%) men were removed from the analysis, as they had only reported insertive anal sex and not reported receptive anal sex in thr computer assisted self-interview (Table28).

Three hundred and forty-six of the remaining 1084 women (32%, 95% CI: 29-35%) and 168 of the remaining 872 MSM (19%, 95% CI: 17-22%) reported having ever experienced stealthing (Table29). Of those who had experienced stealthing, forty-two women (12%, 95% CI: 9-16%) and 23 MSM (14%, 95% CI: 9-20%) presented to the clinic on the day of the questionnaire following a reported stealthing incident (Table 30).

On multivariate analysis, women who had been stealthed were more likely to be a current sex worker than those who had never experienced stealthing (AOR 2.87, 95% CI: 2.01-4.11, p<0.001) (Table29), and MSM who had been stealthed were more likely to report ‘health issues, such as anxiety or depression which may have affected their decision to use condoms for anal sex’ than those who had never experienced stealthing (AOR 2.13, 95% CI: 1.25-3.60, p=0.005) (Table29).

Most women met the male partner who had stealthed them through friends (29%, 95% CI: 24-34%) or sex work (23%, 95% CI: 19-28%). MSM reporting stealthing most commonly described the partner as someone they “did not know well” (61%) and had predominantly met them through geosocial dating applications or online (67%, 95% CI: 59-74%) (Table 30).

At the time of the stealthing incident, 41% (95% CI: 36-47%) of women and 54% (95% CI: 46-62%) of MSM reported being sober, while 57% (95% CI: 51-62%) of women and 41% (95% CI:

33-49%) of MSM had consumed alcohol. Twelve percent of women and 13% of MSM had used other drugs either in addition to or without alcohol (Table 30). The majority of women reported their partner had consumed alcohol (68%, 95% CI: 62-73%) and/or other drugs (19%), with only 27% (95% CI: 22-33%) stating the partner had been sober when the incident occurred. Many MSM believed their partner to be sober (53%, 95% CI: 44-62%), with 40% (95% CI: 31-50%) of partners under the influence of alcohol, and 12% using additional/or other drugs (Table 30).

The majority of women (61%) and MSM (55%) discussed the removal of the condom with their partners after the event. Over half of the participants reported being emotionally stressed following the incident. Eight percent of women and five percent of MSM reported they thought they had acquired an STI following the event. One percent of women and two percent of MSM believed they had acquired HIV as a consequence of being stealthed (Table 30). Only 1% of people stealthed reported this experience to the police (Table 30).

Both female and MSM participants who had experienced stealthing were less likely to consider it to be sexual assault than participants who had not experienced stealthing. Amongst women, 62% (95% CI: 56-67%) of those stealthed considered it to be assault, compared to 85% (95% CI: 82-87%) of those not stealthed (OR 0.29, 95%CI: 0.22-0.4, $p<0.001$). Amongst men, 61% (95% CI: 53-69%) of those stealthed considered it to be assault versus 84% (95% CI: 81-86%) of those not stealthed (OR 0.31, 95%CI: 0.21-0.45, $p<0.001$).

Table 27. Demographics and epidemiological features of respondents versus non-respondents to survey on rates of non-consensual removal of condoms (stealthing) in a sexually transmitted infections clinic (N=6322)

	Female non-respondents n=1694 (%; 95% CI) or median [range]		Female respondents n=1189 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio[a] (95% CI)		p-value
Age	27	[16-74]	26	[18-64]						
Employment										
Employed	958	(60; 57,62)	689	(60; 57,62)	1					
Not in the labour force[b]	641	(40; 38,43)	467	(40; 38,43)	1.01	(0.87,1.18)	0.87			
Aboriginal and/or Torres Strait Islander peoples										
No	1479	(99; 98,99)	1074	(99; 98,99)	1					
Yes	16	(1; 0,2)	14	(1; 1,2)	1.20	(0.59,2.48)	0.613			
Sex overseas										
No	817	(60; 57,62)	485	(48; 45,52)	1			1		
Yes	552	(40; 38,43)	517	(52; 48,55)	1.58	(1.35,1.86)	<0.001	1.49	(1.26,1.77)	<0.001
Injecting drug use										
Never injected	1420	(98; 97,99)	1023	(98; 97,99)	1					
Ever injected	26	(2; 1,3)	22	(2; 1,3)	1.17	(0.66,2.08)	0.582			
Current sex worker										
No	1095	(76; 74,78)	856	(82; 79,84)	1			1		
Yes	348	(24; 22,26)	191	(18; 16,21)	0.70	(0.58,0.86)	<0.001	0.78	(0.63,0.96)	0.020
Condom Use in the last 3mo with male partners										
Not always	1014	(83; 81,85)	765	(82; 80,85)	1					
Always	204	(17; 15,19)	163	(18; 15,20)	1.06	(0.84,1.33)	0.619			
Number of male sexual partners in the last 3mo	1	[0-50]	1	[0-15]						
	Male non-respondents n=2376 (%; 95% CI) or median [range]		**Male respondents n=1063 (%; 95% CI) or median [range]**		**Unadjusted Odds Ratio (95% CI)**		**p-value**	**Adjusted Odds Ratio[c] (95% CI)**		**p-value**

Age	30	[16-82]	30	[18-75]						
Employment										
Employed	1480	(67; 65,69)	644	(64; 61,67)	1					
Not in the labour force[b]	742	(33; 31,35)	361	(36; 33,39)	1.12	(0.96,1.31)	0.161			
Aboriginal and/or Torres Strait Islander peoples										
No	2114	(99; 98,99)	978	(99; 99,100)	1			1		
Yes	26	(1; 1,2)	5	(1; 0,1)	0.42	(0.16,1.09)	0.073	0.64	(0.21,1.97)	0.441
Sex overseas										
No	1365	(70; 69,72)	542	(61; 58,64)	1			1		
Yes	587	(30; 28,32)	345	(39; 36,42)	1.48	(1.25,1.75)	<0.001	1.70	(1.37,2.11)	<0.001
Injecting drug use										
Never injected	2048	(96; 96,97)	914	(97; 96,98)	1					
Ever injected	75	(4; 3,4)	28	(3; 2,4)	0.84	(0.55,1.3)	0.428			
Current sex worker										
No	2126	(>99; 99,100)	933	(99; 98,99)	1			1		
Yes	9	(<1; 0,1)	10	(1; 1,2)	2.53	(1.03,6.25)	0.044	2.72	(0.97,7.59)	0.057
Condom Use in the last 3mo with male partners										
Not always	1379	(74; 72,76)	616	(71; 68,74)	1					
Always	492	(26; 24,29)	246	(29; 26,32)	1.12	(0.93,1.34)	0.220			
HIV status										
Negative	1279	(91; 90,93)	558	(95; 92,96)	1			1		
Positive	119	(9; 7,10)	32	(5; 4,8)	0.62	(0.41,0.92)	0.019	0.61	(0.38,0.97)	0.038
Use of prep										
No	1844	(81; 79,82)	861	(83; 81,85)	1					
Yes	436	(19; 18,21)	174	(17; 15,19)	0.85	(0.70,1.04)	0.112			
Number of male sexual partners in the last 3mo	3	[0-100]	3	[0-140]						

Abbreviations: n=number; CI=confidence interval; mo= months; HIV=human immunodeficiency virus; PrEP=HIV pre-exposure prophylaxis

Notes: [a]Adjusted model for females includes: sex overseas and current sex worker; [b]Not in the labour force includes both those who are unemployed and/or students [c]Adjusted model for males includes: Aboriginal and/or Torres Strait Islander peoples, sex overseas, current sex worker and HIV status.

Data missing for: <5% of PrEP data; <5%-10% of employment data; 5-15% of Aboriginal and/or Torres Strait Islander peoples data; 10-15% of current sexworker data; 10%-20% of sex overseas data and injecting drug use data; 15- ≥20% of condom use data; and >20% of HIV data. Proportions are calculated using available data.

Table 28. Reported events of non-consensual removal of condoms (stealthing) amongst patients presenting to a sexually transmitted infections clinic (N=2252)[a]

	Female respondents n=1189 (%; 95% CI)		Male respondents n=1063 (%; 95% CI)	
Classified as not experiencing 'stealthing'				
Never stealthed	420	(35; 33,38)	496	(47; 44,50)
Condom removed w permission	455	(38; 35,41)	315	(30; 27,32)
Condom removed w/o permission but continued willingly	104	(9; 7,10)	77	(7; 6,9)
Classified as experiencing 'stealthing'				
Condom removed w/o permission, and continued unwillingly	108	(9; 8,11)	52	(5; 4,6)
Condom removed w/o permission, and stopped	135	(11; 10,13)	65	(6; 5,8)
Condom removed w/o permission, but didn't realise until afterwards	147	(12; 11,14)	60	(6; 4,7)
Condom never put on but had been requested	84	(7; 6,9)	41	(4; 3,5)
Removed from further analysis				
Not applicable[b]	45	(4; 3,5)	127	(12; 10,14)
Decline answer	*60*	*(5; 4,6)*	*64*	*(6; 5,8)*

Abbreviations: n=number; CI=confidence interval; w=with; w/o=without.

Notes: [a]Patients could select multiple options, to report multiple events occurring, i.e. events are not mutually exclusive, therefore percentages do not sum to 100. Percentages represent the proportion of participants who have reported the event. If reporting multiple events, patients were classified in the analysis based off the highest numbered event they reported, if 1 is Never and 7 is 'Condom never put on even though requested'. [b]Not applicable refers to patients who have not/do not engaged in penetrative penile sex, includes 97 MSM who responded to survey but reported no receptive anal sex and 30 who selected not applicable.

Table 29. Risk factors associated with non-consensual removal of condoms (stealthing) in patients presenting to a sexually transmitted infections clinic (N=2042)

	Women who have not had been stealthed n=738 (%; 95% CI) or median [range]		Women who have have been stealthed n=346 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio (95% CI)[a]		p-value
Age	26	[18-58]	26	[18-55]						
Number of male sexual partners in the last 3mo	2	[0-15]	1	[0-15]						
Employment[b]										
Employed	439	(61; 57,65)	189	(56; 51,62)	1					
Not in the labour force	281	(39; 35,43)	146	(44; 38,49)	1.21	(0.93,1.57)	0.161			
Education level										
Did not complete high school	18	(2; 1,4)	13	(4; 2,6)	1					
High school/Certificate/Diploma	238	(33; 29,36)	134	(39; 34,45)	0.78	(0.37,1.64)	0.512			
University degree	475	(65; 61,68)	195	(57; 52,62)	0.57	(0.35,1.47)	0.131			
Aboriginal and/or Torres Strait Islander peoples										
No	672	(99; 98,99)	319	(98; 96,99)	1					
Yes	8	(1; 1,2)	5	(2; 1,4)	1.31	(0.43,4.06)	0.632			
Australian/New Zealander										
No	441	(63; 59,66)	166	(51; 45,56)	1					
Yes	264	(37; 34,41)	160	(49; 44,55)	1.61	(1.23,2.09)	<0.001	1.26	(0.94,1.70)	0.122
Current sex worker										
No	573	(87; 85,90)	215	(71; 65,76)	1					
Yes	83	(13; 10,15)	89	(29; 24,35)	2.86	(2.04,4.01)	<0.001	2.87	(2.01,4.11)	<0.001
Injecting drug use										
Never injected	644	(98; 97,99)	295	(97; 95,99)	1					
Ever injected	11	(2; 1,3)	8	(3; 1,5)	1.59	(0.63,3.99)	0.325			
Sex overseas										

No	303	(47; 44,51)	136	(47; 41,53)	1			
Yes	335	(53; 49,56)	153	(53; 47,59)	1.02	(0.77,1.34)	0.903	
Use other contraceptives in addition to condoms[c]								
No	293	(46; 42,50)	112	(47; 40,53)	1			
Yes	339	(54; 50,58)	128	(53; 47,60)	0.94	(0.733,1.33)	0.936	

	MSM who have not been stealthed n=704 (%; 95% CI) or median [range]		**MSM who have been stealthed n=168 (%, 95% CI) or median [range]**		**Unadjusted Odds Ratio (95% CI)**	**p-value**	**Adjusted Odds Ratio (95% CI)[d]**	**p-value**
Age	30	[18-75]	29	[18-58]				
Number of male sexual partners in the last 3mo	3	[0-140]	3	[0-100]				
Employment								
Employed	435	(65; 61,69)	98	(61; 53,68)	1			
Not in the labour force	232	(35; 31,39)	63	(39; 32,47)	1.20	(0.85,1.72)	0.302	
Education level								
Did not complete high school	24	(3; 2,5)	7	(4; 2,8)	1			
High school/Certificate/Diploma	183	(26; 23,30)	34	(20; 14,27)	0.64	(0.25,1.60)	0.336	
University degree	494	(70; 67,74)	127	(76; 68,82)	0.88	(0.37,2.09)	0.775	
Aboriginal and/or Torres Strait Islander peoples								
No	701	(100; 99,100)	166	(99; 97,100)	1			
Yes	2	(0; 0,1)	1	(1; 0,3)	2.11	(0.19,23.42)	0.543	
Australian/New Zealander								
No	343	(50; 46,54)	83	(49; 42,57)	1			
Yes	342	(50; 46,54)	85	(51; 43,58)	1.03	(0.73,1.44)	0.877	
Current sex worker								
No	622	(99; 98,100)	151	(99; 95,100)	1			

Yes	5	(1; 0,2)	2	(1; 0,5)	1.65	(0.32,8.57)	0.553			
Injecting drug use										
Never injected	611	(97; 96,98)	145	(96; 92,99)	1					
Ever injected	18	(3; 2,4)	6	(4; 1,8)	1.4	(0.55,3.60)	0.480			
Sex overseas										
No	354	(60; 56,64)	82	(57; 49,66)	1					
Yes	237	(40; 36,44)	61	(43; 34,51)	1.11	(0.77,1.61)	0.577			
HIV status										
No	375	(95; 93,97)	96	(2; 85,97)	1					
Yes	19	(5; 3,7)	8	(8; 3,15)	1.64	(0.70,3.87)	0.255			
Use of prep										
No	582	(84; 81,87)	126	(78; 71,84)	1			1		
Yes	110	(16; 13,19)	35	(22; 16,29)	1.47	(0.96,2.25)	0.077	1.16	(0.70,1.92)	0.567
Drugs use with anal sex w/o a condom in the last 12mo[e]										
No	222	(58; 53,63)	58	(55; 45,64)	1					
Yes	162	(42; 37,47)	48	(45; 36,55)	1.13	(0.74,1.75)	0.569			
Drunk during anal sex w/o a condom in the last 12mo[e]										
No	219	(57; 52,62)	53	(50; 41,60)	1					
Yes	166	(43; 38,48)	52	(50; 40,59)	1.29	(0.84,1.99)	0.242			
Anal sex w/o a condom with known HIV positive in the last 12mo[e]										
No	319	(83; 79,87)	83	(82; 73,89)	1					
Yes	65	(17; 13,21)	18	(18; 11,27)	1.06	(0.60,1.89)	0.832			
Anal sex w/o a condom with someone of unknown HIV status in the last 12mo[e]										
No	189	(50; 45,55)	39	(38; 29,48)	1			1		
Yes	190	(50; 45,55)	63	(62; 52,71)	1.61	(1.03,2.51)	0.038	1.51	(0.96,2.39)	0.075
Self-reported health issues, such as anxiety or depression, which may have affected your decision to use condoms for anal sex?[e]										
No	318	(85; 81,89)	74	(73; 63,81)	1			1		
Yes	55	(15; 11,19)	28	(27; 19,37)	2.19	(1.30,3.68)	0.003	2.13	(1.25,3.6)	0.005

Abbreviations: n=number; CI=confidence interval; mo=months; MSM= men who have sex with men; HIV=human immunodeficiency virus; PrEP=HIV pre-exposure prophylaxis; w/o=without

Notes: [a]Adjusted model for females includes: Australian and current sex worker; [b]Not in the labour force includes both those who are unemployed and/or students [c]Women who reported not using contraception due to pregnancy were excluded (2 females who did not have condoms removed, and 10 who did). [d]Adjusted model for males includes: use of prep, condom use with someone of uncertain HIV status, health issues (anxiety & depression) affecting decisions to use condoms. [e]These questions were asked only to patients who had reported unprotected anal sex since their last HIV test as part of their routine computer assisted self-interviewing.
Data missing for: <5% of employment data, education data and PrEP data; <5%-10% of Aboriginal and/or Torres Strait Islander peoples data and Australian data; 10%-15% of injecting drug use data and current sex worker data, 10%-20% sex overseas data; 10- ≥20% contraception data; and ≥20% of HIV status and questions on issues affecting decisions to use condoms. Proportions are calculated using available data.

Table 30. Situational factors surrounding non-consensual removal of condoms (stealthing) reported by patients presenting to a STI clinic (N=523)

	Women n=346 (%; 95% CI)		MSM n=168 (%; 95% CI)	
When the incident occurred				
Here today for this reason	42	(12; 9,16)	23	(14; 9,20)
In the last 3mo	59	(17; 13,22)	20	(12; 7,18)
3-12 mo ago	78	(23; 18,28)	35	(21; 15,28)
More than 12 months ago	120	(35; 30,40)	78	(46; 39,54)
More than 1 occasion	43	(13; 9,17)	12	(7; 4,12)
Relationship				
Did not know him well	101	(30; 25,36)	102	(61; 54,69)
Friend	33	(10; 7,14)	10	(6; 3,11)
Friend with benefits/ Sex buddy	51	(15; 12,20)	30	(18;13,25)
Casually dating	54	(16; 12,21)	22	(13; 8,19)
Relationship	25	(8; 5,11)	2	(1; 0,4)
Client (of sex worker)	69	(21; 16,25)	0	(0; 0,2)[a]
Relationship duration				
Less than a day (<24hrs)	126	(38; 33,44)	85	(52; 44,59)
One day to one month	95	(29; 24,34)	39	(24; 17,31)
More than one month	107	(33; 28,28)	41	(25; 18,32)
Met through				
Smartphone dating app/Internet	64	(20; 15,24)	110	(67; 59,74)
(Gay) bar or party	50	(15; 12,20)	20	(12; 8,18)
Gay sauna, beats of SOPV, sex party	2	(1; 0,2)	24	(15; 10,21)
Friend, or friend of friend	94	(29; 24,34)	6	(4; 1,8)
Co-workers	22	(7; 4,10)	3	(2; 0,5)
Sex work	76	(23; 19,28)	0	(0; 0,2)[a]
Travel	15	(5; 3,7)	0	(0; 0,2)[a]
Other (café, park etc.)	4	(1; 0,3)	1	(1; 0,3)
Drugs used by partner[bc]				
None	75	(27; 22,33)	63	(53; 44,62)
Alcohol	188	(68; 62,73)	48	(40; 31,50)
Cannabis/marijuana/hash	28	(10; 7,14)	4	(3; 1,8)
Ecstasy	12	(4; 2,7)	4	(3; 1,8)
Speed/ice/meth	5	(2; 1,4)	6	(5; 2,11)
GHB	2	(1; 0,3)	3	(2; 1,7)
Cocaine	10	(4; 2,7)	3	(2; 1,7)
Heroin	1	(<1; 0,2)	0	(0; 0,3)[a]
Other	1	(<1; 0,2)	3	(2; 1,7)
Drugs used by respondent[bd]				
None	135	(41; 36,47)	87	(54; 46,62)
Alcohol	186	(57; 51,62)	65	(41; 33,49)
Cannabis/marijuana/hash	21	(6; 4,10)	3	(2; 0,5)
Ecstasy	9	(3; 1,5)	4	(3; 1,6)
Speed/ice/meth	5	(2; 0,4)	8	(5; 2,9)
GHB	2	(1; 0,2)	3	(2; 0,5)

Cocaine	8	(2; 1,5)	4	(3; 1,6)
Heroin	1	(<1; 0,2)	0	(0; 0,2)[a]
Other	0	(0; 0,1)[a]	4	(3; 1,6)
Condom removal discussed with partner				
No	128	(39; 33,44)	74	(45; 37,52)
Yes	204	(61; 56,67)	92	(55; 48,63)
Consequences of condom removal[b]				
None	85	(25; 21,30)	62	(38; 30,46)
Emotional stress	190	(56; 51,62)	86	(52; 45,60)
Caught an STI	26	(8; 5,11)	9	(5; 3,10)
Contracted HIV	2	(1; 0,2)	3	(2; 0,5)
Fight	49	(14; 11,19)	15	(9; 5,15)
Relationship broke up	30	(9; 6,12)	6	(4; 1,8)
Other	42	(12; 9,16)	12	(7; 4,12)
Reported to the police				
No	336	(99; 97,100)	163	(98; 95,100)
Yes	3	(1; 0,3)	3	(2; 0,5)

Abbreviations: n=number; CI=confidence interval; MSM=men who have sex with men; mo=months; SOPV=sex on premises venue; GHB= Gamma-hydroxybutyrate; STI=sexually transmitted infection; HIV=human immunodeficiency virus

Notes: [a]one-sided, 97.5% confidence interval [b]Patients could select multiple options, to report multiple events occurring, i.e. events are not mutually exclusive, therefore percentages do not sum to 100. Percentages represent the proportion of participants who have reported the event. [c]64 women (19%) and 47 MSM (28%) were unsure as to whether or not their partner had used any alcohol and/or other drugs and were removed from the analysis. [d]11 women (3%) and 6 MSM (4%) were unsure as to whether or not they had used any alcohol and/or other drugs and were removed from the analysis.

Data missing from up to 5% of female respondents and up to 3% of male respondents; proportions are calculated using available data

9.6 Discussion

Although increasingly discussed in international media, there is little scientific research on non-consensual removal of condoms, popularly termed ‘stealthing’. To our knowledge this is the first study investigating how common stealthing is, the context in which it occurred, the impact on individuals, and how those stealthed perceive the event. A surprising proportion of clients attending a sexual health centre in Melbourne (32% of women and 19% of MSM) reported removal of a condom in a situation where they would not have willingly engaged in sexual intercourse without one - in other words, a violation of their consent (Brodsky 2017).

These data need to be interpreted in the context of a STI clinic population which is generally a higher risk group than the general population. Our data show that 4% of women and 3% of MSM presenting to our clinic during the study period were attending following a stealthing incident. This equates to over 1200 consultations per year (Chow *et al.* 2014). These data suggest that stealthing is common and should be considered when assessing patients in STI services.

Female respondents were less likely to be a current sex worker and MSM respondents were less likely to be HIV positive, compared to non-respondents. It is possible that both sex workers and HIV positive men were less likely to complete the survey due to privacy concerns, especially with regards to condom use and their legal obligations, which vary state by state in Australia. In Victoria, sex workers are legally required to use condoms with clients, and while those who are HIV positive are not legally required to disclose their HIV status, they must take reasonable precautions to prevent HIV transmission to those they are engaging in penetrative sex with (Kidd). Reasonable precaution refers to correct use of condoms and lube during intercourse. While female sex workers were less represented in respondents than non-respondents, 18% of participants were sex workers and we still observed an association between being a sex worker and being more likely to be stealthed. Low numbers of HIV positive men participating may have limited our ability to examine any association between stealthing and HIV status. Lastly, both women and MSM who had been overseas recently were more likely to respond to our survey. This may bias our findings towards individuals who may have participated due to recent high risk sexual encounters, in the context of overseas travel (Sundbeck *et al.* 2017).

Women who experienced stealthing were three times more likely to be sex workers compared to those who had not. In the Law and Sex Worker Health (LASH) Survey conducted in Australia, 8% of respondents reported assault by clients (Donovan 2012). However the LASH survey did not compare rates of assault to the general population or differentiate between physical and sexual assault, and only examined assault in the workplace. Perkins' (1991) research with Sydney-based

brothel workers found that 20% of sex workers experienced rape while working. Outside the workplace sex workers experienced higher levels of sexual assault compared with non-sex workers, with 46.9% reporting rape, compared to 21.9% of health workers and 12.7% of students (Perkins). Our data are consistent with these findings that sex workers are at increased risk of non-consensual sex acts.

Sixty-seven percent of MSM who had experienced stealthing met the partner via geosocial dating applications, for example Grindr, Tinder or Scruff. This is comparable to the number of MSM meeting partners through dating applications (70%) (Chow *et al.* 2016). Sexual encounters initiated online are more likely to include unprotected anal intercourse (Horvath *et al.* 2008), however it has also been found that meeting partners online increases the likelihood of discussion between partners of preferred sexual practices compared to meeting partners offline (Horvath *et al.* 2008; Carballo-Diéguez *et al.* 2006). MSM who had been stealthed were twice as likely to report having anxiety or depression. Depressive symptoms and anxiety are predictive of condom non-use (Lehrer *et al.* 2006) and higher levels of depression are related to lower levels of self-efficacy for sexual safety (Alvy *et al.* 2011). MSM who have anxiety or depression may be vulnerable to stealthing for this reason.

In this study, the majority of women (73%) believed the partner who had stealthed them to be under the influence of alcohol and/or other drugs. In heterosexual relations, the link between alcohol consumption and committal of sexual assault is well documented (Abbey 2002; Abbey *et al.* 2004). Condom resistance tactics and sexual aggression with female partners are more commonly employed by men with history of sexual aggression and alcohol intoxication (Davis *et al.* 2016; Davis 2010). Additionally, both alcohol consumption (Rubin *et al.* 1976) and condom use (Musacchio *et al.* 2006; Graham *et al.* 2006) have been associated with erectile dysfunction. Men with erection issues are more likely to engage in unprotected sex, misuse condoms (Musacchio *et al.* 2006; Graham *et al.* 2006), and are more likely to remove condoms before sex is over ($p=0.001$) (Graham *et al.* 2006). Literature supports our finding that heterosexual men who have consumed alcohol may be at increased risk of committing nonconsensual sex acts, and may be removing the condom to maintain an erection.

Whilst the majority of those reporting stealthing considered it sexual assault, they were three times less likely to consider stealthing sexual assault than those who had never experienced it. The US National Crime Victimization Survey found 20% of female victim narratives contained excuses for offenders' behaviour, denials of injury, or justification of the incident as the victims' fault (Weiss 2009). This allowed the women to avoid the distress of labelling themselves victims of a crime, or

their partners as criminals (Weiss 2009). Victims of stealthing may also not yet view themselves as sexual assault victims as stealthing is a relatively new topic. Sexual assault is a term with many connotations and there are cultural myths as to who is a 'real' sexual victim (Lievore 2003), with the type of violence experienced influencing society's view as to whether a woman is a victim (Healicon 2016). Our current language around sexual assault (and in this case, stealthing) may require expansion- until an act is named as assault it cannot be viewed as such, and cannot be reported or legislated against (Grady 2017). A limitation of this study is that we did not ask respondents why they did not consider stealthing to be sexual assault.

Stealthing has potentially serious consequences. The majority of patients reported consequences following the stealthing incident, with over half experiencing emotional stress. Although literature contains estimates as to the rate of STI and HIV transmission during sexual assault, it is difficult to establish if an STI has been acquired from a specific event. The CDC guidelines recommend testing all people for STIs following sexual assault (Jiang *et al.* 2015), with the caveat that many positive tests will be from a pre-existing STI (Sachs *et al.* 2018). MSM patients with condom malfunction or condom-less sex presenting in a 72 hour window fulfil criteria for HIV non-occupational post exposure prophylaxis (McDougal *et al.* 2014; Pinkerton *et al.* 2004), and therefore MSM who present reporting non-consensual condom removal should be prescribed it.

This study has several limitations. Firstly, this study was offered in English only, which means it cannot be generalised to attendees who are not fluent in English. Secondly, this study may be subject to responder bias, as those who have experienced stealthing may have been more likely to answer the survey. Given this is a retrospective survey, participant responses may be subject to recall bias, and specific contextual situational factors and outcomes were asked about one event only for those stating it had happened on more than one occasion. While some participants within our study attributed the acquisition of STIs to being stealthed, this cannot be verified. According to attribution theory (Kelley 1967) following an adverse event people will make attributions to understand and control their environment (Wong *et al.* 1981) , with situational factors often exaggerated when there is a negative outcome (Heider 1958), and thus patients could be incorrectly attributing contracting a STI to the stealthing event.

Despite these limitations, this study has a large sample size with over two thousand responses. Accurate statistics describing the prevalence and incidence of sexual assault are difficult to obtain since the majority of assaults are not reported to authorities and victims often do not access services (Lievore 2003). Only 1% of patients reporting stealthing in this study reported the event to the police. Although this study may be subject to recall bias, population surveys are the best means of

learning the true extent and nature of these crimes, rather than relying on crime statistics. This is the first study to describe how commonly this practice is occurring.

In summary, stealthing was commonly experienced by our clinic population, with a third of women and a fifth of MSM reporting it, with situational contexts often involving alcohol and/or drugs in women, and geosocial networking applications in MSM. Sex work was a clear risk factor identified among women, and risk factors for MSM included anxiety and depression. Knowledge of these risk factors can enable services to ask about stealthing in target groups and offer specific support and counselling. Further community-based research would help determine the prevalence in the broader population and studies that link behavioural measures to biological outcomes would help to quantify the STI risk associated with this practice.

SECTION C

THESIS CONCLUSIONS

10. Outlook and Concluding Remarks

10.1 *Mycoplasma genitalium*

10.1.1 General Discussion of *Mycoplasma Genitalium*

M.genitalium is bacterial STI that is becoming increasingly complex to treat due to increasing antimicrobial resistance. The need to treat is also impacted by the lack of clarity around its natural history. Since the introduction of commercial diagnostic assays, *M.genitalium* testing is now increasingly available. As such, it has become increasingly important to examine the evidence underpinning *M.genitalium* testing and treatment. This is of particular importance in MSM, as a population at high risk of STIs, and in women, as the population most significantly affected by STI sequelae. This thesis sought to determine the prevalence of *M.genitalium* in specific populations and anatomical sites, and to define the contribution of *M.genitalium* to genital symptoms and syndromes in women.

The systematic review and meta-analysis in Chapter Three provided the first estimates of the prevalence of *M.genitalium* by anatomical site in MSM. Including data from 46 studies, representing nearly 24,000 samples, we found the overall summary estimate for *M.genitalium* in MSM to be 5.8% (95%CI: 4.5-7.3), with a prevalence of 6.2% (95%CI: 4.6-8.1) at the rectum, 5.0% (95%CI: 3.5-6.8) in the urethra and 1.0% (95%CI: 0.0-5.1; I^2=96.0) in the pharynx, though high heterogeneity was recorded in most of the summary estimates. These data suggest the pharynx does not play a significant role in the transmission of *M.genitalium*. Urethral and rectal *M.genitalium* were more common in MSM with symptoms, supporting current recommendations that *M.genitalium* testing should be undertaken in men with urethral symptoms, and suggesting that *M.genitalium* is associated with rectal symptoms in MSM. *M.genitalium* was more common at the urethra in MSM living with HIV, although whether this represented behavioural and/or biological factors was not possible to determine. The highest prevalence estimates came from the Western Pacific region, though there were limited studies from Africa, South East Asia and the Eastern Mediterranean, potentially biasing regional estimates. Estimating the prevalence of *M.genitalium* in MSM assists in providing an evidence base to inform testing and clinical practice, and highlights the need for further research in this population, to understand the pathogenic role and natural history of *M.genitalium* in MSM.

Chapter Four examined rectal co-infections of *M.genitalium* with *C.trachomatis* or *N.gonorrhoeae* in MSM, as well as providing a prevalence estimate of pharyngeal- *M.genitalium* in MSM attending our clinical service, Melbourne Sexual Health Centre. We found high rates of rectal coinfection, with *M.genitalium* present in 13% (95%CI: 9-18) of MSM with rectal- *C.trachomatis* and 14%

(95%CI: 9-19) with rectal- *N.gonorrhoeae*. These findings indicate that 1 in 7 MSM infected with rectal *C.trachomatis* or *N.gonorrhoeae* are co-infected with *M.genitalium,* and that a substantial number of undiagnosed rectal *M.genitalium* infections are often inadvertently exposed to macrolide antibiotics, which is likely to be contributing to increasing macrolide resistance. Pharyngeal-*M.genitalium* was uncommonly detected in 2% (95%CI: 1-3) of MSM.

M.genitalium is a recognised cause of urethritis in men, however it has taken much longer for a pathogenic role in women to be established. In Chapter 5, we determined estimates for the prevalence of *M.genitalium* in women attending the Melbourne Sexual Health Centre, and examined associations with self-reported symptoms and clinician reported signs. *M.genitalium* was detected in 6% (95%CI: 5%–8%) of women attending our service (n=1318), while *C.trachomatis* was detected in 8% (95%CI: 6-9). *M.genitalium* was not associated with any common genital symptoms, which is helpful in informing additional indications for *M.genitalium* testing in women at our service. In contrast, *C.trachomatis* was associated with self-reported vaginal discharge, and dyspareunia in women with vulvovaginal candidiasis. *M.genitalium* was significantly associated with cervicitis but no other clinical signs, providing support for routine testing for *M.genitalium* in women with clinical signs of cervicitis on examination. *C.trachomatis* was associated with vaginal discharge, mucopurulent cervicitis, and ≥5 PMNL/hpf on microscopy of vaginal secretions. Importantly, 1 in 2 *M.genitalium* infections in women were macrolide-resistant, highlighting the value of testing using assays which convey microbial susceptibilities, in order to individualise therapy where possible. As recruitment occurred at a single sexual health clinic prevalence estimates are not generalisable to the community.

While it has taken considerable time for the role of *M.genitalium* in PID to be recognised, *M.genitalium* has been associated with a 2-fold increased odds of PID by meta-analysis (Lis et al. 2015). In Chapter Six we found that the most commonly reported symptom in women with PID and M.genitalium was abdominal pain which occurred in 86% (95%CI: 77-92) of women. Our study did not find clinically discernible differences between *M.genitalium*-PID and *C.trachomatis*-PID to assist clinicians in differentiating between these two aetiological agents at presentation. *M.genitalium*-PID was more likely to be associated with abdominal tenderness than *C.trachomatis*-PID, but any clinical relevance of this finding is not unclear. Women with *C.trachomatis*-PID were more likely to have post-coital bleeding and elevated cervico-vaginal PMNL counts, supporting the role of *C.trachomatis* as an established cause of PID and morbidity. An important limitation of both Chapters Five and Six is that outpatient sexual health services are likely to see milder presentations of STI related symptoms and syndromes, compared to studies conducted at hospitals or emergency services. Chapter Six showed that moxifloxacin is highly effective in achieving microbiological

cure of *M.genitalium* in women with PID (95%), but that 2 in 5 women experienced predominantly mild adverse effects. Since publication, moxifloxacin has been widely recommended for the treatment of *M.genitalium*-PID, particularly in settings where fluoroquinolone resistance is not common. This remains the largest case-series to date of *M.genitalium*-PID.

10.1.2 Implications for Clinical Practice

There were several implications for clinical practice identified through the studies presented in the thesis.

Chapters Three and Five reported on the prevalence of *M.genitalium*, in MSM and women which was comparable to *C.trachomatis* in these populations. The two STIs were found to be clinically indistinguishable in women in Chapters Five and Six, in both women with milder symptoms, and those with PID. While the two STIs have similarities in prevalence and presentation, screening for *M.genitalium* is not recommended in any population or at any anatomical site, due to lack of evidence regarding the natural history of *M.genitalium* and increasing complexities surrounding treatment due to widespread antimicrobial resistance in *M.genitalium*.

Our data confirms that screening and testing are not indicated at the pharynx. Chapters Three and Four both examined the prevalence of pharyngeal *M.genitalium.* The meta-analysis of seven studies, all which used PCR assays, found the estimated prevalence to be 1.0% (95%CI: 0.0-5.1). Chapter Four utilised a highly sensitive TMA assay, and found the pharyngeal prevalence of *M.genitalium* amongst MSM presenting to a sexual health clinic to be 2% (95%CI: 1-3). This data supports prior research in concluding that the pharynx is not a significant site of *M.genitalium* infection.

There are several clinical situations in which testing for *M.genitalium* is recommended. Our meta-analysis in Chapter Three found the highest prevalence of *M.genitalium* occurred at the rectum in MSM, with a summary estimate of 6.1% (95%CI: 4.5-7.9), and prevalence was significantly higher amongst men with rectal symptoms (p=0.039). Chapter Four also reported high rates of *M.genitalium* rectal co-infection. *M.genitalium* testing at the rectum has been controversial, but overall estimates for pooled data from published studies supports testing for *M.genitalium* infection amongst men with symptoms of proctitis. These data do not inform whether first line testing or testing in men with persistent symptoms who test negative for *C.trachomatis* and *N.gonorrhoeae* is indicated. British and Australian guidelines currently contain this recommendation (ASHA 2020; BASHH 2018), and do recommend testing in symptomatic men who have tested negative for *C.trachomatis* and *N.gonorrhoeae,* or have persistent symptoms following treatment.

Testing may also need to be prioritised in MSM living with HIV who have rectal or urethral symptoms. In Chapter Three, urethral prevalence of *M.genitalium* was significantly higher among MSM living with HIV compared to HIV-negative MSM (p=0.006). Chapter Four reported that MSM with *N.gonorrhoeae*/*M.genitalium* rectal co-infection were significantly more likely to be living with HIV, than those who had rectal *N.gonorrhoeae* monoinfection (OR 2.96, 95%CI: 1.21-7.26). Clinicians may have a higher burden of suspicion of *M.genitalium* infection amongst men living with HIV, and may consider testing in the presence of other symptoms or clinical indications for testing.

Testing for *M.genitalium* is currently recommended in women with cervicitis in a number of international guidelines. Chapter Five reported that *M.genitalium* was not associated with specific genitourinary symptoms in women, however we did confirm it was significantly associated with cervicitis (AOR=4.38, 95%CI: 1.69–11.33) in our clinic population. This study provided evidence to support restricting testing to women presenting with cervicitis and not undertaking testing, certainly first line, in women with common genital symptoms such as dysuria or vaginal discharge.

It is well documented that *M.genitalium* is becoming increasingly macrolide resistant. In Chapter Five, we detected macrolide-resistance in 1 in 2 women. This data supports the need for *M.genitalium* testing to be performed using an assay that conveys the resistance profile, to enable first line use of resistance-guided therapy to avoid inappropriate use of antimicrobials.

Treatment of *M.genitalium*-PID is particularly important, as prompt treatment of PID can help prevent long term sequelae. Although moxifloxacin has been recommended in international guidelines for the treatment of *M.genitalium*-PID, the evidence to support this was generated by one trial with three *M.genitalium*-PID cases (Ross *et al.* 2006). In Chapter Six, we showed that moxifloxacin was a highly effective agent in treating *M.genitalium*-PID, with 95% (95%CI: 82-99) of women achieving microbiological cure. Clinical cure (i.e. resolution of symptoms) was statistically more common amongst women who received moxifloxacin, compared to standard PID treatment (p=0.004). Although these data are limited to a case series, they confirm that moxifloxacin should be prescribed in the treatment of *M.genitalium*-PID where available. It is important to note 2 in 5 women had predominately mild adverse effects following moxifloxacin, and therefore patients should be informed and monitored for side effects.

10.1.3 Areas for future research

There were several areas identified, in both the literature review and my studies, which will require further investigation particularly around the prevalence and incidence of *M.genitalium* in specific

populations, changes in antimicrobial resistance over time, and a better understanding of the relationship between *M.genitalium* and HIV.

Prevalence of *M.genitalium* and of antimicrobial resistance needs to be continually assessed through the development of surveillance programmes which can inform timely changes in treatment strategies in specific populations. In regional prevalence estimates in Chapter Three, there was a deficiency of studies from Africa, South East Asia and the Eastern Mediterranean, which may have biased regional prevalence estimates. Studies of MSM in these regions have been limited due to sociocultural and legal constraints, and further research is required before true prevalence can be determined in these locations.

As noted above, pharyngeal *M.genitalium* infection was uncommon. It was unclear if the low positivity reflected passive infection/deposition, rather than active infection as has been hypothesized for pharyngeal- *C.trachomatis*. Further investigation could establish whether the pharynx is a site of low, but active *M.genitalium* infection, or whether *M.genitalium* has been detected at the pharynx following passive deposition only.

Strong evidence exists for the relationship between *M.genitalium* and non-gonococcal urethritis, PID and cervicitis. However Chapter Two highlighted the need for further evidence to establish the association between *M.genitalium* and proctitis, but also balanitis, posthitis, prostatitis, epididymitis and infertility in men, and important sequelae in women such as preterm birth, spontaneous abortion, stillbirth, ectopic pregnancy and infertility. Chapter Five describes the association between *M.genitalium* and cervicitis; it was noted in the discussion the lack of consistent criteria for the diagnoses of cervicitis and urethritis in women in both guidelines and the published literature. This is of relevance, as it impacts on the validity of studies using variable cut-offs, and limits the comparability of prior and future research. Our study in Chapter Five reflected *M.genitalium* symptoms in women attending STI clinics and outpatient services; further research should be conducted amongst those attending hospital services, to assess *M.genitalium*'s capacity to cause symptoms of greater severity, particularly severe PID.

Chapter Six described the largest published case series of *M.genitalium*-PID. While there is strong evidence regarding the association between *M.genitalium* and PID, rates of long-term sequalae such as ectopic pregnancy and chronic pelvic pain following *M.genitalium*-PID are limited. Given the difficulty in effectively treating *M.genitalium*, and early treatment a factor in helping preventing serious sequalae, this is priority area for future research. This study highlighted that in spite of being recommended by most international guidelines, there are limited data supporting the use of

moxifloxacin for *M.genitalium*-PID. While we found it to be highly effective, further higher level evidence is required to support our findings.

There are limited data on the relationship between *M.genitalium* and HIV. It was noted throughout the thesis that although there is an established relationship between these infections, the temporality and nature of the relationship remains to be defined. Further research is needed to conclude whether this relationship represents behavioural and/or biological factors. This relationship should be examined particularly in populations at risk of HIV, such as MSM. Chapter Three noted a meta-analysis on HIV and *M.genitalium* contained only two studies specific to MSM. In Chapter Four, we observed that rectal *M.genitalium/N.gonorrhoeae* co-infection was more common amongst individuals living with HIV. We could not assess this association amongst patients with *C.trachomatis*, and this relationship could be examined by further research.

Overall and importantly, there is a lack of knowledge around the natural history of *M.genitalium* infection in men and women, particularly asymptomatic *M.genitalium* infection. Filling this knowledge gap could allow for a 'no treatment' option or a 'wait and watch' approach when *M.genitalium* is detected. This may become increasingly necessary in some populations given *M.genitalium* has shown marked a propensity for development of antimicrobial resistance. The development of the next generation of resistance assays will promote more appropriate use of antibiotics in the future (Nijhuis *et al.* 2015). Assays will have to continually adapt, as additional mutations to existing and new agents are identified.

10.2 'Stealthing'

10.2.1 General Discussion of 'Stealthing'

Although increasingly discussed in international media, there is very little research on 'stealthing'. To my knowledge this is the first study investigating the proportion of individuals who have experienced it, the context in which it occurred and the alleged outcomes such as stress and STI acquisition. 'Stealthing' was common amongst clients attending Melbourne Sexual Health Centre, with 32% of women (n=1189) and 19% of MSM (n=1063) reporting removal of a condom in a situation where they would not have willingly engaged in sexual intercourse without one. Situational contexts often involved alcohol and/or drugs where women were 'stealthed', and undertaking sex work was a significant risk factor among women. Amongst MSM, geosocial networking applications were commonly how MSM met the person who 'stealthed' them, and risk factors included anxiety and depression. These data need to be interpreted in the context of a STI clinic population being a higher risk group than the general population.

10.2.2 Implications for Clinical Practice

Several implications for clinical practice were identified during the course of the study. Our study reported that of those who had experienced 'stealthing', 4% of women and 3% of MSM presented to the clinic directly following a 'stealthing' incident. These data suggest that 'stealthing' is not uncommon amongst patients attending our service, and likely other similar sexual health services. Clinicians at our practice and other sexual health practices, should consider the possibility of patients presenting following 'stealthing', particularly with patients in high-risk groups, such as sex workers.

When it is known that a patient is presenting following an incident of 'stealthing', clinicians should consider referral to counselling services, as over 50% of those who had been 'stealthed' in our study reported emotional distress following the incident. Clinicians should also consider STI screening. While STI transmission was reported in our study, this data could not be confirmed and may be subject to recall bias. STI transmission following sexual assault is uncommon, however patients presenting could be offered opportune STI screening. Clinicians may also consider the need for HIV non-occupational post exposure prophylaxis in MSM presenting following stealthing. MSM who have experienced condom malfunction or condom-less sex presenting in the 72 hours prior to presentation fulfil the criteria for HIV non-occupational post exposure prophylaxis. Therefore, MSM who present following a 'stealthing' incident within this timeframe should be prescribed PrEP, in accordance with the guidelines.

10.2.3 Areas for future research

The initial literature search revealed several areas requiring further research, particularly in the field of consent. The literature concerning consent has predominately been conducted using the framework of sexual script theory, developed in the 1970's, with the man as the initiator and the woman as the receiver of sex (Gagnon *et al.* 1973). While this has been a useful framework, sexual attitudes rapidly evolve, and this theory requires regular assessment as to its current validity in the twenty-first century amongst heterosexuals. Further research on sexual initiation and consent is required amongst groups that are excluded by sexual script theory, such as MSM, WSW, and other non-cis identifying partners. Majority of research exploring sexual initiation and consent has focused on college students, meaning the research needs further testing in a more age diverse population (Hickman *et al.* 1999; McCormick 1979). The literature concerning consent has also predominately viewed consent as a static process, with few papers referring to consent as an evolving process, or as an evaluation of a partners' behaviour as it progresses throughout a sexual encounter. Further research is required to understand consent in a realistic manner, as it progresses throughout a sexual encounter.

Stealthing is a recently described sexual practice, with our study the first to examine how commonly it occurs amongst Melbourne Sexual Health Centre attendees, and further investigation is required to confirm the validity of our findings. Our study was conducted amongst a potentially high risk population. Research should be conducted amongst varied populations in the general community to ascertain the true prevalence of this practice. Our study examined situational factors associated with 'stealthing' incidents. However, some incidents were not recent, and may have been subject to recall bias. Further research should be conducted on the risk factors for 'stealthing', which may also vary in other populations.

Continued research is required into the consequences of 'stealthing'. While some participants within our study attributed the acquisition of STIs to being 'stealthed', this could not be verified. This may be difficult to ascertain due to recall biases and attribution biases, however given the potential for serious consequences following 'stealthing', efforts should be made to ascertain this data. Further community-based research would help link behavioural measures to biological outcomes, and would help to quantify the STI risk associated with this practice.

Our study did not have the capacity to examine why men may 'stealth' someone. While prior literature suggests it is for birth control sabotage or intentional HIV transmission, anecdotal evidence and the sheer prevalence of this act suggest otherwise. Future research should focus on

determining why someone may do this, as understanding an individual's motivations will aid in the development of prevention strategies.

10.3 Thesis conclusion

The studies in this thesis contribute to the literature on two emerging issues in sexual health; *M.genitalium*, a relatively recently discovered STI, and 'stealthing', a recently discussed sexual practice. As part of my thesis I conducted a meta-analysis on the prevalence of *M.genitalium* in MSM; an investigation as to the prevalence of *M.genitalium* in the pharynx of MSM; a study determining rates of *M.genitalium* co-infection with *C.trachomatis* and *N.gonorrhoeae* at the rectum in MSM; an examination of the prevalence and genitourinary features of *M.genitalium* in women; and exploration of *M.genitalium*-PID and effective treatment. In addition, I conducted the first study to estimate the prevalence of 'stealthing' and associated risk factors. My body of work highlights the importance continual repletion of research in sexual health, as a dynamic and ever evolving area, dependent on societal practices and changing patterns of diseases. *M.genitalium* is increasingly complex to treat due to the lack of clarity around its natural history, and widespread microbial resistance, with rates of resistance changing rapidly. 'Stealthing' was a previously undescribed phenomenon in the medical literature, and clinicians should be aware of changing practices, in order to provide optimal care for patients. Overall, my thesis has contributed to the international literature on the contribution of *M.genitalium* to clinical presentations in men and women, including PID and co-infection, and provides the first estimates of 'stealthing' in a large population of sexual health attendees.

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Appendices

Appendix A. Additional information for Chapter 3.

A1. PDF of published study from Chapter 3.

Review

Prevalence of *Mycoplasma genitalium* by anatomical site in men who have sex with men: a systematic review and meta-analysis

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ABSTRACT

Objective To systematically review and appraise published data, to determine the prevalence of *Mycoplasma genitalium* (MG) in men who have sex with men (MSM) tested at each anatomical site, that is, at the urethra, rectum and/or pharynx.

Design Systematic review and meta-analysis.

Data sources Ovid Medline, PubMed, Embase were searched for articles from 1st January 1981 (the year MG was first identified) to 1st June 2018.

Review methods Studies were eligible for inclusion if they reported MG prevalence in MSM tested at the urethra, rectum and/or pharynx, in at least 50 MSM, using nucleic acid amplification testing. Data were extracted by anatomical site, symptom and HIV status. Summary estimates (95% CIs) were calculated using random-effects meta-analysis. Subgroup analyses were performed to assess heterogeneity between studies.

Results Forty-six studies met inclusion criteria, with 34 reporting estimates of MG prevalence at the urethra (13 753 samples), 25 at the rectum (8629 samples) and 7 at the pharynx (1871 samples). MG prevalence was 5.0% (95% CI 3.5 to 6.8; I^2=94.0) at the urethra; 6.2% (95% CI 4.6 to 8.1; I^2=88.1) at the rectum and 1.0% (95% CI 0.0 to 5.1; I^2=96.0) at the pharynx. The prevalence of MG was significantly higher at urethral and rectal sites in symptomatic versus asymptomatic MSM (7.1% vs 2.2%, p<0.001; and 16.1% vs 7.5%, p=0.039, respectively). MG prevalence at the urethra was significantly higher in HIV-positive compared with HIV-negative MSM (7.0% vs 3.4%, p=0.006).

Conclusion MG was common in MSM, particularly at urethral and rectal sites (5% to 6%). MG was more commonly detected in symptomatic men at both sites, and more common in HIV-positive men at the urethra. MG was uncommonly detected in the pharynx. Site-specific estimates are similar to those for chlamydia and will be helpful in informing testing practices in MSM.

PROSPERO registration number CRD42017058326.

INTRODUCTION

Mycoplasma genitalium (MG) is a STI, with prevalence estimates in the community ranging from 1.3% to 3.9%.[1] The majority of published data provides estimates of urethral infection with less data available on MG infection in gay, bisexual and other men who have sex with men (MSM), particularly for rectal and pharyngeal sites. There has been one prior meta-analysis of community-based studies (n=8) that estimated the overall prevalence of MG in MSM at 3.2% (95% CI 2.1 to 5.1), but clinic studies were excluded, and estimates were predominantly derived from urine samples.[1]

International guidelines recommend screening for *Chlamydia trachomatis* (*C. trachomatis*) and *Neisseria gonorrhoeae* (*N. gonorrhoeae*) in MSM,[2–4] although there is ongoing debate as to the relative contribution of these STIs at extragenital sites to transmission.[5] Guidelines do not recommend screening for MG at any site,[2 3 6] as the situation is more complicated than for *C. trachomatis* or *N. gonorrhoeae* due to lack of clarity around the natural history of MG, and increasing challenges with treatment due to antimicrobial resistance.[7] Testing for MG in men with urethritis is recommended by international guidelines,[2 8] as its pathogenic role in this syndrome is well established.[9] MG's association with rectal symptoms and the syndrome of proctitis has been inconsistent across published studies,[7 10] with UK and Australian guidelines recommending consideration of testing for MG in men with sexually acquired proctitis.[6 8] Site-specific prevalence estimates in MSM are needed to understand the contribution of each anatomical site to MG transmission, and to inform testing practices in MSM.

We undertook a systematic review and meta-analysis of published studies in order to determine the prevalence of MG at the urethra, rectum and pharynx of MSM tested for infection at each site, and to examine the association with symptoms, HIV status and other factors on site-specific prevalence, to inform testing and clinical practice in MSM.

METHODS

The study protocol was registered on Prospero (ID CRD42017058326).

Search strategy and selection criteria

This systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (online supplementary table 1). Two authors (RLL, HSS) independently searched for published peer-reviewed studies reporting the prevalence of MG in MSM by anatomical site, from 1st January 1981 (the year MG was first identified) to 1st August 2017, with the search updated on the 1st June 2018.

We included cross-sectional, longitudinal and cohort studies, and randomised controlled trials where baseline MG prevalence was reported, and conference abstracts from major STI conferences between 1st January 2015 and 1st June 2018 for studies that may not have been published yet. The search was performed using Ovid Medline, PubMed and Embase using search terms (('mycoplasma genitalium' or 'mycoplasma infections' or 'mycoplasmosis') and/or ('men adj3 sex' or 'males adj3 sex' or 'homosexual' or 'homosexuality')) (online supplementary table 2). Medical Subject Headings (MeSH) were used where possible. Reference lists of included studies were reviewed to identify other relevant studies. Studies were eligible for inclusion if they were published in English and reported MG prevalence at the urethra, rectum and/or pharynx, in at least 50 MSM, to reduce small study bias. Prevalence at each anatomical site was defined as the prevalence among MSM tested for MG at each anatomical site. Definition of MSM varied between studies. Participants were assumed to be MSM if studies stated that men either had male sexual partners, or if men had a rectal swab collected.[11 12]

Two reviewers (RLL, HSS) independently screened studies for eligibility using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). Abstracts and titles were screened, and then articles reviewed. Differences were resolved by discussion with other reviewers (CSB, LAV and/or DAM). When multiple articles reported on the same study population, we either included the most comprehensive article or the original article published.

Data extraction

Variables extracted included: name of first author, publication year, study design, geographical location (using WHO regions),[13] study period, study setting, median or mean age, HIV status, symptom status, symptom location, clinical diagnoses, number of participants, laboratory testing methods and number positive for MG by specimen type (urethral swab, first-void urine, rectal swab, and/or pharyngeal swab). Prevalence estimates with 95% CIs were also extracted. One reviewer (RLL) extracted the data, and a second reviewer (HSS) checked data for transcription errors. Any disagreements were discussed between the two reviewers, with differences resolved by discussion with CSB. Authors were contacted for data where estimates were not stratified by anatomical site, HIV or symptom status, or if the study design suggested that additional information was available. Where data was only presented as percentages and authors did not provide raw data on contact, numerical values were generated from percentages if sufficient data were available. Where CIs were not reported, these were calculated using binomial methods.

Primary outcome

The primary outcome was the prevalence of MG in MSM at the urethra, rectum and/or pharynx. This was calculated as the number of men who tested positive for MG at each site (defined as a positive test using nucleic acid amplification testing) divided by the total number tested for MG at the site.

Secondary outcome

Secondary outcomes included the prevalence of MG either by symptom status (asymptomatic vs symptomatic), HIV status (HIV-negative vs HIV-positive) or recruitment setting or location. Analyses were dependent on sufficient data being available.

Analysis

Summary prevalence estimates were calculated using random-effects meta-analysis with Freeman-Tukey double arcsine transformation, and study-specific 95% CIs computed using score method.[14] Included studies were examined using forest plots. I^2 statistics were calculated to assess between-study heterogeneity when more than two studies were included, with values of <25%, 25%–75% and >75% representing low, medium and high heterogeneity, respectively. The χ^2 statistic was used to assess the strength of the evidence for heterogeneity.

We undertook subgroup analyses and univariable random-effects meta-regression by symptom status, HIV status and broad WHO geographic region,[13] to investigate potential sources of heterogeneity. Sensitivity analyses were conducted to determine the effect on summary estimates of removal of either: i) outlier studies, or ii) studies at high risk of being non-representative of MSM. Studies were considered outliers if they fell outside the overall pattern of distribution for prevalence at each site. Studies were considered at risk of not representing the wider MSM community if a) the sample was not a true representation of the broader MSM population, for example select HIV patients, or b) MSM were not clearly defined as men who had penetrative sex in the past year with another man. Data were analysed using Stata (V.14.0, StataCorp, Austin, Texas, USA).

Assessment of bias and quality

The potential presence of publication and small study bias was assessed using funnel plots of proportions against study samples size and the Egger test.[15] To evaluate within study bias, we adapted an instrument designed by Hoy *et al*, which examines both the internal and external validity of selected studies (online supplementary table 3).[16] The tool consisted of eight questions and two reviewers (RLL, HSS) independently assessed each study as being low, medium or high risk of bias for each item, with differences resolved by discussion with CSB.

RESULTS

Study selection

The search process identified 4518 records and 2595 duplicates were removed. Titles and abstracts of 1923 records were screened and 1586 excluded, leaving 337 records for full-text screening. Forty-six records were included in the final analysis (figure 1, included studies described in online supplementary table 4).

Study characteristics

Of the 46 included studies, 34 reported MG prevalence at the urethra (n=13 753 samples), 25 at the rectum (n=8629 samples) and 7 at the pharynx (n=1871 samples) (table 1). Sample sizes ranged from 51 to 6293 (online supplementary table 4). Nineteen studies provided information on the presence of symptoms,[10 17–34] and 29 on HIV status.[10 18–22 24–30 32 33 35–48] Most studies (n=20 (42%)) were from Europe,[18 23 26 29 33 34 37 38 40 42 44–46 49–55] 13 (28%) were from the Western Pacific, although all were from Australia and China,[10 17 20 28 30–32 35 41 47 48 56 57] 12 (26%) were from the Americas, predominately the USA,[19 21 22 24 25 27 36 39 58–60 W1] and the WHO Africa region contained only one South African study (2%),[43] (table 1, online supplementary table 4). Overall, 36 (78%) studies were cross-sectional,[10 17–26 28 33 35–37 39–42 44–49 51–59 W1] 6 (13%) were retrospective cohorts[29–31 34 50 60] and 4 (9%) were prospective cohort studies[27 32 38 43] (table 1, online supplementary table 4).

Mycoplasma genitalium prevalence at the urethra

Prevalence of MG at the urethra (n=34 studies) ranged from 0% to 25.5%, with a summary estimate of 4.6% (95% CI 3.0 to 6.4; I^2=94.4%) (table 2).

Of the 15 studies reporting symptom status at the urethra, 12 included symptomatic men and 9 asymptomatic men. Prevalence

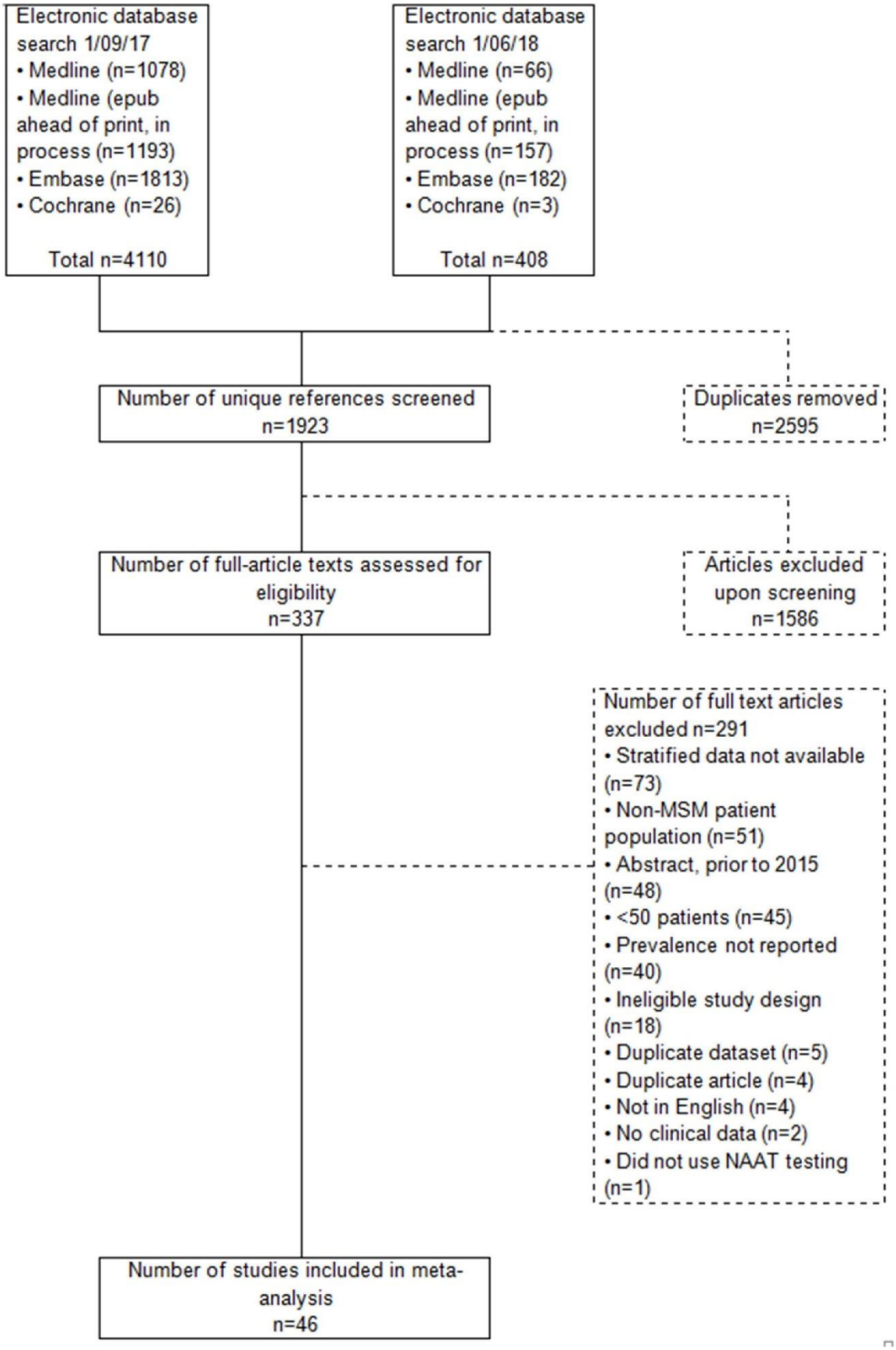

Figure 1 Flow diagram of studies included in meta-analysis of the prevalence of *Mycoplasma genitalium* among men who have sex with men (MSM). epub, electronic publication; n, number; NAAT, nucleic acid amplification testing.

Table 1 Characteristics of included studies (n=46)

	All n=46	Urethra n=34 (total sample=13 753*)	Rectum n=25 (total sample=8629)	Pharynx n=7 (total sample=1871)
Publication year (range)	2006–2018	2006–2018	2008–2018	2009–2018
Period of sample collection†	2002–2017	2002–2017	2002–2017	2002–2017
Symptom status				
Asymptomatic	12	9 (3449)	4 (2150)	1 (54)
Symptomatic	18	12 (1494)	5 (436)	0 (0)
HIV status				
Negative	21	14 (4026)	7 (1058)	1 (17)
Positive	27	18 (1889)	11 (1274)	2 (156)
WHO geographic region				
Africa	1	1 (78)	1 (78)	0 (0)
Europe	20	14 (5329)	10 (3534)	2 (349)
The Americas	12	8 (3808)	7 (1866)	1 (57)
Western Pacific	13	11 (4538)	7 (3151)	4 (1465)
Recruitment setting				
Laboratory	2	0 (0)	2 (156)	0 (0)
Community‡	8	9 (4245)	3 (1054)	2 (621)
Clinic§	36	26 (8998)	8 (7355)	4 (735)
Type of study				
Cross-sectional	36	28 (10 594)	19 (5988)	6 (1817)
Prospective cohort	4	3 (1278)	3 (1512)	1 (54)
Retrospective cohort	6	3 (1881)	3 (1129)	0 (0)

*Includes 13 751 first pass urines and 2 urethral smears.
†Five studies missing date of sample collection.
‡Includes one study conducted at a sex on premises venue.
§Includes 28 studies which recruited from STI clinics, 5 studies which recruited from HIV clinics, 1 mixed methods study (Cosentino) which predominately recruited from a HIV clinic, 1 study which recruited HIV-positive men in an anal cancer screening programme (Fuchs) and 1 study conducted at primary healthcare centres which target MSM (Peters).
n, number.

of MG was higher among men with urethral symptoms (7.1% (95% CI 4.7 to 9.7; I^2=67.2)) than among asymptomatic men (2.2% (95% CI 1.0 to 3.7; I^2=81.8)) (p<0.001) (table 2, online supplementary figure 1).

Twenty studies examining prevalence of MG at the urethra reported HIV status; 18 studies included men living with HIV and 14 included HIV-negative men. Urethral prevalence of MG was higher among men living with HIV (7.0% (95% CI 3.0 to 12.2; I^2=86.3)) than among HIV-negative men (3.4% (95% CI 1.8 to 5.5; I^2=82.6)) (p=0.006) (table 2, online supplementary figure 2).

Prevalence of urethral MG varied across geographical regions (table 2), although assessment of geographical regions was impacted by the limited number of countries providing data in each region. Prevalence of urethral MG was 1.3% (95% CI 0.2 to 6.9) in the single African study, 2.2% (95% CI 1.3 to 3.3; I^2=75.3) across European studies (n=14 studies), 7.4% (95% CI 4.8 to 10.6; I^2=86.5) in studies from the Americas (n=8 studies) and 8.2% (95% CI 4.0 to 13.6; I^2=97.1) across studies from the Western Pacific (n=11 studies). Prevalence of MG did not differ by recruitment setting (table 2).

Mycoplasma genitalium prevalence at the rectum

Prevalence of MG at the rectum (n=25 studies) ranged from 0% to 29.9%, with a summary estimate of 6.1% (95% CI 4.5 to 7.9; I^2=89.0) (table 2). One outlier study by Ong *et al* reported a prevalence of 29.9%.[30] A sensitivity analysis excluding this study resulted in a summary estimate of 5.4% (95% CI 4.2 to 6.8; I^2=81.9) (online supplementary table 6).

Of the seven studies reporting symptom status at the rectum, five included symptomatic men and four asymptomatic men. Prevalence of MG was higher among men with rectal symptoms (16.1% (95% CI 7.2 to 27.5; I^2=82.9)) than asymptomatic men (7.5% (95% CI 5.4 to 10.0; I^2=72.5)) (p=0.039) (Table 2, online supplementary figure 3).

Eleven studies examining prevalence of MG at the rectum reported HIV status; all included men living with HIV, with seven also including HIV-negative men. Rectal prevalence of MG was not different among men living with HIV (10.6% (95% CI 5.5 to 17.0; I^2=89.4)) compared with HIV-negative men (6.8% (95% CI 1.2 to 15.8; I^2=94.4)) (p=0.456) (Table 2, online supplementary figure 4).

The prevalence of rectal MG varied across geographical regions (table 2). Prevalence of rectal MG was 0.0% (95% CI 0.0 to 4.7) in the single African study, 4.3% (95% CI 3.1 to 5.7; I^2=56.0) across European studies (n=10 studies), 6.3% (95% CI 4.1 to 8.9; I^2=65.0) in studies from the Americas (n=7 studies) and 9.5% (95% CI 5.1 to 15.1; I^2=95.5) across studies from the Western Pacific (n=7 studies). Prevalence of MG did not differ by recruitment setting (table 2).

Mycoplasma genitalium prevalence at the pharynx

Prevalence of MG at the pharynx (n=7 studies) ranged from 0% to 13.4%, with a summary estimate of 1.0% (95% CI 0.0 to 5.1; I^2=96.0) (table 2, online supplementary figure 5). Six of the seven studies reported pharyngeal prevalence between 0% and 2%. One outlier study by Jiang *et al* reported a prevalence of 13.4%.[57] A sensitivity analysis excluding this study resulted in a summary estimate of 0.0% (95% CI 0.0 to 0.3; I^2=36.6) (online supplementary table 6). As symptom status was only reported in one study,[32] subgroup analysis by symptoms was not performed.

Table 2 Analyses assessing prevalence of *Mycoplasma genitalium* by anatomical site

	Urethra*				Rectum				Pharynx				
Analysis	**No**	**SE, % (95% CI)**		**I^2 %†**	**P value‡**	**No**	**SE, % (95% CI)**		**I^2 %†**	**P value‡**	**No**	**SE, % (95% CI)**		**I^2 %†**	**P value‡**
Overall prevalence	34	4.6	(3.0 to 6.4)	94.4		25	6.1	(4.5 to 7.9)	89.0		7	1.0	(0.0 to 5.1)	96.0	
Symptom status															
Asymptomatic	9	2.2	(1.0 to 3.7)	81.8		4	7.5	(5.4 to 10.0)	72.5		1	1.9	(0.0 to 7.8)	–	
Symptomatic	12	7.1	(4.7 to 9.7)	67.2	<0.001	5	16.1	(7.2 to 27.5)	82.9	0.039	0	–	–	–	–
HIV status															
Negative	14	3.4	(1.8 to 5.5)	82.6		7	6.8	(1.2 to 15.8)	94.4		1	0.0	(0.0 to 18.4)		
Positive	18	7.0	(3.0 to 12.2)	86.3	0.006	11	10.6	(5.5 to 17.0)	89.4	0.456	2	0.3	(0.0 to 2.2)		0.819
WHO geographic region															
Africa	1	1.3	(0.2 to 6.9)	–	Ref§	1	0.0	(0.0 to 4.7)	–	Ref§††	0	–	–	–	–
Europe	14	2.2	(1.3 to 3.3)	75.3	0.698	10	4.3	(3.1 to 5.7)	56.0	0.006	2	0.0	(0.0 to 5.2)	–	Ref§
The Americas	8	7.4	(4.8 to 10.6)	86.5	0.021	7	6.3	(4.1 to 8.9)	65.0	0.001	1	1.8	(0.3 to 9.3)	–	0.086
Western Pacific	11	8.2	(4.0 to 13.6)	97.1	0.030	7	9.5	(5.1 to 15.1)	95.5	<0.001	4	1.8	(0.0 to 10.3)	97.9	0.273
Recruitment setting															
Laboratory	–	–	–	–	–	2	3.6	(1.1 to 7.4)	–	Ref§	–	–	–	–	–
Community¶	8	4.2	(1.2 to 8.9)	97.2		3	4.5	(0.4 to 12.3)	–	0.915	3	1.9	(0.0 to 13.7)	–	–
Clinic**	26	5.4	(3.7 to 7.5)	92.7	0.580	20	6.9	(4.8 to 8.6)	88.6	0.184	4	0.2	(0.0 to 1.7)	57.8	0.599

*Includes 13 751 first pass urines and 2 urethral smears.
†I^2 statistic to estimate the proportion of total variability in summary estimates attributed to heterogeneity other than that due to chance. All I^2 statistic values had a p value <0.001.
‡P values reflect random-effects meta-analyses to determine differences in pooled summary estimates between subgroups.
§Reference refers to reference group for statistical comparison between subsequent groups.
¶Includes one study conducted at a sex on premises venue.
**Includes 28 studies which recruited from STI clinics, 5 studies which recruited from HIV clinics, 1 mixed methods study (Cosentino) which predominately recruited from a HIV clinic, 1 study which recruited HIV-positive men in an anal cancer screening programme (Fuchs) and 1 study conducted at primary healthcare centres which target MSM (Peters).
††Meta-analysis unable to run with zero value in reference group, Peters *et al* paper prevalence altered to 0.5%.
No, number of studies; Ref, reference group; SE, summary estimate.

Two studies examining prevalence of MG at the pharynx reported HIV status. One study included MSM living with HIV only, and the other included both men living with and without HIV, with no difference in the prevalence of pharyngeal MG by HIV status (p=0.891) (table 2).

The prevalence of pharyngeal MG did not differ by geographical region, ranging from 0.0% (95% CI 0.0 to 5.2) across European studies (n=2 studies), to 1.8% (95% CI 0.3 to 9.3) in studies from the Americas (n=1 study) and 1.8% (95% CI 0.0 to 10.3) across studies from the Western Pacific (n=4 studies) (table 2). Prevalence of MG did not differ by recruitment setting (table 2).

Between-study bias

Assessment of funnel plots indicated no small study effects (online supplementary table 6-8), although inclusion criteria required studies to contain ≥50 MSM. There was no evidence of publication bias by the Egger test, with a coefficient of 0.23 (95% CI −0.65 to 1.11; p=0.573).

Within-study bias

Online supplementary table 5 provides a summary of risk of bias for included studies, using the adapted tool from Hoy *et al* (online supplementary table 3). Twenty-one studies (46%) were deemed to be at low risk of bias, 23 (50%) at medium risk and 2 studies (4%) at high risk of bias.[60 W1] Only one study was considered as low risk of bias across all domains.[40] Thirteen studies randomly or consecutively selected participants or specimens.[10 18 22–25 32 40 44 49 52 55] Seven studies did not state the study population to be MSM but used rectal samples from men,[49 50 52 54 55 60 W1] which for the purpose of this meta-analysis were assumed to be from MSM.[1112 W2] Four papers did not provide a numerator or denominator, instead presenting percentage data.[41 53 57 60] Sensitivity analyses which removed studies deemed to be at high risk of representative bias or outlier studies did not show any major shift in summary estimates (online supplementary table 6,7).

DISCUSSION

To our knowledge, this is the first meta-analysis to determine the prevalence of MG by anatomical site in MSM tested for infection at each site, and to explore the association between MG and specific factors such as symptom and HIV status. We combined data from 46 studies, representing nearly 24 000 samples. Prevalence of MG in MSM ranged from 0% to 29.9%, with summary estimates of 6.2% (95% CI 4.6 to 8.1; I^2=88.1) at the rectum, 5.0% (95% CI 3.5 to 6.8; I^2=94.0) in the urethra and 1.0% (95% CI 0.0 to 5.1; I^2=96.0) in the pharynx, but estimates were limited by high heterogeneity. MG was more common in MSM with symptoms at the urethra or rectum and more common at the urethra in MSM living with HIV.

The prevalence of MG in this study was similar to *C. trachomatis* prevalence estimates in MSM, which have been reported in the order of 3.5%–3.7% at the urethra, 5.6% (95% CI 4.8 to 6.3) at the rectum and 0.5% (95% CI 0.2 to 0.9) at the pharynx.[W3-W5] Screening for *C. trachomatis* and *N. gonorrhoeae* at urethral, rectal and pharyngeal sites in MSM is recommended in many countries.[2–4] However, WHO states a number of key principles must be satisfied when screening for an infection,[W6] including: 1) the natural history of the condition should be understood and 2) the cost-benefit of finding and treating cases should be balanced.[W6] MG meets neither criteria, with limited knowledge of the natural history of asymptomatic MG infection, particularly in MSM, and increasing antimicrobial resistance resulting in greater use of costly antimicrobials that can be associated with serious side effects.[7]

Our meta-analysis confirmed MG to be uncommonly detected in the pharynx of MSM (1%), although estimates were limited by small numbers of included studies. When the outlier study by Jiang *et al* was excluded, the overall estimate declined to 0.0% (95% CI 0.0 to 0.3; I^2=36.6).[57] In contrast to *N. gonorrhoeae*, where pharyngeal prevalence is 5.5%–8.3%,[W7 W8] pooled estimates of MG appear similar to those for chlamydia at 0.5%.[W5] These data suggest the pharynx does not play a significant role in transmission of MG.

While MG is an established cause of urethritis, its contribution to proctitis has been less clear. Several studies have reported no association between rectal MG and proctitis.[7 22 46] However in this meta-analysis MG was associated with symptoms at both the urethra and rectum. These data support recommendations that MG testing should be undertaken in men with urethral symptoms,[2 8] and suggest that MG is associated with rectal symptoms in MSM, supporting UK and Australian guidelines that recommend consideration of MG testing in men with proctitis.[6 8]

Our meta-analysis found the prevalence of MG to be higher at the urethra among men living with HIV compared with HIV-negative men. The pattern was similar at the rectum, but there were fewer studies, limiting this comparison. A prior meta-analysis reported an association between MG and HIV (OR=2.01, 95% CI 1.44 to 2.79),[W9] but only two studies with data specific to MSM were included.[W8] While our data suggests MG may be more common at the urethra and rectum in HIV-positive men, specific recommendations around testing for MG in men living with HIV cannot be inferred from these results due to the high heterogeneity and limited number of studies. Overall however, these data add plausibility to a relationship between MG and HIV, as has been previously reported.[W9]

There were several important limitations to this study. Random-effects meta-analysis takes into consideration real or larger heterogeneity between studies, but despite this we recorded high heterogeneity in most of the summary estimates. Symptoms and HIV status were only known for a subset of studies, reducing precision around these estimates. Assessment of the influence of geographical region on MG prevalence was greatly impacted by the limited number of countries providing regional data. While the prevalence of MG appeared highest in the Western Pacific, studies from this region only originated from Australia and China. Similarly, only one South African study contributed to African estimates, and there were no studies from South East Asian or Eastern Mediterranean regions. High heterogeneity may have resulted from a combination of all three of the risk factors explored (ie, symptoms, HIV status and geographical location), but the small number of studies available for each end point limited our ability to perform multivariable meta-regression analyses.[14 W10] As information on potentially important factors such as age and risk behaviour were not consistently reported, we could not assess the impact of these on heterogeneity. There was limited power to look at the influence of time (ie, year of study) within different subgroups. Few of the included studies were from systematically sampled populations, as screening is not recommended for MG, and testing has generally been limited to symptomatic individuals and specialist services with access to research assays, resulting in over-representation of STI clinics compared with other sites. Thus, estimates may represent a select population, contributing to the significant heterogeneity and limited generalisability of the findings. Finally, this review

was limited to studies published in English only, which also limits the generalisability of our findings; however, only four studies were excluded on this basis.

Our meta-analysis found the prevalence of MG to be similar to that of *C. trachomatis* in MSM tested across all three anatomical sites, with associations seen between symptoms and HIV-positivity in subgroup analyses. This meta-analysis provides site-specific estimates for MG in MSM that, despite high heterogeneity, represent biologically plausible patterns of infection. These data provide an evidence base to inform testing and clinical practice, and highlight the need for further research in this population to understand the pathogenic role and natural history of MG in MSM.

Key messages

- Prevalence of *Mycoplasma genitalium* (MG) at the urethra, rectum and pharynx was estimated in men having sex with men (MSM) tested for infection at each site.
- MG prevalence was 5.0% (95% CI 3.5 to 6.8; I^2=94.0) at the urethra; 6.2% (95% CI 4.6 to 8.1; I^2=88.1) at the rectum and 1.0% (95% CI 0.0 to 5.1; I^2=96.0) at the pharynx.
- The prevalence of MG was higher at urethral and rectal sites in symptomatic versus asymptomatic MSM.
- MG prevalence at the urethra was higher in HIV-positive compared with HIV-negative MSM.

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Appendix B. Additional information for Chapter 4.

B1. PDF of published study from Chapter 4.

ORIGINAL ARTICLE

Extragenital *Mycoplasma genitalium* infections among men who have sex with men

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ABSTRACT

Objectives There are limited data on the prevalence of *Mycoplasma genitalium* (Mgen) coinfection with rectal chlamydia (*Chlamydia trachomatis* (CT)) and rectal gonorrhoea (*Neisseria gonorrhoeae* (NG)) infections and few studies examining the prevalence of pharyngeal Mgen in men who have sex with men (MSM). Using transcription-mediated amplification assay, this study aimed to determine the proportion of rectal CT and rectal NG infections in MSM who are coinfected with rectal Mgen, and the proportion of MSM with Mgen detected in the pharynx in order to inform clinical practice.

Methods This was a cross-sectional study conducted at Melbourne Sexual Health Centre in Australia. Consecutively collected rectal swabs from MSM that tested positive for CT (n=212) or NG (n=212), and consecutively collected pharyngeal samples (n=480) from MSM were tested for Mgen using the Aptima *Mycoplasma genitalium* Assay (Hologic, San Diego). Samples were linked to demographic data and symptom status.

Results Rectal Mgen was codetected in 27 of 212 rectal CT (13%, 95% CI 9 to 18) and in 29 of 212 rectal NG (14%, 95% CI 9 to 19) samples, with no difference in the proportion positive for Mgen. MSM with rectal CT/Mgen coinfection had more sexual partners than those with rectal CT monoinfection (mean 6 vs 11, p=0.06). MSM with rectal NG/Mgen coinfection were more likely to be HIV-positive than those with rectal NG monoinfection (OR=2.96, 95% CI 1.21 to 7.26, p=0.023). MSM with rectal CT/Mgen coinfection were more likely to be using pre-exposure prophylaxis than MSM with rectal NG/Mgen coinfection (OR 0.25, 95% CI 0.10 to 0.65, p=0.002). Pharyngeal Mgen was uncommon and detected in 8 of 464 samples (2%, 95% CI 1% to 3%). Pharyngeal Mgen was associated with having a rectal STI (OR=10.61, 95% CI 2.30 to 48.87, p=0.002), and there was a borderline association with being HIV-positive (p=0.079).

Conclusion These data indicate one in seven MSM treated for rectal CT or rectal NG will have undiagnosed Mgen that is potentially exposed to azithromycin during treatment of these STIs. Rectal Mgen coinfection was associated with specific risk factors which may inform testing practices. Pharyngeal Mgen was uncommon.

INTRODUCTION

Mycoplasma genitalium (Mgen) is an established cause of urethritis[1]; however, there are limited data on the prevalence of Mgen at anatomical sites other than the urethra. A recent meta-analysis estimated the prevalence of Mgen in men who have sex with men (MSM) in the community at 3.2% (95% CI 2.1 to 5.1).[2] Studies in urban STI clinics in Melbourne and Sydney estimate the overall prevalence of Mgen in MSM between 9.5% and 13.4%, respectively, with a prevalence of 7.0%–8.9% in the rectum and 2.7%–4.9% in the urethra.[3 4] There are limited data on the prevalence of Mgen coinfection with the two most common STIs, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). In a study of clinical samples collected at STI clinics in London, Mgen was found to be a coinfection in 13.0% of CT and 2.4% of NG infections in all people at the clinic, including women, using PCR.[5] Screening and/or testing for CT and NG at extragenital sites is commonly performed in MSM in line with international guidelines[6]; however, this is not currently recommended for Mgen.

Both CT and NG have been commonly treated with macrolides, in accordance with international treatment guidelines.[6 7] Britain has recently moved away from azithromycin for first-line treatment of CT and NG due to growing concerns over macrolide resistance.[8 9] One gram (1g) of azithromycin has also commonly been recommended for treatment of Mgen[6 7]; however, macrolide resistance is now detected in at least 40%–60% of Mgen infections in many countries,[10 11] and treatment failure following azithromycin currently exceeds 60% in Melbourne, Australia. Treatment with 1g azithromycin leads to the selection of macrolide resistance in at least 12% of infections.[12 13] The inadvertent exposure of Mgen to azithromycin during treatment of CT or NG infections may lead to the selection of macrolide resistance and contribute to the rising rates of macrolide resistance seen in Mgen globally over the past decade.[12]

Screening for NG and CT in the pharynx is widely recommended in MSM.[14] There are few studies on the prevalence of Mgen in the pharynx, with conflicting data from published studies. In two Australian studies Mgen was not detected in the pharynx of MSM.[3 15] However another study presented at the STI & HIV World Congress in 2015 showed a high prevalence of pharyngeal Mgen (13.5%) among 388 MSM recruited from gay bars in five cities across China.[16] All studies to date have used PCR-based assays. We used a highly sensitive transcription-mediated amplification

(TMA) assay to determine the prevalence of Mgen in MSM attending Melbourne Sexual Health Centre (MSHC), given the high background prevalence of rectal (7%) and urethral (3%) Mgen infection in asymptomatic MSM at MSHC.[4]

This study aimed to determine the proportion of rectal chlamydial and gonococcal infections in MSM coinfected with rectal Mgen and predictors of coinfection, as well as the proportion of MSM infected with Mgen in the pharynx and the risk factors associated with pharyngeal Mgen.

MATERIALS AND METHODS

This was a cross-sectional study among MSM attending MSHC in Victoria, Australia, between May 2017 and January 2018. Patients presenting to MSHC routinely complete a computer-assisted self-interview (CASI) about their sexual history, including gender, number of partners and condom use over the last 3 months, HIV status, and use of pre-exposure prophylaxis (PrEP) for HIV. MSM were identified as men attending MSHC who reported having anal sex with another man in the previous year via CASI. MSHC is the largest public sexual health service in Victoria, Australia. The centre provides around 50 000 consultations every year, with 36% of consultations for MSM.

We examined the prevalence of Mgen rectal coinfection with either CT or NG, and the prevalence of Mgen pharyngeal infection, in serially collected specimens. Clients attending MSHC are offered free screening for STIs, including rectal and pharyngeal CT and NG, with swabs tested using Aptima Combo 2 TMA Assay (Hologic, San Diego, California, USA).

Rectal coinfection

Consecutive swabs that tested positive for CT or NG were stored at room temperature in Aptima specimen transport tubes until further testing, and samples obtained from MSM were identified. Rectal samples that tested positive for CT from HIV-positive patients at MSHC were sent to an external laboratory for Lymphogranuloma venereum (LGV) testing and were therefore not available for this study. Samples from MSM were stored until batches of 100 samples were formed. Samples were stored and tested within 60 days. Samples were assessed prior to further testing to determine if there was sufficient remaining buffer; a minimum of 1.7 mL of buffer was required for Mgen testing and samples without sufficient buffer were discarded. The remaining samples were tested for Mgen using the Aptima *Mycoplasma genitalium* Assay (Hologic). Equal numbers of sequential CT and NG positive samples were selected.

Pharyngeal infection

All consecutive pharyngeal swabs with sufficient remaining buffer following testing for CT and NG were stored at room temperature, and swabs from MSM were identified. Samples from MSM were stored until batches of 100 samples were formed. Samples were stored and tested within 60 days. Samples determined to have adequate buffer remaining were tested for Mgen using the Aptima *Mycoplasma genitalium* Assay (Hologic).

Data extraction

Samples were linked to demographic and epidemiological data (eg, age, number of male partners, condom and PrEP use, and HIV status), as well as clinical diagnosis, presence of any symptoms and reason for presentation, and then irreversibly deidentified.

Statistical analysis

All analyses were performed using Stata IC V.14 (StataCorp, College Station, Texas). We estimated that if 5% of 225 CT or NG samples were positive for Mgen, this would yield CIs of 2.5% to 8.6%. We used univariable and multivariable logistic regression analyses to determine risk factors associated with rectal coinfection with CT/Mgen and rectal coinfection with NG/Mgen, and pharyngeal Mgen monoinfection. Variables were included in multivariable models if the p value was ≤ 0.05; strongly correlated variables were excluded from multivariable analyses. Ninety-five per cent binomial CIs were calculated for all proportions.

RESULTS

Characteristics of rectal samples examined for coinfection

Four hundred and eighty rectal samples, positive for either CT or NG, were selected for testing for Mgen. Fifty-five (11%) samples yielded invalid results due to insufficient buffer despite careful selection. Valid results were obtained for 212 rectal samples that were positive for CT and 212 rectal samples that were positive for NG. One sample which was positive for both CT and NG and excluded from analyses tested negative for Mgen.

Characteristics of cases with rectal CT compared with rectal NG

Compared with MSM with rectal NG, MSM with rectal CT were more likely to be presenting to MSHC as a contact with partners diagnosed with an STI (p=0.07) and to be using PrEP (p=0.084), although these did not reach significance (table 1). MSM with rectal NG were more likely to be symptomatic compared with those with rectal CT (OR=2.54, 95% CI 1.71 to 3.77, p<0.001; table 1). MSM with rectal NG were more likely to be HIV-positive compared with men with rectal CT (OR=5.40, 95% CI 2.32 to 12.52, p<0.001) (table 1), although this finding is likely to have been influenced by the removal of a significant proportion of rectal CT samples from HIV-positive men for LGV testing. Both groups were similar in terms of age, number of sexual partners and condom use.

A relatively high proportion of MSM with rectal CT also had urethral CT (41/211 (19%, 95% CI 14 to 25)) and pharyngeal CT (25/212 (12%, 95% CI 8 to 17)) (table 1). In patients who had rectal NG, 66 of 208 (32%, 95% CI 25 to 39) also had urethral NG, and 85 of 209 (41%, 95% CI 34 to 48) had pharyngeal NG (table 1).

Characteristics of rectal CT and rectal NG cases that were coinfected with rectal Mgen

Overall, Mgen was detected in 27 of 212 rectal CT samples (13%, 95% CI 9 to 18) and in 29 of 212 rectal NG samples (14%, 95% CI 9 to 19) (table 1), with no difference in the proportion of cases with Mgen coinfection (p=0.774) (table 1).

MSM with rectal CT/Mgen coinfection had more sexual partners than those who had rectal CT monoinfection (mean 6 vs 11, p=0.06); however, this was of borderline significance (table 2). There were no significant differences in terms of age, condom use in the last 3 months, symptom status, whether they were an STI contact or STIs at other anatomical sites (table 2).

MSM with rectal NG/Mgen coinfection were more likely to be HIV-positive than those with rectal NG monoinfection (OR=2.96, 95% CI 1.21 to 7.26, p=0.023) (table 2). There were no significant differences in terms of age, number of sexual partners, PrEP usage, symptom status or reason for presentation (table 2).

Table 1 Demographics and epidemiological characteristics of *Neisseria gonorrhoeae* cases compared with *Chlamydia trachomatis* cases (N=424)

	C. trachomatis, n=212 (%, 95% CI) or mean (range)	*N. gonorrhoeae*, n=212 (%, 95% CI) or mean (range)	OR (95% CI)	P value*
Age				
	33 (20–84)	32 (21–59)	0.99 (0.97 to 1.01)	0.360
Number of male sexual partners in the last 3 months				
	6 (0–100)	9 (1–270)	1.01 (0.99 to 1.03)	0.213
Condom use with male partners in the last 3 months				
Not always	169 (84, 78 to 89)	157 (81, 75 to 86)	1	
Always	32 (16, 11 to 22)	37 (19, 14 to 25)	1.24 (0.74 to 2.09)	0.410
HIV status†				
Negative	204 (97, 93 to 97)	178 (84, 79 to 89)	1	
Positive	7 (3, 1 to 7)	33 (16, 11 to 21)	5.40 (2.32 to 12.52)	**<0.001**
Using PrEP‡				
No	139 (68, 62 to 75)	136 (76, 69 to 82)	1	
Yes	64 (32, 25 to 38)	42 (24, 18 to 31)	0.67 (0.43 to 1.06)	0.084
Symptomatic§				
No	144 (68, 61 to 74)	96 (46, 39 to 53)	1	
Yes	68 (32, 26 to 39)	115 (54, 47 to 61)	2.54 (1.71 to 3.77)	**<0.001**
Anal symptoms¶				
No	201 (95, 91 to 97)	180 (85, 79 to 89)	1	
Yes	11 (5, 3 to 9)	32 (15, 11 to 21)	3.25 (1.59 to 6.63)	**0.001**
STI contact				
No	176 (83, 77 to 88)	189 (89, 84 to 93)	1	
Yes	36 (17, 12 to 23)	23 (11, 7 to 16)	0.59 (0.33 to 1.04)	0.070
Mycoplasma genitalium detected in the rectum				
Negative	185 (87, 82 to 91)	183 (86, 81 to 91)	1	
Positive	27 (13, 9 to 18)	29 (14, 9 to 19)	1.09 (0.62 to 1.91)	0.774
C. trachomatis detected in the urethra				
No	170 (81, 75 to 86)	199 (96, 92 to 98)	1	
Yes	41 (19, 14 to 25)	9 (4, 2 to 8)	0.19 (0.09 to 0.40)	**<0.001**
N. gonorrhoeae detected in the urethra				
No	209 (99, 97 to 100)	142 (68, 61 to 75)	1	
Yes	2 (1, 0 to 3)	66 (32, 25 to 39)	48.57 (11.71 to 201.51)	**<0.001**
C. trachomatis detected in the pharynx				
No	187 (88, 83 to 92)	207 (99, 97 to 100)	1	
Yes	25 (12, 8 to 17)	2 (1, 0 to 3)	0.07 (0.02 to 0.31)	**<0.001**
N. gonorrhoeae detected in the pharynx				
No	197 (93, 89 to 96)	124 (59, 52 to 66)	1	
Yes	15 (7, 4 to 11)	85 (41, 34 to 48)	9.00 (4.97 to 16.29)	**<0.001**

Data missing for up to 8% of condom data, up to 2% of *C. trachomatis* and *N. gonorrhoeae* detected in other sites, and up to 1% of all other variables (excluding age).
*P value calculated using logistic regression, and bold indicates significant findings.
†Chlamydia samples from HIV-positive patients were removed for lymphogranuloma venereum testing.
‡Individuals with HIV were excluded for this variable.
§Symptomatic was defined as the presence of any symptom, including symptoms at sites other than the rectum.
¶Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus.
PrEP, HIV pre-exposure prophylaxis.

Table 2 Characteristics of cases coinfected with Mgen compared with rectal chlamydia or rectal gonococcal monoinfections (N=424)

Chlamydial rectal infections (n=212)*				
	CT cases only, n=185 (%, 95% CI) or mean (range)	**CT cases with Mgen detected, n=27 (%, 95% CI) or mean (range)**	**OR (95% CI)**	**P value†**
Age	33 (20–84)	32 (22–52)	0.99 (0.95 to 1.03)	0.658
Number of male sexual partners in the last 3 months				
	6 (0–30)	11 (1–100)	1.04 (1.00 to 1.09)	0.064
Condom use with male partners in the last 3 months				
Not always	145 (83, 76 to 88)	24 (92, 75 to 99)	1	
Always	30 (17, 12 to 24)	2 (8, 1 to 25)	0.40 (0.09 to 1.80)	0.233
Using PrEP‡				
No	124 (70, 63 to 77)	15 (56, 35 to 75)	1	
Yes	52 (30, 23 to 37)	12 (44, 35 to 65)	1.91 (0.84 to 4.35)	0.125
STI detected in the urethra§				
No	146 (79, 73 to 85)	22 (81, 62 to 94)	1	
Yes	38 (21, 15 to 27)	5 (19, 6 to 38)	0.87 (0.31 to 2.46)	0.797
STI detected in the pharynx¶				
No	152 (82, 76 to 87)	22 (81, 62 to 94)	1	
Yes	33 (18, 13 to 24)	5 (19, 6 to 38)	1.05 (0.37 to 2.97)	0.931
Symptomatic**				
No	125 (68, 60 to 74)	19 (70, 50 to 86)	1	
Yes	60 (32, 26 to 40)	8 (30, 14 to 50)	0.88 (0.36 to 2.12)	0.771
Anal symptoms††				
No	176 (95, 91 to 98)	25 (93, 76 to 99)	1	
Yes	9 (5, 2 to 9)	2 (7, 1 to 24)	1.56 (0.32 to 7.66)	0.597
STI contact				
No	153 (83, 76 to 88)	23 (85, 66 to 96)	1	
Yes	32 (17, 12 to 24)	4 (15, 4 to 34)	0.83 (0.27 to 2.57)	0.749
Gonorrhoeal rectal infections (n=212)				
	NG cases only, n=183 (%, 95% CI) or mean (range)	**NG cases with Mgen detected, n=29 (%, 95% CI) or mean (range)**	**OR (95% CI)**	**P value***
Age	32 (21–59)	31 (21–56)	0.98 (0.92 to 1.03)	0.351
Number of male sexual partners in the last 3 months				
	9 (1–270)	8 (1–25)	1 (0.98 to 1.02)	0.872
Condom use with male partners in the last 3 months				
Not always	136 (80, 74 to 86)	21 (84, 64 to 95)	1	
Always	33 (20, 14 to 26)	4 (16, 5 to 36)	0.78 (0.25 to 2.44)	0.669
HIV status				
Negative	158 (87, 81 to 91)	20 (69, 49 to 85)	1	

Continued

Table 2 Continued

Chlamydial rectal infections (n=212)*	CT cases only, n=185 (%, 95% CI) or mean (range)	CT cases with Mgen detected, n=27 (%, 95% CI) or mean (range)	OR (95% CI)	P value†
Positive	24 (13, 9 to 19)	9 (31, 15 to 51)	2.96 (1.21 to 7.26)	**0.023**
Using PrEP‡				
No	122 (77, 70 to 84)	14 (70, 46 to 88)	1	
Yes	36 (23, 16 to 30)	6 (30, 12 to 54)	1.45 (0.52 to 4.05)	0.485
STI detected in the urethra‡‡				
No	116 (64, 57 to 71)	19 (68, 48 to 84)	1	
Yes	64 (36, 29 to 43)	9 (32, 16 to 52)	0.86 (0.37 to 2.01)	0.725
STI detected in the pharynx§§				
No	106 (59, 51 to 66)	16 (57, 37 to 76)	1	
Yes	75 (41, 34 to 49)	12 (43, 24 to 63)	1.06 (0.47 to 2.37)	0.887
Symptomatic**				
No	81 (45, 37 to 52)	15 (52, 33 to 71)	1	
Yes	101 (55, 48 to 63)	14 (48, 29 to 67)	0.75 (0.34 to 1.64)	0.469
Anal symptoms††				
No	156 (85, 79 to 90)	24 (83, 64 to 94)	1	
Yes	27 (15, 10 to 21)	5 (17, 6 to 36)	1.2 (0.42 to 3.43)	0.732
STI contact				
No	164 (90, 84 to 94)	25 (86, 68 to 96)	1	
Yes	19 (10, 6 to 16)	4 (14, 4 to 32)	1.38 (0.43 to 4.39)	0.585

Chlamydia rectal infections: data missing for up to 5% condom use data; 1% of HIV status, PrEP use and STI detected in the urethra data.
Gonorrhoea rectal infections: data missing for up to 14% of condom use data; 3% of STI detected in the urethra and or pharynx; and 1% of HIV, PrEP and symptom status data.
*Due to the removal of the majority of CT-positive samples from individuals with HIV, the association between CT, CT/Mgen and HIV could not be assessed.
†P value calculated using logistic regression, and bold indicates significant findings.
‡Individuals with HIV were excluded for this variable.
§CT monoinfection cases, infections in the urethra: CT=37, NG=1, Mgen=1. CT and Mgen coinfection cases, infections in the urethra: CT=4, NG=1, Mgen=1.
¶CT monoinfection cases, infections in the pharynx: CT=21, NG=14. CT and Mgen coinfection cases, infections in the pharynx: CT=4, NG=1.
**Symptomatic was defined as the presence of any symptoms, including symptoms at sites other than the rectum.
††Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus.
‡‡NG monoinfection cases, infections in the urethra: CT=9, NG=58, Mgen=1. NG and Mgen coinfection cases, infections in the urethra: CT=1, NG=7, Mgen=2.
§§NG monoinfection cases, infections in the pharynx: CT=2, NG=73. CT and Mgen coinfection cases, infections in the pharynx: CT=0, NG=12.
CT, *Chlamydia trachomatis*; Mgen, *Mycoplasma genitalium*; NG, *Neisseria gonorrhoeae*; PrEP, HIV pre-exposure prophylaxis.

Overall MSM with rectal NG/Mgen coinfection were less likely to be using PrEP than MSM with rectal CT/Mgen coinfection (OR 0.25, 95% CI 0.10 to 0.65, p=0.002; table 3).

Pharyngeal Mgen infection

Four hundred and eighty pharyngeal samples from MSM were tested for Mgen during the course of the study. Fourteen samples yielded invalid results due to insufficient buffer, with valid results obtained for 464 pharyngeal samples. Eight of 464 samples were positive for Mgen (2%, 95% CI 1% to 3%), 7 of 464 were positive for CT (2%, 95% CI 1% to 3%), and 23 of 464 were positive for NG (5%, 95% CI 3% to 7%).

Pharyngeal Mgen was associated by univariable analyses with being HIV-positive (OR=5.13, 95% CI 1.19 to 22.12), having Mgen detected in the rectum (OR=60.14, 95% CI 3.41 to 1061.47) and having either rectal CT or rectal NG (OR=12.99, 95% CI 3.01 to 56.06) (table 4). Using a composite variable of any rectal STI, in multivariable analyses, MSM with pharyngeal Mgen were more likely to have a rectal STI detected (OR=10.61, 95% CI 2.30 to 48.87; table 4) and have a borderline association with being HIV-positive. Although MSM with pharyngeal Mgen were more likely to have either rectal CT and/or rectal NG, none of the eight patients had an STI detected in their urethra or a coinfection detected in their pharynx. Of note, no male patient with pharyngeal Mgen had pharyngeal symptoms.

As for pharyngeal Mgen, men who tested positive for CT or NG in the pharynx were also more likely to have CT or NG detected in the rectum. Five of seven pharyngeal CT infections

Table 3 Demographics and epidemiological features of *Chlamydia trachomatis* cases coinfected with *Mycoplasma genitalium*, compared with *Neisseria gonorrhoeae* cases coinfected with *M. genitalium*, respectively (n=56)

	C. trachomatis cases with *M. genitalium* detected, n=27 (%, 95% CI) or mean (range)	*N. gonorrhoeae* cases with *M. genitalium* detected, n=29 (%, 95% CI) or mean (range)	OR (95% CI)	P value*
Age	32 (22–51)	30 (20–56)	0.98 (0.92 to 1.05)	0.556
Number of male sexual partners in the last 3 months				
	10.5 (1–100)	8.08 (1–25)	0.99 (0.95 to 1.03)	0.567
Condom use with male partners in the last 3 months				
Not always	24 (92, 75 to 99)	21 (84, 64 to 95)	1	
Always	2 (8, 1 to 25)	4 (16, 5 to 36)	2.29 (0.38 to 13.77)	0.367
Using PrEP†				
No	15 (56, 35 to 75)	14 (70, 46 to 88)	1	
Yes	12 (44, 35 to 65)	6 (30, 12 to 54)	0.25 (0.10 to 0.65)	**0.002**
Symptomatic‡				
No	19 (70, 50 to 86)	15 (52, 33 to 71)	1	
Yes	8 (30, 14 to 50)	14 (48, 29 to 67)	2.22 (0.74 to 6.67)	0.157
Anal symptoms§				
No	25 (93, 76 to 99)	24 (83, 64 to 94)	1	
Yes	2 (7, 1 to 24)	5 (17, 6 to 36)	2.60 (0.46 to 14.73)	0.258
STI contact				
No	23 (85, 66 to 96)	25 (86, 68 to 96)	1	
Yes	4 (15, 4 to 34)	4 (14, 4 to 32)	0.92 (0.21 to 4.11)	0.913

Data missing for up to 14% of condom use in gonorrhoea samples and 4% of condom use in chlamydia samples.
*P value calculated using logistic regression, and bold indicates significant findings.
†Individuals with HIV were excluded for this variable.
‡Symptomatic was defined as the presence of any symptoms, including symptoms at sites other than the rectum.
§Anal symptoms included anal discharge, anal itch, anal pain, anal bleeding, painful bowel motions or tenesmus.
PrEP, HIV pre-exposure prophylaxis.

were associated with concurrent rectal CT (71%, 95% CI 29 to 96, OR=34.14, 95% CI 6.35 to 183.65), and 10 of 23 pharyngeal NG infections were associated with concurrent rectal NG (43%, 95% CI 23 to 66, OR=28.29, 95% CI 10.14 to 78.90).

DISCUSSION

This study examined coinfection of Mgen with CT and NG in the rectum of MSM, as well as the prevalence of pharyngeal Mgen in MSM being screened for CT and NG at the largest urban sexual health centre in Australia. We found high rates of coinfection, with Mgen present in 13%–14% of MSM with rectal CT or rectal NG. Pharyngeal Mgen was uncommon and detected in 2% of MSM, and most cases were associated with rectal CT or rectal NG infection. These findings indicate one in seven MSM infected with CT or NG in the rectum are coinfected with Mgen and that a substantial number of undiagnosed rectal Mgen infections are being inadvertently exposed to macrolide antibiotics, which is likely to be contributing to increasing macrolide resistance in Mgen.

A recent meta-analysis, including five studies mostly testing urine, estimated the prevalence of Mgen in MSM in the community at 3.2% (95% CI 2.1 to 5.1) at any site.[2] Studies have shown Mgen to be most commonly detected in the rectum of MSM, with 40.7% of contacts of the infection positive in the rectal sites.[17] Mgen was detected in 7% of rectal samples from asymptomatic MSM in a recent study of 1001 asymptomatic MSM at MSHC,[4] and 9% of consecutive asymptomatic and symptomatic anorectal samples from 505 MSM in Sydney.[3] The Melbourne study also found that of 89 MSM with Mgen at any site, 17% were coinfected with CT or NG, and in 143 MSM diagnosed with CT or NG at MSHC over the same period, 11% were coinfected with Mgen.[4] Notably a recent Sydney study reported rectal CT to be independently associated with anorectal rectal Mgen (OR=5.0, 95% CI 2.1 to 11.8, p<0.001).[3] A study at our centre did not show this association in the rectum, but that CT and NG were associated with urethral Mgen (p=0.03 and p=0.002, respectively).[4]

Globally for over a decade CT and NG have commonly been treated with regimens that include 1g azithromycin. Recent studies in Australia have shown resistance to azithromycin in Mgen in MSM now exceeds 80%.[3 13 18] A meta-analysis and published data from our group have shown de novo resistance occurs in 12% of Mgen infections following use of 1g azithromycin.[12 19] Data on the effect of extended azithromycin regimens on de

Table 4 Demographics and epidemiological features of *Mycoplasma genitalium*-negative pharyngeal samples versus *M. genitalium*-positive pharyngeal samples from men who have sex with men (n=466)

	***M. genitalium* negative, n=458 (%, 95% CI) or mean (range)**	***M. genitalium* positive, n=8 (%, 95% CI) or mean (range)**	**OR (95% CI)**	**P value***	**Adjusted OR (95% CI)†**	**P value**
Age	34 (18–87)	30 (22–38)	0.96 (0.88 to 1.04)	0.323		
Number of male sexual partners in the last 3 months						
	5 (0–100)	3 (0–8)	0.90 (0.67 to 1.21)	0.484		
HIV status						
Negative	410 (90, 86 to 92)	5 (62.5, 24 to 91)	1			
Positive	48 (10, 8 to 14)	3 (37.5, 9 to 76)	5.13 (1.19 to 22.12)	**0.028**	4.19 (0.85 to 20.81)	0.079
Using PrEP‡						
No	315 (82, 78 to 86)	4 (80, 28 to 99)	1			
Yes	69 (18, 14 to 22)	1 (20, 1 to 72)	1.14 (0.13 to 10.36)	0.907		
STI detected in the rectum						
No	374 (98, 96 to 99)	3 (37.5, 9 to 76)	1			
Yes	7 (2, 1 to 4)	5 (62.5, 24 to 91)	12.99 (3.01 to 56.06)	**0.001**	10.61 (2.30 to 48.87)	**0.002**
STI contact						
No	402 (88, 64 to 91)	7 (87.5, 47 to 100)	1			
Yes	56 (12, 9 to 16)	1 (12.5, 0 to 53)	1.03 (0.12 to 8.49)	0.981		

Data missing for up to 6% of PrEP use, and 17% of men who have sex with men were not tested for STI in the rectum.
*P value calculated using logistic regression, and bold indicates significant findings.
†Variables were included in the adjusted analysis if the p value was less than 0.05.
‡Individuals with HIV were excluded for this variable.
PrEP, HIV pre-exposure prophylaxis.

novo resistance are less clear. A recent meta-analysis containing 82 patients who received 1.5g azithromycin (without preceding doxycycline) found de novo resistance to be 3.7% (95% CI 0.8% to 10.3%), while a prospective study of 106 Mgen-infected patients treated with 1.5g azithromycin found de novo resistance to be similar to 1g (12%, 95% CI 3% to 27%).[12 19] The rising levels of macrolide resistance seen in Mgen is likely to be due to azithromycin use, particularly 1g in the treatment of Mgen, as well as inadvertent exposure during treatment of CT and NG. Macrolide resistance is also rising in gonorrhoea and syphilis.[20 21] Collectively, these data add to a growing body of evidence that suggests azithromycin use should be reduced in the STI field.[9] However, while limited safe and available treatment options exist for macrolide-susceptible Mgen, it seems sensible to use an extended azithromycin regimen with preceding doxycycline as published data suggest this may be associated with low levels of de novo resistance (2.6%, 95% CI 0.3% to 9.2%).[22]

Coinfection with Mgen in MSM with either rectal CT or rectal NG was associated with specific risk factors. Compared with having rectal NG alone, rectal Mgen/NG coinfection was more common among individuals with HIV. This association could not be assessed among patients with CT due to the removal of samples for LGV testing. A recent meta-analysis showed an association between HIV and Mgen in MSM, predominantly reflecting cross-sectional observational data.[23] Prospective studies have suggested an association between Mgen and HIV infection in women in Africa,[23 24] but there are no prospective studies examining this relationship in MSM, and the nature of this relationship remains to be established. Coinfection with Mgen in individuals with either rectal CT or rectal NG was associated with factors that largely reflect increased risk—current use of PrEP, HIV infection and having more male partners. While individually not particularly useful, collectively these risk factors may indicate which individuals are more likely to be rectally coinfected with Mgen. Mgen has been associated with current use of PrEP in previous studies,[3] and the majority of studies have found younger age and increased number of sexual partners to be associated with Mgen.[25 26] Neither coinfection with Mgen in the rectum or infection in the pharynx was associated with site-specific symptoms.

Mgen has been rarely reported in the oropharynx of MSM. Previous studies in Sydney and Melbourne, of 508 and 515 men, respectively, both failed to detect pharyngeal Mgen using PCR. TMA has a higher analytical sensitivity than PCR for Mgen,[27] although the Aptima *Mycoplasma genitalium* Assay (Hologic) has yet to be validated for detection of pharyngeal Mgen. Given the organism load of Mgen is up to 100 times lower than that of CT,[28] and may be at particularly low loads in the pharynx, TMA may be more likely to detect pharyngeal infections. We detected Mgen in 2% of pharyngeal samples, which is similar to the prevalence of CT in the pharynx in MSM attending our clinic.[29] Pharyngeal Mgen was associated with having a concomitant rectal STI (either CT or NG), although a recent study at our centre found only 1.9% of 54 rectal Mgen infections had pharyngeal

Mgen by PCR.[4] Other pharyngeal STIs commonly occur concurrently with rectal infections, with 71% and 43% of CT and NG pharyngeal infections found to have a concurrent rectal infection, respectively. MSM who were HIV-positive were also more likely to have pharyngeal Mgen detected than HIV-negative men. As mentioned previously, there is a known association between HIV and Mgen,[23] and the clearance rate of Mgen appears to be slower among HIV-positive people[30]; however, this association with HIV has not been previously reported with pharyngeal Mgen. There was no difference in the proportion of MSM reporting to be STI contacts between those with Mgen in the pharynx and those without. The transmissibility of pharyngeal Mgen is not known as it is not clear if this low positivity in the pharynx reflects passive infection/deposition, rather than active infection as has been hypothesised for pharyngeal CT. Overall these and other data indicate the pharynx is unlikely to be a significant source of Mgen transmission.

There were several limitations to this study. The Aptima buffer evaporates from the stored rectal and pharyngeal samples when stored at room temperature, which left insufficient remnant buffer for testing for many specimens. Subsequently, the samples were resealed with parafilm, which reduced evaporation. Rectal and pharyngeal samples that were positive for both chlamydia and gonorrhoea were more likely to have insufficient buffer remaining for additional Mgen testing, impacting on our ability in this study to examine cases with triple (Mgen, CT and NG) infections. Removal of CT-positive rectal samples from HIV-positive men being tested for LGV impacted on our ability to examine the association between HIV and rectal CT. This study was conducted at a single sexual health clinic, and so is likely to reflect a higher risk population that may impact on prevalence estimates.

We have demonstrated a high prevalence of coinfection of Mgen with CT and NG in the rectum of MSM attending our service, which highlights how commonly Mgen is being inadvertently exposed to antibiotics in the treatment of other STIs. While the prevalence of Mgen in men with other rectal STIs is high, lack of clarity around the natural history of Mgen in the rectum and concerns around issues of cost, toxicity and antimicrobial resistance in the treatment of macrolide-resistant Mgen make the issue of screening for Mgen in MSM far more complex than it is for CT and NG. Few papers have investigated pharyngeal Mgen in MSM. Our findings are in line with other publications and show Mgen is not commonly detected in the pharynx of MSM being screened for CT and NG, indicating it is not a common site of infection.

Key messages

- One in seven men who have sex with men treated for rectal *Chlamydia trachomatis* or rectal *Neisseria gonorrhoeae* will have undiagnosed *Mycoplasma genitalium* (Mgen).
- Undiagnosed rectal Mgen appears to be commonly exposed to azithromycin during screening and treatment of rectal chlamydia and gonorrhoea.
- Pharyngeal Mgen is uncommon.

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Appendix C. Additional information for Chapter 5.

C1. Patient Questionnaire.

OMG Mycoplasma genitalium

Questionnaire

DATE: _____ STUDY ID: _____

OMG: ONE MINUTE SURVEY

QUESTIONS FOR PATIENTS

Q 1. Have you had any of these symptoms in the past week? Please tick one in each row.

Abdominal (stomach) pain or pelvic pain	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Deep pelvic pain when having sex	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Abnormal discharge from the vagina	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Abnormal vaginal odour	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Bleeding after or during sex	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Vaginal bleeding (or spotting) in between periods	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Vaginal itch	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Burning when you urinate	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Increased urinary urgency or frequency (need to urinate urgently or more often)	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐
Fevers or sweats	None: ☐	Mild: ☐	Moderate: ☐	Severe: ☐

Q 2. These questions are about your history and relationships.

Have you been treated for any of these sexually transmitted infections in the last 6 months: chlamydia, gonorrhoea, or Mycoplasma genitalium?	Yes: ☐	No: ☐	Not sure: ☐
With how many men have you had sex in the past 12 months?	Number: _____		
Did you use condoms the whole time with all of these men?	No, never: ☐	Always: ☐	Not always: ☐
With how many women have you had sex in the past 12 months?	None: ☐	Number: _____	

QUESTIONS FOR DOCTORS/NURSES

Please tick the signs that are present.

Abnormal vaginal discharge	Yes: ☐	No: ☐	
Abnormal vaginal odour	Yes: ☐	No: ☐	
Vulval redness or vulvitis	Yes: ☐	No: ☐	Not done: ☐
Cervicitis	Yes: ☐	No: ☐	Not done: ☐
Cervical or adnexal tenderness	Yes: ☐	No: ☐	Not done: ☐
Cervical contact bleeding	Yes: ☐	No: ☐	Not done: ☐
Please record vaginal pH	pH=_____		

Thanks for helping our research!

C2. Patient information and consent form.

Participant Information Sheet .

Title	Oh MG! The symptoms of *Mycoplasma genitalium* in women.
Short title	OMG
Project Number	100/17
Principal Investigators	Rosie Latimer, A/Prof Catriona Bradshaw, Dr Tim Read
Associate Investigators	Prof Christopher Fairley, Dr Gerald Murray
Location	Melbourne Sexual Health Centre

PART 1 WHAT DOES MY PARTICIPATION INVOLVE?

1. Introduction

We are inviting you to be part of this study (OMG study) because you are presenting to the Melbourne Sexual Health Centre with symptoms, for STI testing. Please ask about anything that you do not understand. Participation in this research is voluntary. If you don't wish to take part, you don't have to. If you decide not to take part this will not affect other aspects of your care at the centre.

2. What is the purpose of this research?

We are researching a sexually transmitted bacterium called *Mycoplasma genitalium*(MG). Currently we are not sure what symptoms MG causes in women. We want to test women with any symptoms to see whether MG might be a cause. We believe MG is similar to chlamydia in the symptoms it causes, and has similar long term complications.

3. What does participation in this research involve?

By telling the nurse or doctor that you will enter the study you are consenting to an extra swab. In addition to being tested for chlamydia and gonorrhoea, you will be tested for MG. This swab will be sent to the Royal Women's Hospital for testing for MG and antibiotic resistance testing (the lab we normally use for this test). This swab will then be stored. You will be asked to complete a 1-page questionnaire which will ask about symptoms and sexual history. Your medical record will be accessed by researchers to record test results and risk factors for infection.

If you test positive for MG we will call you and invite you to return for free treatment which is also available for your sexual partners.

This study will help us decide which women to test for MG in the future. There are no payments or fees involved in this project, which aims to recruit 1000 women.

4. What are the possible risks and benefits of taking part?

There may be some slight discomfort from the vaginal swabs. If you have MG, and the test shows it is resistant to the recommended antibiotic, azithromycin, we will provide an alternative antibiotic. Any antibiotic can sometimes cause side-effects, which in rare cases are serious. If you have antibiotic-resistant MG we will explain your treatment choices. The benefit of taking part is that you get to be tested for MG, which may or may not be the cause of your symptoms, and is not yet a routine test for symptomatic women except for those with specific conditions.

5. What happens when the research project ends?

We hope to complete this project by late 2018 and a summary of the results will be available on the Melbourne Sexual Health Centre website.

PART 2 HOW IS THE RESEARCH PROJECT BEING CONDUCTED

6. What will happen to the information about me?

By telling the nurse or doctor you would like to join the study, you consent to the research team collecting personal information about you for the project. Information obtained in this research project that can identify you will remain confidential and will be stored securely and indefinitely. The database containing the questionnaires and the code linking these to your name will remain at Melbourne Sexual Health Centre and only the study team will have access. Your information will only be used for this research project, or future research at this centre, and will not be disclosed except as required by law.

The results of this research will be published and presented at a conference in such a way that you are not identified. The study is partially funded by the Australian manufacturer of the MG test, SpeeDx which will share with the researchers ownership of the study findings and data, but not your test samples. They will not receive any information that identifies you; they only receive summary data such as averages. In accordance with relevant Australian and/or Victorian privacy and other relevant laws, you have the right to request access to the information about you that is collected and stored by the research team. You also have the right to request that any information with which you disagree to be corrected. Please inform the research team member named at the end of this document if you want to see your information.

7. What will happen to my test samples after testing?

These will be stored at the Royal Women's Hospital after testing, for future research into other infectious causes of genital symptoms in women, resistance to antibiotics and validation of new STI tests.

8. Who is organising and funding the research?

This research project is being conducted by A/Prof Catriona Bradshaw and Dr Tim Read (Sexual Health Physicians), and Rosie Latimer (PhD candidate). Some of the funding for the study comes from Australia's National Health and Medical Research Council and the test kits and funding for a research nurse come from SpeeDx. It is possible that SpeeDx may benefit if the research demonstrates a need to increase MG testing, however there will be no payment to you for participating.

9. Who has reviewed the research project?

The ethical aspects of this research project have been approved by the Human Research Ethics Committee (HREC) of the Alfred Hospital. This project will be carried out according to the National Statement on Ethical Conduct in Human Research (2007). This statement has been developed to protect the interests of people who agree to participate in human research studies.

10. Information, complaints, withdrawing from the project and who to contact

You are free to withdraw from the project at any time and ask for your test sample and information to be destroyed. Note: the MG test will be done within a few days of your visit and the result will go into your medical record. For information or any problems related to the project, **contact the study nurse** or **Rosie Latimer on (03) 9341 6200**. For complaints about the project or questions about being a research participant in general, you may contact the Complaints Officer, Office of Ethics & Research Governance, Alfred Health on 9076 3619 or email research@alfred.org.au (please quote project number 100/17).

CONSENT FORM- *Adult providing own consent*

Title	Oh MG! The symptoms of *Mycoplasma genitalium* in women.
Short title	OMG
Project Number	100/17
Principal Investigators	Rosie Latimer, A/Prof Catriona Bradshaw, Dr Tim Read
Associate Investigators	Prof Christopher Fairley, Dr Gerald Murray
Location	Melbourne Sexual Health Centre

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.
I understand the purposes, procedures and risks of the research described in the project.
I have had an opportunity to ask questions and I am satisfied with the answers I have received.
I consent to researchers accessing my medical records for use as described in the Participant Information Sheet.
I consent to the storage and use of vaginal samples taken from me for use as described in the Participant Information Sheet.
I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.
I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print)_____

Signature_____ Date_____

Declaration by Study Doctor/Research nurse

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Research nurse (please print) _____

Signature_____ Date_____

Study ID:

Attach UR label

Appendix D. Additional information for Chapter 6.

D1. PDF of published study from Chapter 6.

ORIGINAL STUDY

Clinical Features and Therapeutic Response in Women Meeting Criteria for Presumptive Treatment for Pelvic Inflammatory Disease Associated With *Mycoplasma genitalium*

Rosie L. Latimer, Tim R.H. Read, PhD,*† Lenka A. Vodstrcil, PhD,*†*
Jane L. Goller, MPH,‡ Jason J. Ong, MBBS,†§ Christopher K. Fairley, PhD,*†*
Jane S. Hocking, PhD,‡ and Catriona S. Bradshaw, PhD†*

Background: There are limited published data describing clinical features and therapeutic response in women meeting the criteria for presumptive treatment of pelvic inflammatory disease associated with *Mycoplasma genitalium* (MG-PID). The MG-PID has been reported to respond poorly to standard PID treatment regimens and while moxifloxacin is recommended in several treatment guidelines, published data to support its use are scant.

Methods: We conducted a retrospective study of women at Melbourne Sexual Health Centre between 2006 and 2017, who met the Centers for Disease Control and Prevention criteria for presumptive treatment of PID, and had MG detected as the sole pathogen. Clinical and laboratory characteristics of MG-PID were compared to cases of chlamydial PID (CT-PID) by multivariable analysis. Microbiological and clinical cure following moxifloxacin and standard PID treatment was determined for women with MG-PID who returned for test of cure between 14 and 120 days.

Results: Ninety-two patients with MG-PID were compared with 92 women with CT-PID. The MG-PID was associated with increased lower abdominal tenderness (adjusted odds ratio, 2.29; 95% confidence interval [CI], 1.14–4.60), but a lesser vaginal polymorphonuclear response compared to CT-PID by multivariable analysis. Of the 92 women with MG-PID, 54/92 (59%) received moxifloxacin (10–14 days) and 37/54 had a test of cure between 14 and 120 days; 27/37 (73%) cases had a median of 7 days of a standard regimen containing doxycycline and metronidazole +/− azithromycin before moxifloxacin. Microbial cure following moxifloxacin was 95% (95% CI, 82–99%) and did not differ from standard therapy (P = 0.948), however clinical cure was significantly higher following moxifloxacin (89%; 95% CI, 75–97%; P = 0.004)] although adverse effects were more common.

Conclusions: Women meeting Centers for Disease Control and Prevention criteria for presumptive treatment of MG-PID did not significantly differ to those with CT-PID. Moxifloxacin was associated with higher rates of symptom resolution in women with PID, and although microbial cure was high, it did not differ between regimens.

*M*ycoplasma genitalium (MG) has been associated with cervicitis, pelvic inflammatory disease (PID), spontaneous abortion and preterm delivery in a recent meta-analysis.[1,2] However, there are limited published data on the contribution of MG to PID. More data are needed to establish the attributable risk of MG for female genital tract infections.[2] International guidelines for treatment of PID vary, however predominantly recommend presumptive use of antimicrobials, such as tetracyclines, beta-lactams, and nitro-imidazoles, before detection of the causative organism. These regimens aim to treat *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG) and anaerobes, and do not contain highly effective antimicrobials against MG.[3,4] When PID was treated in accordance with US Centers for Disease Control and Prevention (CDC) guidelines with cefoxitin and doxycycline, 41% of women with PID remained MG positive after 30 days.[5] Inadequately treated PID increases the risk of chronic pelvic pain, ectopic pregnancy and infertility.[6]

Mycoplasma genitalium has marked propensity to develop antimicrobial resistance, with macrolide resistance seen in 40% to 60% of infections in many countries.[7,8] Moxifloxacin has been highly effective against macrolide-resistant MG,[9] is active against CT and NG,[10,11] and was as effective as ofloxacin and metronidazole for all cause PID in a randomized controlled trial (RCT).[11] Although only 3 patients in this trial had MG-PID, all were cured.[11] The US and UK guidelines now recommend 14 days of moxifloxacin for MG-PID,[2,12] although efficacy data are limited,[11] and a recent meta-analysis has shown a decline in cure to 89% (95% CI, 82–94%) since 2010.[13]

With recent regulatory approval of MG assays in many countries, testing for MG in presumptive PID may become increasingly common. However, little has been published on clinical characteristics of MG-PID and whether they differ from those associated with CT-PID. In our study, we compared women who met CDC criteria for presumptive treatment of PID with MG to women with CT-PID to investigate whether MG-PID is associated with distinct clinical and laboratory characteristics. In addition, we report the clinical and microbiological outcomes following moxifloxacin and standard antimicrobial therapy.

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Conflict of Interest: Melbourne Sexual Health Centre receives funding from SpeeDx Pty Ltd (Australia) for research projects on *M. genitalium,* however no funding was received or used to support this project.

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MATERIALS AND METHODS

Study Design and Participants

We undertook a retrospective study of women with MG and presumptive PID (henceforth MG-PID) between February 2006 and March 2017, at Melbourne Sexual Health Centre (MSHC), Victoria, Australia. Women were included if they were 18 years or older, PID presumptively diagnosed using CDC criteria, and MG was the sole pathogen (ie, negative for CT and NG). Testing for MG in women with presumptive PID was at clinician discretion from 2006 to 2010, and from 2011 recommended in MSHC protocols. MG-PID cases were women with presumptive PID at presentation (87%), who underwent sexually transmitted infection (STI) testing and were commenced on recommended therapy for PID (henceforth standard treatment). In a minority of cases (13%), women developed symptoms and signs after testing and were diagnosed with presumptive PID at follow up.

We compared characteristics of MG-PID to those of CT-PID (chlamydia sole pathogen), and treatment outcomes (clinical and microbial cure) after standard PID therapy and moxifloxacin. All cases diagnosed with MG-PID between February 2006 and March 2017 were extracted from the clinic database. Cases of CT-PID were selected over the same period from the clinic database.

Definitions and Data Collection

Presumptive PID was diagnosed using CDC criteria, which recommends treatment be initiated in sexually-active women at risk of STIs who are experiencing pelvic or lower abdominal pain and have one of the following: uterine, cervical motion, or adnexal tenderness.[3] These criteria are designed to maximize sensitivity for PID diagnosis and err on the side of over rather than undertreatment of PID. Only approximately half of women meeting these criteria have been shown to have laparoscopic evidence of salpingitis or plasma cell endometritis,[14,15] although the positive predictive value for these criteria are higher in a STI clinic compared with a community setting.[3] All women included in the analysis were sexually active and presented with abdominal pain or pelvic pain, and had either lower abdominal/pelvic tenderness or cervical/adnexal motion tenderness on examination. All reference to MG-PID and CT-PID refers to presumptive PID diagnosis using CDC criteria. *Mycoplasma genitalium* was detected on vaginal or cervical swab, or first pass urine, using an in-house PCR assay targeting the 16S rRNA gene of MG.[16] *Chlamydia trachomatis* was detected using strand displacement amplification (Becton Dickinson) before 2015, and Aptima transcription-mediated amplification assay from 2015 (Hologic, Marlborough). Bacterial vaginosis (BV) was diagnosed using Amsel's criteria.[17] Trichomoniasis was diagnosed by wet preparation and culture. Vaginal and cervical polymorphonuclear leukocyte counts (PMN) on Gram stain were grouped in the following categories: vaginal PMN/high power field (hpf): less than 1, 1 to 4, and 5 or greater; and cervical PMN/hpf: less than 5, 5 to 8, greater than 8.

Clinical Characteristics

Epidemiological, clinical, and laboratory data were extracted from paper-based and electronic records for women with MG-PID and CT-PID. Clinical information recorded included: (i) symptoms: lower abdominal pain, dyspareunia, vaginal discharge, fever, intermenstrual or postcoital bleeding; (ii) signs: lower abdominal tenderness, cervical motion or adnexal tenderness, mucopurulent cervicitis, cervical-contact bleeding, abnormal vaginal discharge; (iii) laboratory and microbiological results: vaginal and cervical PMN count, detection of CT, NG, *Trichomonas vaginalis*, and BV; and (iv) antimicrobial regimens prescribed including duration, adherence and adverse effects.

Patients were excluded if they did not fulfill presumptive PID criteria, were coinfected with another STI, or if symptoms were subsequently attributed to another condition.

Treatment Outcomes

Women with presumptive PID were tested and treated with a standard regimen containing metronidazole 400 mg twice daily and doxycycline 100 mg twice daily for 14 days +/− azithromycin 1 g.[18] The MG test results were available 48 hours after testing. Beginning in 2011 all MG-positive women were recalled and the standard regimen replaced with moxifloxacin 400 mg once daily for 14 days, in accordance with MSHC guidelines. Before 2011, patients often received standard PID regimens only. Treatment outcomes in all women receiving moxifloxacin and standard regimens were analyzed.

Microbiological cure was defined as a negative test of cure (TOC) between 14 and 120 days. Patients were excluded if: (i) TOC was not performed or outside 14 to 120 days after treatment, (ii) they received less than 7 days of moxifloxacin, (iii) TOC was performed after more than 2 courses of antibiotics.

Clinical cure was defined as resolution of all PID-related symptoms. Antibiotic adherence, partner treatment, reinfection risk, and microbiological and clinical outcomes were recorded, where available. Reinfection risk was defined as none (no sex), possible (sex with a new or treated partner), or probable (sex with a regular partner who had not been tested or treated).

The Alfred Hospital Research Ethics Committee approved this study (project number 304/15).

Statistical Analysis

Data were analyzed using STATA (v14). Univariate and multivariable logistic regression analysis were performed to determine factors associated with MG-PID compared with CT-PID. Variables were included in multivariable models if the P value was 0.05 or less; if correlated, the variable most strongly associated with the outcome was used. Models were built in a forward-stepwise fashion, using the likelihood ratio test to determine the significance of the contribution of each variable to avoid overfitting the model. Ninety-five percent CIs were calculated for proportions. As vaginal and cervical PMN counts were correlated and vaginal PMN count is a minor CDC criterion, only vaginal PMN was included. Logistic regression was used to assess differences in outcomes by treatment group for those with MG-PID.

RESULTS

During the study period (2006–2017), 149 records of women with MG-PID were identified, and MG accounted for 5.5% of PID cases at MSHC during this timeframe. Ninety-two women fulfilled the criteria for presumptive PID and had MG with no other pathogen detected; 57 women were excluded for reasons listed in Figure 1. Ninety-two CT-PID cases were randomly selected, as described in Materials and Methods. Eight-seven percent of patients had PID diagnosed at their initial consultation, and 13% on return to clinic.

Demographic and Behavioral Characteristics

There was no significant difference between median ages of women with MG-PID and CT-PID (25 years, interquartile range [IQR], 21–29; and 24 years, IQR, 21–28, respectively; Table 1). In

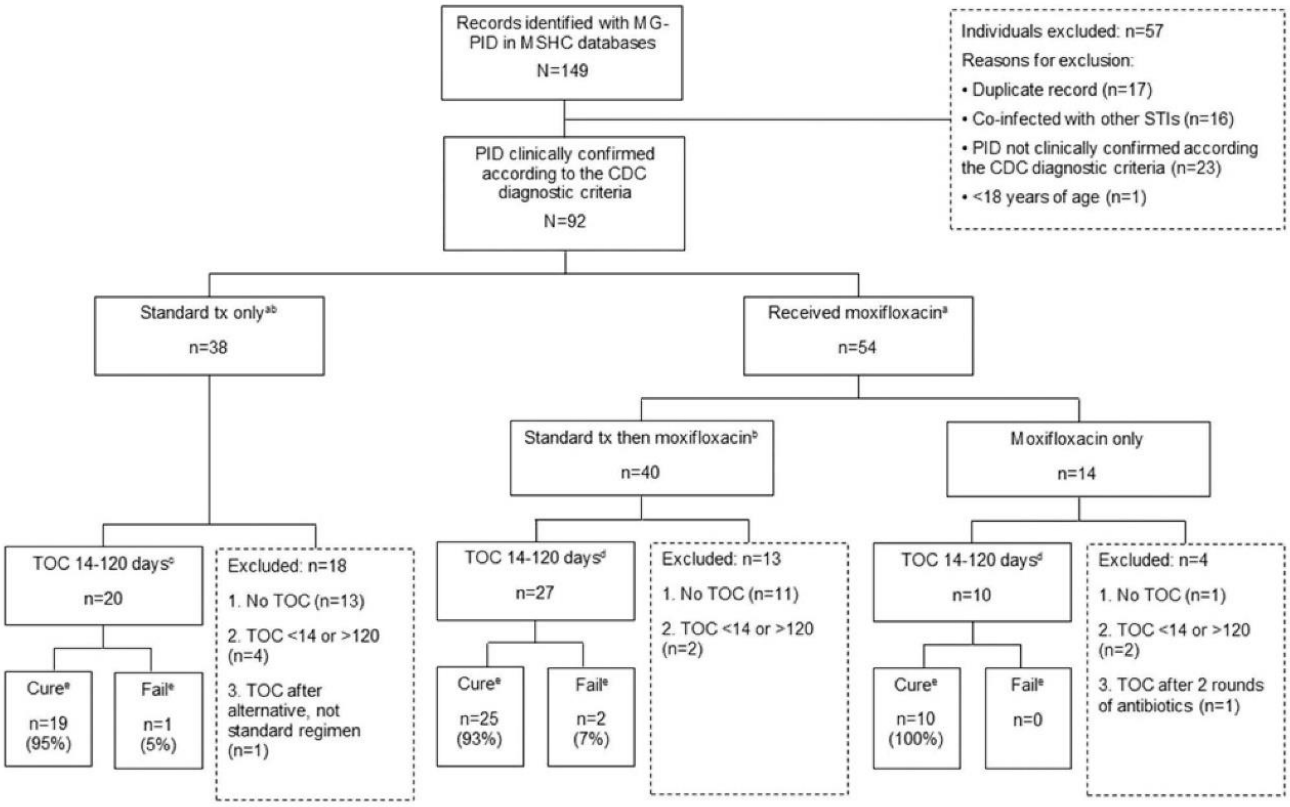

Figure 1. Selection of patients for analysis of effectiveness of moxifloxacin and standard treatment of *Mycoplasma genitalium* associated pelvic inflammatory disease. [a]Patients were not randomized, selection of moxifloxacin versus standard therapy was at clinician discretion; [b]Standard therapy includes varied combinations of azithromycin 1 g single dose, doxycycline 100 mg twice daily and metronidazole 400 mg twice daily for 14 days; [c]20 patients analyzed for effectiveness of standard treatment; [d]27 patients who received standard therapy and moxifloxacin and 10 patients who received moxifloxacin only combined for analyses of effectiveness of moxifloxacin; [e]Cure refers to a negative test of cure, fail refers to a positive test of cure. n, number; tx, treatment.

unadjusted analyses, compared to CT-PID, women with MG-PID were more likely to be sex workers (odds ratio [OR] 3.07; 95% CI, 1.33–7.08), and to have fewer recent male partners (excludes sex work clients) (MSP > 1; OR, 0.54; 95% CI, 0.30–0.97).

Clinical Characteristics

Univariate analyses showed women with MG-PID had similar clinical characteristics to women with CT-PID, although were less likely to report postcoital bleeding (OR, 0.42; 95% CI, 0.18–0.98; Table 1). On clinical examination, MG-PID was more likely to be associated with lower abdominal tenderness (OR, 2.36; 95% CI, 1.29–4.28).

Laboratory Characteristics

Women were tested for MG by cervical swab (64%), vaginal swab (20%), and first-pass urine (16%). On unadjusted analyses, women with MG-PID were less likely than women with CT-PID to have elevated vaginal or cervical PMN counts (Table 1).

Associations With MG-PID Compared With CT-PID by Multivariable Analyses

Women with MG-PID were more likely to have lower abdominal tenderness (adjusted OR, 2.29; 95% CI, 1.14–4.60), but less likely to have a modest elevation in vaginal PMN counts, compared to women with CT-PID (Table 2).

Treatment Outcomes in MG-PID

Of the 92 women with MG-PID, 54 (59%) received moxifloxacin and 38 (41%) a standard regimen only (Fig. 1). Thirty-seven of 54 women who received moxifloxacin returned for TOC at 14 to 120 days (Fig. 1). Of these women, 10 of 37 (27%) received moxifloxacin only and 27 of 37 (73%)started a standard regimen before being recalled for moxifloxacin. Premoxifloxacin, standard therapy in 23 (85%) of 27 women was azithromycin followed by doxycycline and metronidazole and in 4 (15%) of 27 women was doxycycline and metronidazole without azithromycin. Ceftriaxone, which has no effect on MG, was only dispensed presumptively for individuals considered at risk of gonorrhea. Median duration of standard therapy premoxifloxacin was 7 days (range, 2–14 days). Median time to TOC was 32 days (IQR, 24–41 days). A higher proportion of women receiving moxifloxacin returned for TOC at 14 to 120 days (37/54 [69%]) in contrast to standard treatment (20/38 [53%]) (Fig. 1).

Of the 37 women with MG-PID who had moxifloxacin and a 14 to 120 day TOC, 35/37 [95% (95% CI 82–99)] were microbiologically cured (Table 2, Fig. 1). There was no significant difference in microbiological cure between the moxifloxacin group and the standard treatment group (P = 0.948). Of note, both of the moxifloxacin failures received standard PID treatment before moxifloxacin. Clinical cure, defined as resolution of all pelvic symptoms, was significantly higher in women treated with moxifloxacin (33/37 [89%]; 95% CI, 75–97) compared with standard

TABLE 1. MG-PID Compared With CT-PID: An Analysis of Demographics, Behavioral and Clinical Characteristics (N = 184)

	Chlamydia PID Cases n = 92 (%, 95% CI) or Median (IQR)		MG-PID Cases n = 92 (%, 95% CI) or Median (IQR)		Unadjusted OR (95% CI)		*P*	Adjusted* OR (95% CI)		*P*
Age	24	(21–28)	25	(21–29)						
No. MSP last 3 mo†‡										
≤1	35	(38, 28–49)	49	(53, 43–64)	1.0			1.0		
>1	57	(62, 51–72)	43	(47, 36–57)	0.54	(0.30–0.97)	0.039	0.54	(0.27–1.06)	0.071
Consistency of condom usage†										
Not always	79	(94, 87–98)	72	(85, 75–92)	1.0					
Always	5	(6, 2–13)	13	(15, 8–25)	2.85	(0.97–8.40)	0.057			
Current sex worker										
No	83	(90, 82–95)	69	(75, 65–83)	1.0			1.0		
Yes	9	(10, 5–18)	23	(25, 17–35)	3.07	(1.33–7.08)	0.008	1.92	(0.75–4.97)	0.176
Sex within Australia only†										
No	36	(42, 32–54)	23	(28, 19–39)	1.0					
Yes	49	(58, 46–68)	59	(72, 61–81)	1.88	(0.99–3.60)	0.054			
Reported symptoms										
Abdominal pain§										
No	23	(25, 17–35)	13	(14, 8–23)	1.0					
Yes	69	(75, 65–83)	79	(86, 77–92)	2.03	(0.95–4.30)	0.066			
Dyspareunia§										
No	43	(47, 36–57)	45	(49, 38–60)	1.0					
Yes	49	(53, 43–64)	47	(51, 40–62)	0.92	(0.51–1.63)	0.768			
Postcoital bleeding										
No	73	(79, 70–87)	83	(90, 82–95)	1.0			1.0		
Yes	19	(21, 13–30)	9	(10, 5–18)	0.42	(0.18–0.98)	0.044	0.40	(0.15–1.12)	0.082
Intermenstrual bleeding										
No	77	(84, 75–91)	71	(77, 67–85)	1.0					
Yes	15	(16, 9–25)	21	(23, 15–33)	1.52	(0.73–3.17)	0.267			
Dysuria										
No	64	(70, 59–79)	72	(78, 68–86)	1.0					
Yes	28	(30, 21–41)	20	(22, 14–32)	0.63	(0.33–1.23)	0.181			
Urinary frequency‖										
No	72	(78, 68–86)	82	(89, 81–95)	1.0			1.0		
Yes	20	(22, 14–32)	10	(11, 5–19)	0.44	(0.19–1.00)	0.050	0.56	(0.22–1.46)	0.234
Vaginal discharge (symptom)										
No	36	(39, 29–50)	40	(43, 33–54)	1.0					
Yes	56	(61, 50–71)	52	(57, 46–67)	0.84	(0.46–1.50)	0.549			
Recorded clinical signs										
Lower abdominal tenderness§										
No	49	(53, 43–64)	30	(33, 23–43)	1.0			1.0		
Yes	43	(47, 36–57)	62	(67, 57–77)	2.36	(1.29–4.28)	0.005	2.29	(1.14–4.60)	0.020
Cervical or adnexal motion tenderness§										
No	15	(16, 9–25)	13	(14, 8–23)	1.0					
Yes	77	(84, 75–91)	79	(86, 77–92)	1.18	(0.53–2.65)	0.682			
Mucopurulent cervicitis										
No	57	(62, 51–72)	62	(67, 57–77)	1.0					
Yes	35	(38, 28–49)	30	(33, 23–43)	0.79	(0.43–1.44)	0.441			
Cervical contact bleeding										
No	78	(85, 76–91)	81	(88, 80–94)	1.0					
Yes	14	(15, 9–24)	11	(12, 6–20)	0.76	(0.32–1.77)	0.520			
Vaginal discharge (sign)										
No	42	(46, 37–56)	43	(46, 37–56)	1.0					
Yes	50	(54, 44–63)	49	(54, 44–63)	0.96	(0.54–1.71)	0.882			
Bacterial Vaginosis†										
Not detected	49	(53, 43–64)	51	(55, 45–66)	1.0					
Detected	33	(36, 26–47)	32	(35, 25–45)	0.93	(0.50–1.74)	0.824			
Vaginal PMN count†¶**										
< 1	23	(28, 19–39)	38	(46, 35–58)	1.0					
1–4	19	(23, 15–34)	12	(15, 8–24)	0.38	(0.16–0.93)	0.034	0.34	(0.13–0.89)	0.027
≥5	40	(49, 38–60)	32	(39, 28–50)	0.48	(0.24–0.97)	0.041	0.72	(0.34–1.54)	0.400

Continued next page

treatment (10/19 [53%]; 95% CI, 29–76; $P = 0.004$; Table 2). Of the 4 patients in the moxifloxacin group with persistent symptoms, all had abdominal pain and 2 reported dyspareunia. Of the 9 patients in the standard treatment group with ongoing symptoms, 7 had persistent abdominal pain, and 3 had persistent dyspareunia.

TABLE 1. *(Continued)*

	Chlamydia PID Cases n = 92 (%, 95% CI) or Median (IQR)		MG-PID Cases n = 92 (%, 95% CI) or Median (IQR)		Unadjusted OR (95% CI)		P	Adjusted* OR (95% CI)	P
Cervical PMN count†¶**									
<5	5	(11, 4–25)	9	(24, 12–41)	1.0				
5–8	6	(14, 5–27)	14	(38, 22–55)	1.29	(0.30–5.54)	0.726		
>8	33	(75, 60–87)	14	(38, 22–55)	0.24	(0.24–0.15)	0.024		

*Adjusted model includes: number of male sexual partners, current sex worker, postcoital bleeding, urinary frequency, lower abdominal tenderness, and vaginal PMN count.

†Denominator varied due to exclusion of patients with unrecorded data.

‡Male sexual partners does not include commercial partner numbers.

§All women included in the analysis had presumptive-PID based on CDC criteria: were sexually active and presented with pelvic pain, defined as either abdominal pain or deep dyspareunia (pelvic pain elicited during sexual intercourse) and had lower abdominal/pelvic tenderness or cervical motion/adnexal tenderness on examination.

||Urinary frequency was also included in the multivariate analysis based on its contribution to the model using the likelihood ratio statistic.

¶Vaginal PMN from high vaginal swab.

** Vaginal and cervical PMN counts were correlated and since vaginal PMN count is a minor CDC criterion for PID, only vaginal PMN were included in the multivariable model.

mo, months; MSP, male sexual partner; n, number; PMN, polymorphonuclear lymphocyte.

Adherence to Antimicrobial Therapy and Reinfection Risk

Adherence to therapy was documented in 32 of 37 (86%) patients who had moxifloxacin and returned for a TOC visit, with 27 of 32 (84%) (95% CI, 67–95) reporting 100% adherence and 5 of 32 reporting <100% adherence (all took >7 days) (Table 2). Of the 2 microbiological failures following moxifloxacin, one had sex with an untreated partner before TOC and was at high risk of reinfection; the other did not have reinfection risk or adherence documented.

Adherence to standard treatment was documented in 16 of 20 patients returning for TOC with 12 of 16 (75%) (95% CI, 48–93) reporting 100% adherence. There was no difference in adherence between the 2 groups ($P = 0.436$).

Adverse Effects

Adverse effects were significantly more common among those who took moxifloxacin ($P = 0.026$), with 15/37 (41%) women who received moxifloxacin reporting side effects (Table 2). The

TABLE 2. Clinical and Microbiological Outcomes, Adherence and Adverse Effects of Moxifloxacin Compared to Standard Treatment for MG-PID*† (N = 57)

	Standard treatment‡ n = 20 (%, 95% CI)	Moxifloxacin§ n = 37 (%, 95% CI)	P
Adherence||			
Incomplete	4 (25, 7–52)	5 (16, 5–33)	
Complete	12 (75, 48–93)	27 (84, 67–95)	0.436
Reinfection risk||, ¶			
None	6 (55, 23–83)	12 (41, 24–61)	
Possible	3 (27, 6–61)	7 (24, 10–44)	0.856
Probable	2 (18, 2–52)	10 (34, 18–54)	0.320
Reported side effects			
No	18 (90, 68–99)	22 (59, 42–75)	
Yes	2 (10, 1–32)	15 (41, 25–58)	0.026
Microbiological outcome			
Fail	1 (5, 1–25)	2 (5, 1–18)	
Cure	19 (95, 75–100)	35 (95, 82–99)	0.948
Resolution of symptoms||			
No	9 (47, 24–71)	4 (11, 3–25)	
Yes	10 (53, 29–76)	33 (89, 75–97)	0.004

*Patients were excluded if no TOC was performed (n = 25), the TOC occurred <14 day or >120 days after treatment (n = 8), TOC occurred after an alternative, not standard regimen (n = 1), or the TOC was performed after multiple rounds of antibiotics (n = 2).

†Patients were not randomized, selection of moxifloxacin versus standard therapy was at clinician discretion.

‡Standard treatment group includes 22 patients who had a TOC at 14–120 days and includes varied combinations of azithromycin 1 g single dose, doxycycline 100 mg twice daily and metronidazole 400 mg twice daily for 14 days.

§Twenty-seven patients who had standard therapy and moxifloxacin and TOC 14–120 days; 10 patients who had moxifloxacin only and TOC 14–120 days.

||Denominator varied due to exclusion of patients with unrecorded data.

¶Reinfection risk was defined as none (no sex), possible (sex with a new or treated partner), or probable (sex with a regular partner who has not yet been tested or treated).

n, number; TOC, test of cure.

most common side effects were nausea (7 of 15), diarrhea (3 of 15), and candidiasis (2 of 15); 1 patient reported tendon pain. No serious adverse effects were reported but 4 of 5 patients who ceased moxifloxacin early experienced side effects. Of the 20 women who received standard treatment, only 2 (10%) reported any adverse effects, the nature of which was not recorded.

DISCUSSION

This study of women meeting the criteria for presumptive treatment of PID associated with *Mycoplasma genitalium,* found a similar clinical presentation to that of CT-PID. Among 37 evaluable women, moxifloxacin microbiologically cured 95% of MG infections but this did not differ to standard treatment. It is interesting that moxifloxacin was associated with significantly higher resolution of clinical symptoms (89%) compared to standard treatment (53%), although side effects were common with moxifloxacin.

There are limited published data examining the association between MG and PID. While cases included in this dataset fulfilled the CDC criteria for presumptive treatment for PID, past studies found that up to half of all cases of presumptive PID did not have histological endometritis or salpingitis.[14] Mild abdominal pain has previously been reported in MG-associated acute endometritis.[19] Investigators in 1 study found gonococcal-PID to be more severe than MG-PID, but found no difference in the clinical presentation of CT-PID and MG-PID.[20] Although we found MG-PID was more likely to be associated with abdominal tenderness than CT-PID, the significance of this finding is unclear. Universal testing for MG in PID at MSHC was not recommended until 2011, and although the case definition of presumptive PID required the presence of abdominal or pelvic pain and lower abdominal/pelvic tenderness or cervical/adnexal motion tenderness on examination, it is possible that only more severe cases were tested for MG before 2011. In contrast to the clinical findings, MG-PID was associated with modest reduction in cervicovaginal PMN response compared to CT-PID, supporting the role of CT as an established cause of PID and morbidity. An important limitation of this study is that outpatient sexual health services are likely to see milder PID. Studies in hospitals attended by women with more severe PID may have different findings. While records were reviewed by the same researcher to control for differences in interpretation of records, missing data and clinician variability in documentation are unavoidable limitations of a retrospective case review. Despite these limitations, this remains the largest study to date of MG-PID examining clinical features.

In this study, both regimens achieved high levels of microbial cure, although clinical cure was significantly higher with moxifloxacin, and only 53% of women receiving standard regimens experienced complete resolution of PID-symptoms. This raises the possibility of persistent low load infection, as this pattern occurs in MG-urethritis.[21] However, a direct comparison of treatment outcomes should be interpreted with care due to substantial differences between those who received standard treatment, and those given moxifloxacin. Firstly, treatment regimens were not randomized and were at clinician discretion, with temporal differences between the 2 treatment groups. Women receiving standard treatment only were predominantly treated earlier in the study (60% treated before 2013), while the majority of those in the moxifloxacin group were treated later in the study (85% after 2013). MSHC has experienced an extraordinary rise in macrolide-resistant MG and azithromycin-failure from less than 10% in 2006 to greater than 40% in 2016.[21–23] The group who received standard treatment only predominantly reflected the period when macrolide resistance was uncommon and this regimen may be less effective as high levels of macrolide resistance (>50%) are now seen in 2018.[21] These data suggest that regimens containing azithromycin may be effective in regions with low levels of macrolide resistance. It is possible that cure is also enhanced by the presence of doxycycline, which we have recently shown has a substantial effect on MG load and selection of macrolide resistance.[21] Published data suggest that it is the inclusion of azithromycin that is likely to have affected cure, as when MG-PID was treated with cefoxitin and doxycycline, the microbiological cure rate was only 59%.[5] Further data regarding the efficacy of doxycycline-azithromycin inclusive regimens, particularly with respect to clinical cure, would be of value considering the cost and lower tolerability of moxifloxacin.

Adverse effects were significantly more common in women treated with moxifloxacin than standard therapy. Although this may be directly attributable to moxifloxacin, 73% of women receiving moxifloxacin had also been exposed to a standard PID regimen. Gastrointestinal side effects are common with quinolones; however, rare serious adverse effects, including arrhythmias, neuropathy, and tendon rupture, have also been reported. Risk of adverse effects needs to be balanced with risk of serious sequelae from delaying antimicrobial therapy in PID.[6,24,25] This study was conducted at a time of rising macrolide resistance in Australia and during the emergence of quinolone resistance in the region. A recent meta-analysis reported the efficacy of moxifloxacin for MG to be declining, from 100% (95% CI, 99–100) in studies before 2010, to 89% (95% CI, 82–94) since.[13] Due to the small numbers of treatment failures in this study, temporal trends in efficacy could not be assessed. Of note, a significant proportion of women treated with moxifloxacin had exposure to standard PID regimens before moxifloxacin, which may have improved moxifloxacin cure. Although this may have impacted on the reported efficacy of moxifloxacin, this is also reflection of real world practice in the absence of point of care tests. Moxifloxacin, although costly and associated with more adverse effects, currently remains the only available therapeutic option in most clinical settings for patients with macrolide-resistant MG.

This study reports a case series of women with presumptive PID and MG detected as the sole pathogen. It did not find clinically meaningful differences between women with MG-PID and CT-PID and does not provide prospective data to inform clinicians of the likelihood that MG will lead to PID. The CDC recommended standard PID regimen includes doxycycline but not azithromycin, and has been shown to have low microbiological and clinical cure for MG-PID.[5] Macrolide resistance is becoming increasingly common worldwide and in the majority of high-income countries exceeds 40%, so azithromycin-based regimens can be expected to have a declining efficacy. If MG is identified in a woman presenting with the clinical features of PID and no other pathogen is detected, then this study provides data showing moxifloxacin is highly effective in achieving microbiological cure of MG, but that 2 in 5 women will experience predominately mild adverse effects. The future will see point-of-care assays for MG that incorporate resistance markers, and this will assist researchers in determining the efficacy of specific regimens for MG-associated syndromes and clinicians in selecting appropriate antimicrobials to ensure high-level clinical and microbial cure.

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Appendix E. Additional information for Chapter 9.

E1. PDF of published study from Chapter 9.

PLOS ONE

RESEARCH ARTICLE

Non-consensual condom removal, reported by patients at a sexual health clinic in Melbourne, Australia

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Data Availability Statement: Information obtained in this survey is highly sensitive and confidential and includes patient health record data. Furthermore, we are unable to share data as it would be in breach our ethics approval. Our participant information and consent form specifically states that individual participant information will only be accessible to the study team and will not be disseminated further in an identifiable way, i.e. only presented as aggregated data or statistics - "Information obtained in this research project that can identify you will remain confidential and will be stored securely indefinitely.

Abstract

Background

Non-consensual removal of condoms, colloquially referred to as 'stealthing', is the removal of a condom during sex by a sexual partner when consent has been given for sex with a condom only.

Methods

We conducted a cross-sectional survey to determine how commonly women and men who have sex with men (MSM) attending Melbourne Sexual Health Centre had experienced stealthing, and analysed situational factors associated with the event. Responses were linked to demographic information extracted from patient files.

Results

1189 of 2883 women (41.2%), and 1063 of 3439 MSM (30.9%) attending the clinic during the study period completed the survey. Thirty-two percent of women (95% CI: 29%,35%) and 19% of MSM (95% CI: 17%,22%) reported having ever experienced stealthing. Women who had been stealthed were more likely to be a current sex worker (Adjusted Odds Ratio [AOR] 2.87, 95% CI: 2.01,4.11, p <0.001). MSM who had experienced stealthing were more likely to report anxiety or depression (AOR 2.13, 95% CI: 1.25,3.60, p = 0.005). Both female and male participants who had experienced stealthing were three times less likely to consider it to be sexual assault than participants who had not experienced it (OR 0.29, 95% CI: 0.22,0.4 and OR 0.31, 95% CI: 0.21,0.45 respectively).

Conclusions

A high proportion of women and MSM attending a sexual health service reported having experienced stealthing. While further investigation is needed into the prevalence of stealthing in the general community, clinicians should be aware of this practice and consider

The database containing the questionnaires and the code linking these to your name will remain at Melbourne Sexual Health Centre, on password-protected servers, and only the study team will have access. Your information will only be used for this research project, or future research at this centre, and will not be disclosed except as required by law. The results of this research will be published and presented at conferences in such a way that you are not identified." Therefore we have presented all data collected in an aggregate way in the paper so that it is non-identifiable, as approved by our ethics committee. Data is required to be securely stored in keeping with requirements from Alfred Hospital Ethics Committee who can be contacted through Angela Henjak at research@alfred.org.au for queries regarding accessing the data. Any questions regarding the data itself should be directed to Rosie Latimer at rlatimer@mshc.org.au or A/Professor Catriona Bradshaw at cbradshaw@mshc.org.au.

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integrating this question into their sexual health consultation. Understanding situational factors would assist in the development of preventive strategies, particularly female sex workers and MSM.

Introduction

Non-consensual removal of condoms, colloquially referred to as 'stealthing'[1] or 'stealth-breeding' [2], refers to the practice of a sexual partner covertly removing a condom, when consent has been given for condom protected sex only [1]. Condoms are used as a primary preventative method of protecting against sexually transmitted infections (STI), human immunodeficiency virus (HIV) and pregnancy, being 80 to 98.6% effective [3–5]. Stealthing may result in the transmission of STIs, HIV, or unintended pregnancy, and could have significant personal and public health implications.

Studies of undergraduate students have found consent for sexual intercourse to be mostly communicated through non-verbal means [6, 7], with consent for sexual intercourse often implied in the process of asking for or applying a condom [6]. Brodsky has argued that condom removal without mutual agreement violates consent to sex [1].

In young adult heterosexual relations, it is common for male partners to engage in condom resistance tactics [8]. Several studies have identified stealthing as a method of birth control sabotage [9, 10], as well as a means of intentional HIV transmission [11]. Anecdotal research by Brodsky focusing on heterosexual and heteronormative relations, and theoretical research by Brennan focusing on condom-less sex between men, argue these are not the primary motivators for this act [1, 2].

In spite of public interest in stealthing, there are no scientific articles that investigate how common it is, who is most at risk, and the outcomes for those who report being stealthed. We aimed to investigate the proportion of sexual health centre patients reporting nonconsensual removal of condoms: 1) among heterosexual women and 2) among men who have sex with men, as well as associated risk factors. For the purpose of this study, 'stealthing' was defined as condom removal without consent, where consent to sex was conditional upon use of a condom.

Methods

Population and setting

This was a cross-sectional questionnaire-based study conducted amongst women and gay and other men who have sex with men (MSM) attending the Melbourne Sexual Health Centre (MSHC) in Victoria, Australia, between the 22nd December 2017 and the 22nd February 2018. MSHC is the largest public sexual health service in Victoria, Australia. The centre provides around 50,000 consultations every year, 37% with women and 36% with MSM [12]. Clinic attendees routinely complete a computer assisted self-interview (CASI) about their sexual history prior to seeing a triage nurse.

Study measurement

Women and MSM presenting to MSHC, aged 18 or over, were invited to complete an electronic questionnaire containing questions about stealthing after completing CASI. Participants read a patient information and consent form which detailed the nature of the survey, and

patients could only commence the questionnaire after ticking a box stating 'Yes- willing to help'. Due to the potential of the questionnaire to cause distress when recalling the stealthing event, the participant information included advertisement of free counselling services available at MSHC and elsewhere. The Alfred Hospital Ethics Committee approved the study (number 494/17).

Age, number of sexual partners, and HIV status were extracted electronically from routinely collected clinic records for respondents and non-respondents, de-identified for non-respondents, and linked to questionnaire responses for respondents (Fig 1).

The questionnaire asked whether the participant had ever had a condom removed during sex with or without permission and at what point the participant noticed. Participants could choose from a hierarchy of seven responses describing the circumstances. Multiple responses were allowed for those reporting multiple occurrences, and there was no time limit applied to the reported event. Participants were deemed not to have experienced stealthing if they responded either: 1) they had never had a condom removed during sex, 2) that a condom had been removed with permission, or 3) that a condom was removed without permission but they willingly continued sex. Participants were deemed to have experienced stealthing if they reported: 4) condom removal without permission and sex continued unwillingly, 5) condom removal without permission and sex was discontinued, 6) condom removal during sex but they did not realise until afterwards, or 7) the condom was never put on despite being requested. If a participant only selected options between 1 and 3 they were classified as never having been stealthed. If a participant selected any option between 4–7, regardless of whether they had also selected options between 1 and 3, they were classified as ever having been stealthed (Fig 1).

Participants who reported stealthing were asked further questions about the specific event (Fig 1). Participants who had selected multiple options were asked about the incident with the highest assigned number. For instance if they reported several stealthing events with differing scenarios and selected both response 4 and 5, then specific questions were asked about "event 5" only–i.e. condom removal without permission and sex was discontinued. Questions included: when the incident occurred, how long they had known the partner, how they would describe the relationship, where they had met, whether either person had been using drugs or alcohol, whether the event was reported to the police, and what they perceived were the consequences of the condom removal. All respondents were asked whether they considered the removal of a condom without consent to be sexual assault.

Statistical analysis

All analyses were performed using Stata IC version 14. MSM who reported only insertive anal sex and no receptive anal sex while completing CASI were excluded from the dataset prior to analysis of questionnaire responses, as experiencing stealthing was considered unlikely if the male was only the insertive partner. Risk factors for experiencing stealthing in women and MSM were not compared to each other as they are different populations. Univariable and multivariate analyses were performed to determine the differences in demographics between non-respondents and respondents, and the differences between those who had and had not experienced stealthing. Variables were included in multivariate models if the p-value was ≤ 0.1; if correlated, the variable most strongly associated with the outcome was used. Models were built in a backward-stepwise fashion, using the likelihood ratio test to determine the significance of the contribution of each variable. Ninety-five percent binomial confidence intervals (CIs) were calculated for all proportions. We assumed 100 patients would complete the survey each week and estimated 2% would report ever being stealthed. The 95% confidence interval around an estimated 2% prevalence of stealthing after six weeks (600 responses) would be 1.0%, 3.5%.

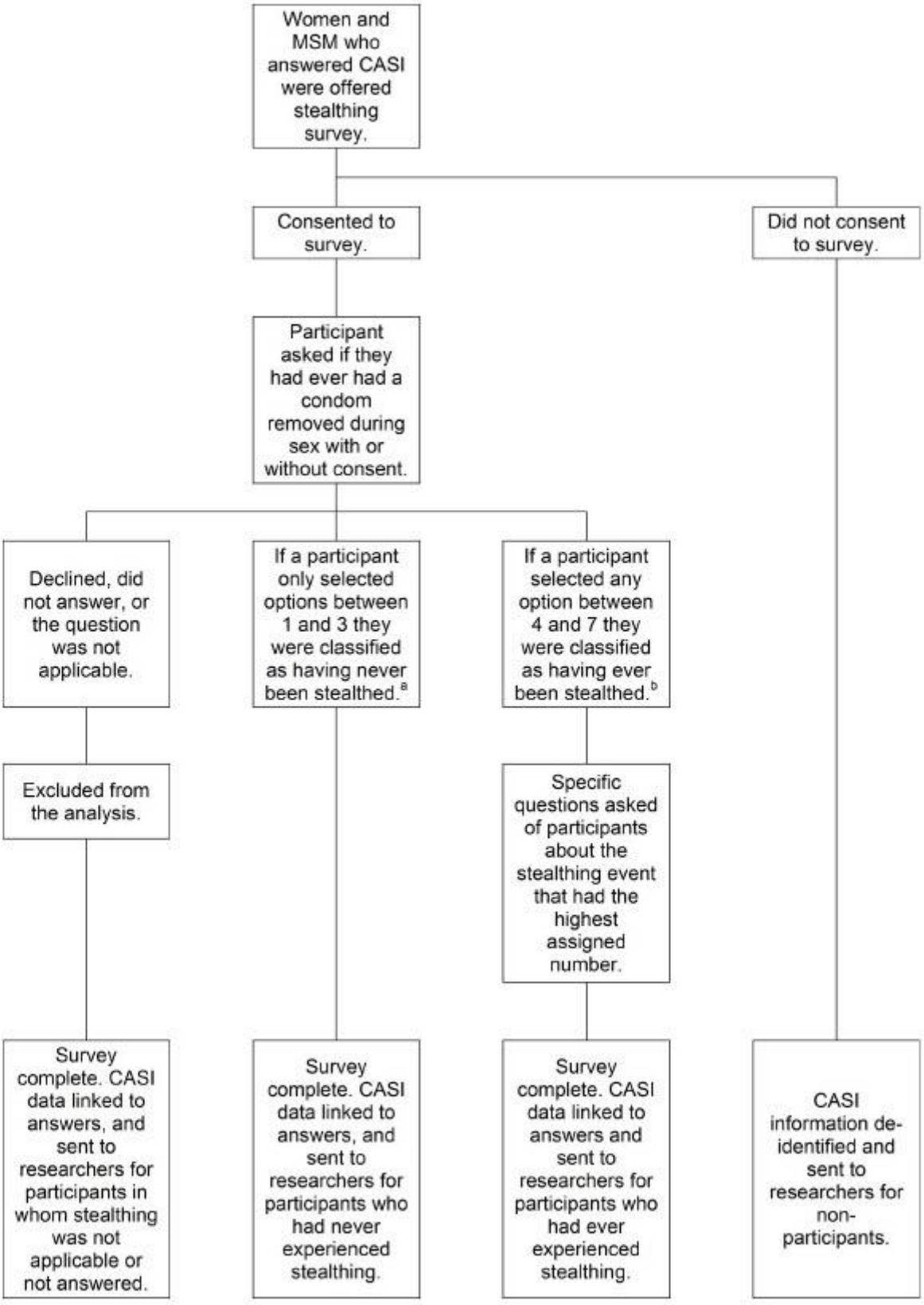
Women and MSM who answered CASI were offered stealthing survey.
Consented to survey.
Did not consent to survey.
Participant asked if they had ever had a condom removed during sex with or without consent.
Declined, did not answer, or the question was not applicable.
If a participant only selected options between 1 and 3 they were classified as having never been stealthed.[a]
If a participant selected any option between 4 and 7 they were classified as having ever been stealthed.[b]
Excluded from the analysis.
Specific questions asked of participants about the stealthing event that had the highest assigned number.
Survey complete. CASI data linked to answers, and sent to researchers for participants in whom stealthing was not applicable or not answered.
Survey complete. CASI data linked to answers, and sent to researchers for participants who had never experienced stealthing.
Survey complete. CASI data linked to answers and sent to researchers for participants who had ever experienced stealthing.
CASI information de-identified and sent to researchers for non-participants.

Fig 1. Possible pathways for patients offered the survey, and the classification for analysis of nonconsensual condom removal. Abbreviations: MSM = men who have sex with men; CASI = computer assisted self-interviewing. [a]Participants were classified as never having experienced stealthing if they responded either: 1) they had never had a condom removed during sex, 2) that a condom had been removed with permission, or 3) that a condom was removed without permission but they willingly continued sex. [b]Participants were deemed to have experienced stealthing if they reported: 4) condom removal without permission and sex continued unwillingly, 5) condom removal without permission and sex was discontinued, 6) condom removal during sex but they did not realise until afterwards, or 7) the condom was never put on despite being requested.

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Results

During the study period, 2883 women and 3439 MSM attended the clinic, of whom 1189 women (41%, 95%CI: 39%,43%) and 1063 MSM (31%, 95%CI: 29%,32%) completed the survey (classified as respondents).

Female respondents were more likely than non-respondents to have had sex overseas in the last twelve months (adjusted odds ratio [AOR] 1.49, 95% CI: 1.26,1.77, $p<0.001$) and were less likely to be a current sex worker (AOR 0.78, 95% CI: 0.63,0.96, $p = 0.02$) (Table 1). Compared to MSM non-respondents, the men who responded were more likely to have had sex overseas in the last twelve months (AOR 1.70, 95% CI: 1.37,2.11, $p<0.001$), and were less likely to be HIV positive (AOR 0.60, 95% CI: 0.38,0.95, $p = 0.029$) (Table 1).

Of the 1189 women and 1063 MSM who consented to the survey and answered the first question: 60 (5%) women and 64 men (6%) declined to answer whether they had experienced stealthing, 45 (4%) women and 37 (3%) men deemed the question to be not applicable to them i.e. they never used condoms, or did not engage in penetrative sex with men and 90 (8%) men were removed from the analysis, as they had only reported insertive anal sex and not reported receptive anal sex in CASI (Table 2).

Three hundred and forty-six of the remaining 1084 women (32%, 95% CI: 29%,35%) and 168 of the remaining 872 MSM (19%, 95% CI: 17%,22%) reported having ever experienced stealthing (Table 3). Of those who had experienced stealthing, forty-two women (12%, 95% CI: 9%,16%) and 23 MSM (14%, 95% CI: 9%,20%) presented to the clinic on the day of the questionnaire following a reported stealthing incident (Table 4).

Data missing from up to 5% of female respondents and up to 3% of male respondents; proportions are calculated using available data.

On multivariate analysis, women who had been stealthed were more likely to be a current sex worker than those who had never experienced stealthing (AOR 2.87, 95% CI: 2.01,4.11, $p<0.001$) (Table 3), and MSM who had been stealthed were more likely to report 'health issues, such as anxiety or depression which may have affected their decision to use condoms for anal sex' than those who had never experienced stealthing (AOR 2.13, 95% CI: 1.25,3.60, $p = 0.005$) (Table 3).

Most women met the male partner who had stealthed them through friends (29%, 95% CI: 24%,34%) or sex work (23%, 95% CI: 19%,28%). MSM reporting stealthing most commonly described the partner as someone they "did not know well" (61%) and had predominantly met them through geosocial dating applications or online (67%, 95% CI: 59%,74%) (Table 4).

At the time of the stealthing incident, 41% (95% CI: 36%,47%) of women and 54% (95% CI: 46%,62%) of MSM reported being sober, while 57% (95% CI: 51%,62%) of women and 41% (95% CI: 33%,49%) of MSM had consumed alcohol. Twelve percent of women and 13% of MSM had used other drugs either in addition to or without alcohol (Table 4). The majority of women reported their partner had consumed alcohol (68%, 95% CI: 62%,73%) and/or other drugs (19%), with only 27% (95% CI: 22%,33%) stating the partner had been sober when the incident occurred. Many MSM believed their partner to be sober (53%, 95% CI: 44%,62%), with 40% (95% CI: 31%,50%) of partners under the influence of alcohol, and 12% using additional/or other drugs (Table 4).

Table 1. Demographics and epidemiological features of respondents versus non-respondents to survey on rates of non-consensual removal of condoms (stealthing) in a STI clinic (N = 6322).

		Female non-respondents n = 1694 (%; 95% CI) or median [range]		Female respondents n = 1189 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio[a] (95% CI)		p-value
Age		27	[16–74]	26	[18–64]						
Employment											
	Employed	958	(60; 57,62)	689	(60; 57,62)	1					
	Not in the labour force[b]	641	(40; 38,43)	467	(40; 38,43)	1.01	(0.87,1.18)	0.87			
Aboriginal and/or Torres Strait Islander peoples											
	No	1479	(99; 98,99)	1074	(99; 98,99)	1					
	Yes	16	(1; 0,2)	14	(1; 1,2)	1.20	(0.59,2.48)	0.613			
Sex overseas											
	No	817	(60; 57,62)	485	(48; 45,52)	1			1		
	Yes	552	(40; 38,43)	517	(52; 48,55)	1.58	(1.35,1.86)	<0.001	1.49	(1.26,1.77)	<0.001
Injecting drug use											
	Never injected	1420	(98; 97,99)	1023	(98; 97,99)	1					
	Ever injected	26	(2; 1,3)	22	(2; 1,3)	1.17	(0.66,2.08)	0.582			
Current sex worker											
	No	1095	(76; 74,78)	856	(82; 79,84)	1			1		
	Yes	348	(24; 22,26)	191	(18; 16,21)	0.70	(0.58,0.86)	<0.001	0.78	(0.63,0.96)	0.020
Condom Use in the last 3mo with male partners											
	Not always	1014	(83; 81,85)	765	(82; 80,85)	1					
	Always	204	(17; 15,19)	163	(18; 15,20)	1.06	(0.84,1.33)	0.619			
Number of male sexual partners in the last 3mo		1	[0–50]	1	[0–15]						
		Male non-respondents n = 2376 (%; 95% CI) or median [range]		Male respondents n = 1063 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio[c] (95% CI)		p-value
Age		30	[16–82]	30	[18–75]						
Employment											
	Employed	1480	(67; 65,69)	644	(64; 61,67)	1					
	Not in the labour force[b]	742	(33; 31,35)	361	(36; 33,39)	1.12	(0.96,1.31)	0.161			
Aboriginal and/or Torres Strait Islander peoples											
	No	2114	(99; 98,99)	978	(99; 99,100)	1			1		
	Yes	26	(1; 1,2)	5	(1; 0,1)	0.42	(0.16,1.09)	0.073	0.64	(0.21,1.97)	0.441
Sex overseas											
	No	1365	(70; 69,72)	542	(61; 58,64)	1			1		
	Yes	587	(30; 28,32)	345	(39; 36,42)	1.48	(1.25,1.75)	<0.001	1.70	(1.37,2.11)	<0.001
Injecting drug use											
	Never injected	2048	(96; 96,97)	914	(97; 96,98)	1					
	Ever injected	75	(4; 3,4)	28	(3; 2,4)	0.84	(0.55,1.3)	0.428			
Current sex worker											
	No	2126	(>99; 99,100)	933	(99; 98,99)	1			1		
	Yes	9	(<1; 0,1)	10	(1; 1,2)	2.53	(1.03,6.25)	0.044	2.72	(0.97,7.59)	0.057
Condom Use in the last 3mo with male partners											
	Not always	1379	(74; 72,76)	616	(71; 68,74)	1					
	Always	492	(26; 24,29)	246	(29; 26,32)	1.12	(0.93,1.34)	0.220			

(*Continued*)

Table 1. (Continued)

HIV status											
	Negative	1279	(91; 90,93)	558	(95; 92,96)	1			1		
	Positive	119	(9; 7,10)	32	(5; 4,8)	0.62	(0.41,0.92)	0.019	0.61	(0.38,0.97)	0.038
Use of prep											
	No	1844	(81; 79,82)	861	(83; 81,85)	1					
	Yes	436	(19; 18,21)	174	(17; 15,19)	0.85	(0.70,1.04)	0.112			
Number of male sexual partners in the last 3mo		3	[0–100]	3	[0–140]						

Abbreviations: n = number; CI = confidence interval; mo = months; HIV = human immunodeficiency virus; PrEP = HIV pre-exposure prophylaxis

[a]Adjusted model for females includes: sex overseas and current sex worker

[b]Not in the labour force includes both those who are unemployed and/or students

[c]Adjusted model for males includes: Aboriginal and/or Torres Strait Islander peoples, sex overseas, current sex worker and HIV status.

Data missing for: <5% of PrEP data; <5%-10% of employment data; 5–15% of Aboriginal and/or Torres Strait Islander peoples data; 10–15% of current sexworker data; 10%-20% of sex overseas data and injecting drug use data; 15- ≥20% of condom use data; and >20% of HIV data. Proportions are calculated using available data.

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The majority of women (61%) and MSM (55%) discussed the removal of the condom with their partners after the event. Over half of the participants reported being emotionally stressed following the incident. Eight percent of women and five percent of MSM reported they thought they had acquired an STI following the event. One percent of women and two percent of MSM believed they had acquired HIV as a consequence of being stealthed (Table 4). Only 1% of people stealthed reported this experience to the police (Table 4).

Table 2. Reported events of non-consensual removal of condoms (stealthing) amongst patients presenting to a STI clinic (N = 2252)[a].

		Female respondents n = 1189 (%; 95% CI)		Male respondents n = 1063 (%; 95% CI)	
Classified as not experiencing 'stealthing'					
	Never stealthed	420	(35; 33,38)	496	(47; 44,50)
	Condom removed w permission	455	(38; 35,41)	315	(30; 27,32)
	Condom removed w/o permission but continued willingly	104	(9; 7,10)	77	(7; 6,9)
Classified as experiencing 'stealthing'					
	Condom removed w/o permission, and continued unwillingly	108	(9; 8,11)	52	(5; 4,6)
	Condom removed w/o permission, and stopped	135	(11; 10,13)	65	(6; 5,8)
	Condom removed w/o permission, but didn't realise until afterwards	147	(12; 11,14)	60	(6; 4,7)
	Condom never put on but had been requested	84	(7; 6,9)	41	(4; 3,5)
Removed from further analysis					
	Not applicable[b]	45	(4; 3,5)	127	(12; 10,14)
	Decline answer	*60*	*(5; 4,6)*	*64*	*(6; 5,8)*

Abbreviations: n = number; CI = confidence interval; w = with; w/o = without.

[a]Patients could select multiple options, to report multiple events occurring, i.e. events are not mutually exclusive, therefore percentages do not sum to 100. Percentages represent the proportion of participants who have reported the event. If reporting multiple events, patients were classified in the analysis based off the highest numbered event they reported, if 1 is Never and 7 is 'Condom never put on even though requested'.

[b]Not applicable refers to patients who have not/do not engaged in penetrative penile sex, includes 97 MSM who responded to survey but reported no receptive anal sex and 30 who selected not applicable.

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Table 3. Risk factors associated with non-consensual removal of condoms (stealthing) in patients presenting to a STI clinic (N = 2042).

		Womenwho have not had been stealthed n = 738 (%; 95% CI) or median [range]		Women who have have been stealthed n = 346 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio (95% CI)[a]		p-value
Age		26	[18–58]	26	[18–55]						
Number of male sexual partners in the last 3mo		2	[0–15]	1	[0–15]						
Employment[b]											
	Employed	439	(61; 57,65)	189	(56; 51,62)	1					
	Not in the labour force	281	(39; 35,43)	146	(44; 38,49)	1.21	(0.93,1.57)	0.161			
Education level											
	Did not complete high school	18	(2; 1,4)	13	(4; 2,6)	1					
	High school/Certificate/Diploma	238	(33; 29,36)	134	(39; 34,45)	0.78	(0.37,1.64)	0.512			
	University degree	475	(65; 61,68)	195	(57; 52,62)	0.57	(0.35,1.47)	0.131			
Aboriginal and/or Torres Strait Islander peoples											
	No	672	(99; 98,99)	319	(98; 96,99)	1					
	Yes	8	(1; 1,2)	5	(2; 1,4)	1.31	(0.43,4.06)	0.632			
Australian/New Zealander											
	No	441	(63; 59,66)	166	(51; 45,56)	1					
	Yes	264	(37; 34,41)	160	(49; 44,55)	1.61	(1.23,2.09)	<0.001	1.26	(0.94,1.70)	0.122
Current sex worker											
	No	573	(87; 85,90)	215	(71; 65,76)	1					
	Yes	83	(13; 10,15)	89	(29; 24,35)	2.86	(2.04,4.01)	<0.001	2.87	(2.01,4.11)	<0.001
Injecting drug use											
	Never injected	644	(98; 97,99)	295	(97; 95,99)	1					
	Ever injected	11	(2; 1,3)	8	(3; 1,5)	1.59	(0.63,3.99)	0.325			
Sex overseas											
	No	303	(47; 44,51)	136	(47; 41,53)	1					
	Yes	335	(53; 49,56)	153	(53; 47,59)	1.02	(0.77,1.34)	0.903			
Use other contraceptives in addition to condoms[c]											
	No	293	(46; 42,50)	112	(47; 40,53)	1					
	Yes	339	(54; 50,58)	128	(53; 47,60)	0.94	(0.733,1.33)	0.936			
		MSM who have not been stealthed n = 704 (%; 95% CI) or median [range]		MSM who have been stealthed n = 168 (%, 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio (95% CI)[d]		p-value
Age		30	[18–75]	29	[18–58]						
Number of male sexual partners in the last 3mo		3	[0–140]	3	[0–100]						
Employment											
	Employed	435	(65; 61,69)	98	(61; 53,68)	1					
	Not in the labour force	232	(35; 31,39)	63	(39; 32,47)	1.20	(0.85,1.72)	0.302			
Education level											
	Did not complete high school	24	(3; 2,5)	7	(4; 2,8)	1					
	High school/Certificate/Diploma	183	(26; 23,30)	34	(20; 14,27)	0.64	(0.25,1.60)	0.336			
	University degree	494	(70; 67,74)	127	(76; 68,82)	0.88	(0.37,2.09)	0.775			
Aboriginal and/or Torres Strait Islander peoples											
	No	701	(100; 99,100)	166	(99; 97,100)	1					
	Yes	2	(0; 0,1)	1	(1; 0,3)	2.11	(0.19,23.42)	0.543			

(*Continued*)

Table 3. (Continued)

		Womenwho have not had been stealthed n = 738 (%; 95% CI) or median [range]		Women who have have been stealthed n = 346 (%; 95% CI) or median [range]		Unadjusted Odds Ratio (95% CI)		p-value	Adjusted Odds Ratio (95% CI)[a]		p-value
Australian/New Zealander											
	No	343	(50; 46,54)	83	(49; 42,57)	1					
	Yes	342	(50; 46,54)	85	(51; 43,58)	1.03	(0.73,1.44)	0.877			
Current sex worker											
	No	622	(99; 98,100)	151	(99; 95,100)	1					
	Yes	5	(1; 0,2)	2	(1; 0,5)	1.65	(0.32,8.57)	0.553			
Injecting drug use											
	Never injected	611	(97; 96,98)	145	(96; 92,99)	1					
	Ever injected	18	(3; 2,4)	6	(4; 1,8)	1.4	(0.55,3.60)	0.480			
Sex overseas											
	No	354	(60; 56,64)	82	(57; 49,66)	1					
	Yes	237	(40; 36,44)	61	(43; 34,51)	1.11	(0.77,1.61)	0.577			
HIV status											
	No	375	(95; 93,97)	96	(2; 85,97)	1					
	Yes	19	(5; 3,7)	8	(8; 3,15)	1.64	(0.70,3.87)	0.255			
Use of prep											
	No	582	(84; 81,87)	126	(78; 71,84)	1			1		
	Yes	110	(16; 13,19)	35	(22; 16,29)	1.47	(0.96,2.25)	0.077	1.16	(0.70,1.92)	0.567
Drugs use with anal sex w/o a condom in the last 12mo[e]											
	No	222	(58; 53,63)	58	(55; 45,64)	1					
	Yes	162	(42; 37,47)	48	(45; 36,55)	1.13	(0.74,1.75)	0.569			
Drunk during anal sex w/o a condom in the last 12mo[e]											
	No	219	(57; 52,62)	53	(50; 41,60)	1					
	Yes	166	(43; 38,48)	52	(50; 40,59)	1.29	(0.84,1.99)	0.242			
Anal sex w/o a condom with known HIV positive in the last 12mo[e]											
	No	319	(83; 79,87)	83	(82; 73,89)	1					
	Yes	65	(17; 13,21)	18	(18; 11,27)	1.06	(0.60,1.89)	0.832			
Anal sex w/o a condom with someone of unknown HIV status in the last 12mo[e]											
	No	189	(50; 45,55)	39	(38; 29,48)	1			1		
	Yes	190	(50; 45,55)	63	(62; 52,71)	1.61	(1.03,2.51)	0.038	1.51	(0.96,2.39)	0.075
Self-reported health issues, such as anxiety or depression, which may have affected your decision to use condoms for anal sex?[e]											
	No	318	(85; 81,89)	74	(73; 63,81)	1			1		
	Yes	55	(15; 11,19)	28	(27; 19,37)	2.19	(1.30,3.68)	0.003	2.13	(1.25,3.6)	0.005

Abbreviations: n = number; CI = confidence interval; mo = months; MSM = men who have sex with men; HIV = human immunodeficiency virus; PrEP = HIV pre-exposure prophylaxis; w/o = without

[a]Adjusted model for females includes: Australian and current sex worker

[b]Not in the labour force includes both those who are unemployed and/or students

[c]Women who reported not using contraception due to pregnancy were excluded (2 females who did not have condoms removed, and 10 who did).

[d]Adjusted model for males includes: use of prep, condom use with someone of uncertain HIV status, health issues (anxiety & depression) affecting decisions to use condoms.

[e]These questions were asked only to patients who had reported unprotected anal sex since their last HIV test as part of their routine computer assisted self-interviewing (CASI).

Data missing for: <5% of employment data, education data and PrEP data; <5%-10% of Aboriginal and/or Torres Strait Islander peoples data and Australian data; 10%-15% of injecting drug use data and current sex worker data, 10%-20% sex overseas data; 10- ≥20% contraception data; and ≥20% of HIV status and questions on issues affecting decisions to use condoms. Proportions are calculated using available data.

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Table 4. Situational factors surrounding non-consensual removal of condoms (stealthing) reported by patients presenting to a STI clinic (N = 523).

		Women n = 346 (%; 95% CI)		MSM n = 168 (%; 95% CI)	
When the incident occurred					
	Here today for this reason	42	(12; 9,16)	23	(14; 9,20)
	In the last 3mo	59	(17; 13,22)	20	(12; 7,18)
	3–12 mo ago	78	(23; 18,28)	35	(21; 15,28)
	More than 12 months ago	120	(35; 30,40)	78	(46; 39,54)
	More than 1 occasion	43	(13; 9,17)	12	(7; 4,12)
Relationship					
	Did not know him well	101	(30; 25,36)	102	(61; 54,69)
	Friend	33	(10; 7,14)	10	(6; 3,11)
	Friend with benefits/ Sex buddy	51	(15; 12,20)	30	(18;13,25)
	Casually dating	54	(16; 12,21)	22	(13; 8,19)
	Relationship	25	(8; 5,11)	2	(1; 0,4)
	Client (of sex worker)	69	(21; 16,25)	0	(0; 0,2)[a]
Relationship duration					
	Less than a day (<24hrs)	126	(38; 33,44)	85	(52; 44,59)
	One day to one month	95	(29; 24,34)	39	(24; 17,31)
	More than one month	107	(33; 28,28)	41	(25; 18,32)
Met through					
	Smartphone dating app/Internet	64	(20; 15,24)	110	(67; 59,74)
	(Gay) bar or party	50	(15; 12,20)	20	(12; 8,18)
	Gay sauna, beats of SOPV, sex party	2	(1; 0,2)	24	(15; 10,21)
	Friend, or friend of friend	94	(29; 24,34)	6	(4; 1,8)
	Co-workers	22	(7; 4,10)	3	(2; 0,5)
	Sex work	76	(23; 19,28)	0	(0; 0,2)[a]
	Travel	15	(5; 3,7)	0	(0; 0,2)[a]
	Other (café, park etc.)	4	(1; 0,3)	1	(1; 0,3)
Drugs used by partner[bc]					
	None	75	(27; 22,33)	63	(53; 44,62)
	Alcohol	188	(68; 62,73)	48	(40; 31,50)
	Cannabis/marijuana/hash	28	(10; 7,14)	4	(3; 1,8)
	Ecstasy	12	(4; 2,7)	4	(3; 1,8)
	Speed/ice/meth	5	(2; 1,4)	6	(5; 2,11)
	GHB	2	(1; 0,3)	3	(2; 1,7)
	Cocaine	10	(4; 2,7)	3	(2; 1,7)
	Heroin	1	(<1; 0,2)	0	(0; 0,3)[a]
	Other	1	(<1; 0,2)	3	(2; 1,7)
Drugs used by respondent[bd]					
	None	135	(41; 36,47)	87	(54; 46,62)
	Alcohol	186	(57; 51,62)	65	(41; 33,49)
	Cannabis/marijuana/hash	21	(6; 4,10)	3	(2; 0,5)
	Ecstasy	9	(3; 1,5)	4	(3; 1,6)
	Speed/ice/meth	5	(2; 0,4)	8	(5; 2,9)
	GHB	2	(1; 0,2)	3	(2; 0,5)
	Cocaine	8	(2; 1,5)	4	(3; 1,6)
	Heroin	1	(<1; 0,2)	0	(0; 0,2)[a]
	Other	0	(0; 0,1)[a]	4	(3; 1,6)

(*Continued*)

Table 4. (Continued)

		Women n = 346 (%; 95% CI)		MSM n = 168 (%; 95% CI)	
Condom removal discussed with partner					
	No	128	(39; 33,44)	74	(45; 37,52)
	Yes	204	(61; 56,67)	92	(55; 48,63)
Consequences of condom removal[b]					
	None	85	(25; 21,30)	62	(38; 30,46)
	Emotional stress	190	(56; 51,62)	86	(52; 45,60)
	Caught an STI	26	(8; 5,11)	9	(5; 3,10)
	Contracted HIV	2	(1; 0,2)	3	(2; 0,5)
	Fight	49	(14; 11,19)	15	(9; 5,15)
	Relationship broke up	30	(9; 6,12)	6	(4; 1,8)
	Other	42	(12; 9,16)	12	(7; 4,12)
Reported to the police					
	No	336	(99; 97,100)	163	(98; 95,100)
	Yes	3	(1; 0,3)	3	(2; 0,5)

Abbreviations: n = number; CI = confidence interval; MSM = men who have sex with men; mo = months; SOPV = sex on premises venue; GHB = Gamma-hydroxybutyrate; STI = sexually transmitted infection; HIV = human immunodeficiency virus

[a]one-sided, 97.5% confidence interval

[b]Patients could select multiple options, to report multiple events occurring, i.e. events are not mutually exclusive, therefore percentages do not sum to 100. Percentages represent the proportion of participants who have reported the event.

[c]64 women (19%) and 47 MSM (28%) were unsure as to whether or not their partner had used any alcohol and/or other drugs and were removed from the analysis.

[d]11 women (3%) and 6 MSM (4%) were unsure as to whether or not they had used any alcohol and/or other drugs and were removed from the analysis.

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Both female and MSM participants who had experienced stealthing were less likely to consider it to be sexual assault than participants who had not experienced stealthing. Amongst women, 62% (95% CI: 56%,67%) of those stealthed considered it to be assault, compared to 85% (95% CI: 82%,87%) of those not stealthed (OR 0.29, 95%CI: 0.22,0.4, $p<0.001$). Amongst men, 61% (95% CI: 53%,69%) of those stealthed considered it to be assault versus 84% (95% CI: 81%,86%) of those not stealthed (OR 0.31, 95%CI: 0.21,0.45, $p<0.001$).

Discussion

Although increasingly discussed in international media, there is little scientific research on non-consensual removal of condoms, popularly termed 'stealthing'. To our knowledge this is the first study investigating how common stealthing is, the context in which it occurred, the impact on individuals, and how those stealthed perceive the event. A surprising proportion of clients attending a sexual health centre in Melbourne (32% of women and 19% of MSM) reported removal of a condom in a situation where they would not have willingly engaged in sexual intercourse without one—in other words, a violation of their consent [1].

These data need to be interpreted in the context of a STI clinic population which is generally a higher risk group than the general population. Our data show that 4% of women and 3% of MSM presenting to our clinic during the study period were attending following a stealthing incident. This equates to over 1200 consultations per year [12]. These data suggest that stealthing is common and should be considered when assessing patients in STI services.

Female respondents were less likely to be a current sex worker and MSM respondents were less likely to be HIV positive, compared to non-respondents. It is possible that both sex workers and HIV positive men were less likely to complete the survey due to privacy concerns,

especially with regards to condom use and their legal obligations, which vary state by state in Australia. In Victoria, sex workers are legally required to use condoms with clients[13], and while those who are HIV positive are not legally required to disclose their HIV status, they must take reasonable precautions to prevent HIV transmission to those they are engaging in penetrative sex with[14]. Reasonable precaution refers to correct use of condoms and lube during intercourse. While female sex workers were less represented in respondents than non-respondents, 18% of participants were sex workers and we still observed an association between being a sex worker and being more likely to be stealthed. Low numbers of HIV positive men participating may have limited our ability to examine any association between stealthing and HIV status. Lastly, both women and MSM who had been overseas recently were more likely to respond to our survey. This may bias our findings towards individuals who may have participated due to recent high risk sexual encounters, in the context of overseas travel [15].

Women who experienced stealthing were three times more likely to be sex workers compared to those who had not. In the Law and Sex Worker Health (LASH) Survey conducted in Australia, 8% of respondents reported assault by clients [16]. However the LASH survey did not compare rates of assault to the general population or differentiate between physical and sexual assault, and only examined assault in the workplace. Perkins' (1991) research with Sydney-based brothel workers found that 20% of sex workers experienced rape while working. Outside the workplace sex workers experienced higher levels of sexual assault compared with non-sex workers, with 46.9% reporting rape, compared to 21.9% of health workers and 12.7% of students [17]. Our data are consistent with these findings that sex workers are at increased risk of non-consensual sex acts.

Sixty-seven percent of MSM who had experienced stealthing met the partner via geosocial dating applications, for example Grindr, Tinder or Scruff. This is comparable to the number of MSM meeting partners through dating applications (70%) [18]. Sexual encounters initiated online are more likely to include unprotected anal intercourse [19], however it has also been found that meeting partners online increases the likelihood of discussion between partners of preferred sexual practices compared to meeting partners offline [19, 20]. MSM who had been stealthed were twice as likely to report having anxiety or depression. Depressive symptoms and anxiety are predictive of condom non-use [21] and higher levels of depression are related to lower levels of self-efficacy for sexual safety [22]. MSM who have anxiety or depression may be vulnerable to stealthing for this reason.

In this study, the majority of women (73%) believed the partner who had stealthed them to be under the influence of alcohol and/or other drugs. In heterosexual relations, the link between alcohol consumption and committal of sexual assault is well documented [23, 24]. Condom resistance tactics and sexual aggression with female partners are more commonly employed by men with history of sexual aggression and alcohol intoxication [25, 26]. Additionally, both alcohol consumption [27] and condom use [28, 29] have been associated with erectile dysfunction. Men with erection issues are more likely to engage in unprotected sex, misuse condoms [28, 29], and are more likely to remove condoms before sex is over ($p = 0.001$) [29]. Literature supports our finding that heterosexual men who have consumed alcohol may be at increased risk of committing nonconsensual sex acts, and may be removing the condom to maintain an erection.

Whilst the majority of those reporting stealthing considered it sexual assault, they were three times less likely to consider stealthing sexual assault than those who had never experienced it. The US National Crime Victimization Survey found 20% of female victim narratives contained excuses for offenders' behaviour, denials of injury, or justification of the incident as the victims' fault [30]. This allowed the women to avoid the distress of labelling themselves

victims of a crime, or their partners as criminals [30]. Victims of stealthing may also not yet view themselves as sexual assault victims as stealthing is a relatively new topic. Sexual assault is a term with many connotations and there are cultural myths as to who is a 'real' sexual victim [31], with the type of violence experienced influencing society's view as to whether a woman is a victim [32]. Our current language around sexual assault (and in this case, stealthing) may require expansion- until an act is named as assault it cannot be viewed as such, and cannot be reported or legislated against [33]. A limitation of this study is that we did not ask respondents why they did not consider stealthing to be sexual assault.

Stealthing has potentially serious consequences. The majority of patients reported consequences following the stealthing incident, with over half experiencing emotional stress. Although literature contains estimates as to the rate of STI and HIV transmission during sexual assault, it is difficult to establish if an STI has been acquired from a specific event. The Centers for Disease Control and Prevention (CDC) guidelines recommend testing all people for STIs following sexual assault [34], with the caveat that many positive tests will be from a pre-existing STI [35]. MSM patients with condom malfunction or condom-less sex presenting in a 72 hour window fulfil criteria for HIV Non-Occupational Post Exposure Prophylaxis (nPEP) [36, 37], and therefore MSM who present reporting non-consensual condom removal should be prescribed it.

This study has several limitations. Firstly, this study was offered in English only, which means it cannot be generalised to attendees who are not fluent in English. Secondly, this study may be subject to responder bias, as those who have experienced stealthing may have been more likely to answer the survey. Given this is a retrospective survey, participant responses may be subject to recall bias, and specific contextual situational factors and outcomes were asked about one event only for those stating it had happened on more than one occasion. While some participants within our study attributed the acquisition of STIs to being stealthed, this cannot be verified. According to attribution theory [38] following an adverse event people will make attributions to understand and control their environment[39], with situational factors often exaggerated when there is a negative outcome [40], and thus patients could be incorrectly attributing contracting a STI to the stealthing event.

Despite these limitations, this study has a large sample size with over two thousand responses. Accurate statistics describing the prevalence and incidence of sexual assault are difficult to obtain since the majority of assaults are not reported to authorities and victims often do not access services [31]. Only 1% of patients reporting stealthing in this study reported the event to the police. Although this study may be subject to recall bias, population surveys are the best means of learning the true extent and nature of these crimes, rather than relying on crime statistics. This is the first study to describe how commonly this practice is occurring.

In summary, stealthing was commonly experienced by our clinic population, with a third of women and a fifth of MSM reporting it, with situational contexts often involving alcohol and/or drugs in women, and geosocial networking applications in MSM. Sex work was a clear risk factor identified among women, and risk factors for MSM included anxiety and depression. Knowledge of these risk factors can enable services to ask about stealthing in target groups and offer specific support and counselling. Further community-based research would help determine the prevalence in the broader population and studies that link behavioural measures to biological outcomes would help to quantify the STI risk associated with this practice.

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E2. Patient Questionnaire on 'stealthing'

Extra Questions on Stealthing – Female Version

Slide 1. Introduction

We would like your help with some voluntary short questions (about 1 minute) to help us understand the rates of non-consensual condom removal during sex, the circumstances in which this is occurring, and the consequences of this action. If you do not want to answer the questions it will not influence your care with us today.

Your answers to this questionnaire will only be used for research. They will **not** appear in your medical record and will **not** be visible to the doctor or nurse you see today. Researchers will not be able to identify you by your answers to the questionnaire.

For more information please see the brochure: "Extra Questions". If you find this questionnaire distressing, please talk to staff for support or for referral to available services.

□ Yes – willing to help

□□No – prefer to stop

Slide 2.

a) What is your highest level of education?

Primary School

Year 11 or below

Year 12

Certificate III / IV

Advanced Diploma / Diploma

Bachelor Degree

Graduate Diploma / Graduate Certificate

Postgraduate Degree

These questions are about sex that you agreed to:

b) Has a male partner <u>ever</u> removed a condom while having sex with you?

Yes

No

Decline answer

Not applicable (no male partners, never use condom s)

c) Has a condom ever been removed <u>without</u> your consent?

Yes

No

Decline answer

Not applicable (no male partners, never use condom s)

d) When did this occur (you may select more than one answer):

I am here today for this reason

In the last 3 months

In the last 12 months

More than 12 months ago

This has happened on more than one occasion

This has never happened to me

Decline answer

Not applicable (no male partners, never use condoms)

Slide 3

a) Was your partner a:

Casual hook-up or sex off dating app

Casual sexual partner

Regular sexual partner

In a relationship with you

Decline answer

b) Had you used any alcohol or drugs when your partner did this?

No

Alcohol

Recreational drugs (marijuana, ecstasy, MDMA etc.)

Injecting drug use

Decline answer

c) Were there any consequences from this incident (you may select more than one answer)?

No

Emotional distress

Caught an STI

Unwanted pregnancy

Other- _____

Decline answer

d) Did you raise or discuss this with your partner at the time?

Yes

No

Decline answer

e) Did you bring this issue to the police or authorities?

Yes

No

Decline answer

f) Do you consider non-consensual condom removal to be sexual assault?

Yes

No

Not sure

Decline answer

Slide 4

Thank you for completing this questionnaire.

If this questionnaire caused you any distress, please discuss it with our staff members, who can arrange counselling or refer you to appropriate services.

- Sexual Assault Crisis Line (Victoria) 1800 806 292
- 1800 RESPECT (Australia) 1800 737 732

Extra Questions on Stealthing – MSM Version

Slide 1. Introduction

We would like your help with some voluntary short questions (about 1 minute) to help us understand the rates of non-consensual condom removal during sex, the circumstances in which this is occurring, and the consequences of this action. If you do not want to answer the questions it will not influence your care with us today.

Your answers to this questionnaire will only be used for research. They will **not** appear in your medical record and will **not** be visible to the doctor or nurse you see today. Researchers will not be able to identify you by your answers to the questionnaire.

For more information please see the brochure: "Extra Questions". If you find this questionnaire distressing, please talk to staff for support or for referral to available services.

☐ Yes – willing to help

□□No – prefer to stop

Slide 2.

a) What is your highest level of education?

Primary School

Year 11 or below

Year 12

Certificate III / IV

Advanced Diploma / Diploma

Bachelor Degree

Graduate Diploma / Graduate Certificate

Postgraduate Degree

These questions are about sex that you agreed to:

b) Has a male partner ever removed a condom while having sex with you?

Yes

No

Decline answer

Not applicable (no male partners, never use condom s)

c) Has a condom ever been removed without your consent?

Yes

No

Decline answer

Not applicable (no male partners, never use condom s)

d) When did this occur (you may select more than one answer):

I am here today for this reason

In the last 3 months

In the last 12 months

More than 12 months ago

This has happened on more than one occasion

This has never happened to me

Decline answer

Not applicable (no male partners, never use condoms)

Slide 3

a) Was your partner a:

Casual hook-up of sex off a dating app

Casual sexual partner

Regular sexual partner

In a relationship with you

Decline answer

b) Had you used any alcohol or drugs when your partner did this?

No

Alcohol

Recreational drugs (marijuana, ecstasy, MDMA etc.)

Injecting drug use

Both (alcohol and drugs)

Decline answer

c) Were there any consequences from this incident (you may select more than one answer)?

No

Emotional distress

Caught an STI

Other- _____

Decline answer

d) Did you raise or discuss this with your partner at the time?

Yes

No

Decline answer

e) Did you bring this issue to the police or authorities?

Yes

No

Decline answer

f) Do you consider non-consensual condom removal to be sexual assault?

Yes

No

Decline answer

Slide 4

Thank you for completing this questionnaire.

If this questionnaire caused you any distress, please discuss it with our staff members, who can arrange counselling or refer you to appropriate services.

- Sexual Assault Crisis Line (Victoria) 1800 806 292
- 1800 RESPECT (Australia) 1800 737 732