The contribution of *Mycoplasma genitalium* to the aetiology of sexually acquired infectious proctitis in men who have sex with men

M. Bissessor[1,2], S. N. Tabrizi[3,4], C. S. Bradshaw[1,5], C. K. Fairley[1,5], J. S. Hocking[2], S. M. Garland[3], J. Twin[4], M. Poljak[4], J. Peel[1] and M. Y. Chen[1,5]

1) *Melbourne Sexual Health Centre, Alfred Health,* 2) *Melbourne School of Population and Global Health, University of Melbourne, Melbourne,* 3) *Department of Obstetrics and Gynaecology, University of Melbourne,* 4) *Department of Microbiology, Infectious Diseases, The Royal Women's Hospital, Murdoch Children's Research Institute, Parkville and* 5) *Central Clinical School, Monash University, Melbourne, Vic., Australia*

Abstract

This study examined the contribution of *Mycoplasma genitalium* to sexually acquired infectious proctitis in men who have sex with men (MSM). MSM with symptomatic proctitis between May 2012 and August 2013 were tested for rectal sexually transmitted infections including chlamydia, gonorrhoea, herpes simplex virus (HSV) and *M. genitalium*. The load of rectal *M. genitalium* in men with symptomatic proctitis was compared with a separate group of men who had rectal *M. genitalium* but no symptoms of proctitis. Among 154 MSM with proctitis, rectal *M. genitalium* was detected in 18 men (12%, 95% CI 6.9–17.1) and was significantly more common among human immunodeficiency virus (HIV) -positive men (21%, 95% CI 9.5–32.6) than HIV-negative men (8%, 95% CI 2.9–13.1; prevalence ratio 3.2, 95% CI 1.2–8.8). Among HIV-positive men the detection of *M. genitalium* was comparable to that for chlamydia (21%, 95% CI 9.5–32.5), gonorrhoea (25%, 95% CI 16.2–41.8) and HSV (19%, 95% CI 7.9–30.1). Rectal *M. genitalium* load was significantly higher among the 18 men with symptomatic *M. genitalium*-associated proctitis than among a separate group of 18 men with asymptomatic rectal *M. genitalium* infection (60 000 copies of organism/swab versus 10 744 copies of organism/swab, p 0.023). Comprehensive testing for rectal pathogens in MSM with proctitis should include testing for *M. genitalium*.

Keywords: Human immunodeficiency virus, men who have sex with men, *Mycoplasma genitalium*, proctitis, sexually transmitted infections
Original Submission: 4 September 2015; **Revised Submission:** 5 November 2015; **Accepted:** 28 November 2015
Editor: S.J. Cutler
Article published online: 12 December 2015

Corresponding authors: M. Bissessor and M. Chen, 580 Swanston Street, Carlton, Vic 3053, Australia
E-mails: mbissessor@mshc.org.au **(M. Bissessor),** mchen@mshc.org.au **(M.Y. Chen)**

Introduction

Rectal infections among men who have sex with men (MSM) are caused by a range of sexually transmitted pathogens. Many of these rectal infections are asymptomatic whereas others present with symptoms of proctitis, typically rectal pain and/or discharge [1]. Rectal infections with chlamydia and gonorrhoea have been shown to increase the risk for human immunodeficiency virus (HIV) transmission between men, ostensibly from the attraction of HIV-susceptible cells to the rectal mucosa [2,3]. Guidelines recommend at least annual screening of MSM for rectal chlamydia and gonorrhoea [4,5].

Mycoplasma genitalium is a sexually transmitted pathogen that is a cause of urethritis in men and genital tract infection in women including pelvic inflammatory disease and associated female reproductive sequelae [6–8]. Several previous studies have shown prevalence rates of rectal *M. genitalium* infection in MSM ranging between 1.6% and 5.0% [9–13]. These previous studies consisted of men who were mainly asymptomatic and who had been screened for rectal *M. genitalium*, with only a small proportion of men with rectal symptoms. No previous

http://dx.doi.org/10.1016/j.cmi.2015.11.016

studies have sought to establish how common rectal *M. genitalium* infection is among MSM presenting with clinical proctitis.

To investigate whether *M. genitalium* is a cause of symptomatic proctitis, we conducted a prospective study of MSM presenting with symptomatic proctitis to a sexually transmitted infections (STI) clinic. The primary aim of the study was to determine the prevalence of rectal *M. genitalium* infection among MSM with proctitis and to compare these between HIV-positive and HIV-negative men. The secondary aim was to ascertain whether the load of *M. genitalium* was higher among men with symptomatic rectal *M. genitalium* infection compared with a separate group of men with asymptomatic rectal *M. genitalium*.

Methods

Study population and recruitment

This study was conducted at the Melbourne Sexual Health Centre, the main public STI clinic in Victoria, Australia. The clinic operated a walk-in STI clinic where MSM were screened for STIs according to national guidelines [5].

From May 2012 all MSM presenting to the STI and HIV clinics with symptomatic proctitis were tested for rectal *M. genitalium* infection in addition to other rectal pathogens: gonorrhoea, chlamydia and herpes simplex virus (HSV). The diagnosis of proctitis was a clinical one made by a specialist sexual health physician based on the presence of rectal pain, rectal discharge, or both of these. Because proctoscopy can be painful for patients with proctitis the procedure was not routinely performed and was undertaken at the clinician's discretion. Included in this study were MSM aged $\geq$18 years who presented to the Melbourne Sexual Health Centre with rectal pain and/or discharge and who were given a clinical diagnosis of proctitis. Heterosexual men were excluded. Treatment of men presenting with proctitis was empirical and was commenced before test results became available.

For the primary aim, the prevalence of rectal *M. genitalium* in consecutive MSM who presented to the clinic between May 2012 and August 2013 with symptomatic proctitis was compared with the prevalence of other rectal pathogens among HIV-positive and HIV-negative men.

To address the secondary aim, we measured the organism load in men with *M. genitalium*-associated symptomatic proctitis and compared this with the organism load of *M. genitalium* in a separate group of men with asymptomatic rectal *M. genitalium* infection who were screened rectally because they were sexual contacts of men with urethral *M. genitalium*. For this comparison group, men with asymptomatic rectal *M. genitalium* were selected into the study from the beginning of the study period in consecutive order of presentation until there was one case of asymptomatic rectal *M. genitalium* infection for each case of *M. genitalium*-associated symptomatic proctitis.

Specimen collection and laboratory methods

Men with symptomatic proctitis had four anal swabs taken using dacron swabs inserted at least 2 cm into the anus. One of the swabs was directly plated onto modified Thayer Martin media for gonorrhoea culture. Inoculated culture plates were taken to the clinic's onsite laboratory and incubated at 36°C in 5% CO_2 for 48 h. Presumptive *Neisseria gonorrhoeae* colonies were selected and a smear was prepared using Gram staining. Colonies were oxidase tested and speciation was confirmed by carbohydrate reaction tests [14].

A second anal swab was placed in the manufacturer's collection device and transported as per the manufacturer's instruction for *Chlamydia trachomatis* testing by strand displacement amplification [15]. Chlamydia-positive samples were subjected to genotyping to identify lymphogranuloma venereum using an in-house *omp1* gene Sanger DNA sequencing method [16].

A third anal swab was collected for detection of *M. genitalium* using quantitative PCR targeting a 517-bp region of the 16S rRNA gene [17]. The swab for *M. genitalium* quantitative PCR was rotated ten times in 400 µl of PBS; 200 µl of the PBS containing swab cells was then extracted using MagNA pure 96 platform (Roche Diagnostics, Mannheim, Germany) and eluted in 100 µl of MagNA pure elution buffer. The *M. genitalium* load in each sample was determined by comparing the crossing points of each sample to a standard curve constructed by amplifying a range of known copy *M. genitalium* (ATCC 33530) DNA and prepared pooled DNA from *M. genitalium* negative samples. All assays were performed on the LC480 real-time instrument (Roche Diagnostics) using 5 µl DNA in a 20-µl reaction using SensiFAST™ Probe enzyme (Bioline Australia, Alexandria, Australia).

A fourth anal swab was taken for HSV-1 and HSV-2 PCR testing using an in-house herpes multiplex PCR assay amplifying the glycoprotein B gene of HSV-1 and HSV-2. Testing for *Treponema pallidum* by PCR [18] was performed at clinician discretion in cases where perianal ulcers were present using a TaqMan real-time PCR assay. Men were tested for HIV by immunoassay (DiaSorin, Gerenzano, Italy).

Sample size and statistical analysis

For the overall study a sample size of 150 men was sought based on 95% confidence intervals around anticipated *M. genitalium* rates of 6%–10%. For the primary aim, we ascertained the prevalence of each rectal pathogen including

