Author/s:
Chow, EPF;Lee, D;Bond, S;Fairley, CK;Maddaford, K;Wigan, R;Fehler, G;Lange, SA;De Petra, V;Bissessor, M;Bradshaw, CS;Howden, BP;Hocking, JS;Williamson, DA;Chen, MY

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MAJOR ARTICLE

Nonclassical Pathogens as Causative Agents of Proctitis in Men who Have Sex With Men

Eric P. F. Chow,[1,2,3,a,©] Darren Lee,[4,a] Stephanie Bond,[2] Christopher K. Fairley,[1,2] Kate Maddaford,[2] Rebecca Wigan,[2] Glenda Fehler,[2] Sigrid A. Lange,[4] Vesna De Petra,[4] Melanie Bissessor,[2] Catriona S. Bradshaw,[1,2,3] Benjamin P. Howden,[4] Jane S. Hocking,[3] Deborah A. Williamson,[4,5,b] and Marcus Y. Chen[1,2,b]

[1]Central Clinical School, Monash University, Melbourne, Victoria, Australia, [2]Melbourne Sexual Health Centre, Alfred Health, Melbourne, Victoria, Australia, [3]Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia, [4]Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity at The University of Melbourne, Melbourne, Victoria, Australia, and [5]Department of Microbiology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

Background. This study aimed to identify enteric and sexually acquired rectal pathogens, other than chlamydia and gonorrhea, associated with symptomatic proctitis in men who have sex with men (MSM).

Methods. Anorectal swab samples were obtained from MSM presenting with rectal symptoms and a clinical diagnosis of proctitis at the Melbourne Sexual Health Centre between January 2017 and March 2019. Samples that tested positive for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were excluded. As a comparison group, anorectal samples were also obtained from MSM not reporting symptoms of proctitis between November 2018 and February 2019. Samples from both groups were tested for 15 viral, bacterial, and protozoal enteric pathogens using polymerase chain reaction.

Results. Anorectal samples from 499 men with symptomatic proctitis and 506 asymptomatic men were analyzed. Age, HIV status, and pre-exposure prophylaxis (PrEP) use did not differ between men with proctitis and asymptomatic men. *Treponema pallidum* was more common in men with proctitis (risk difference [RD], 3.6%; 95% CI, 2.0%–5.2%). Most men with anorectal *T. pallidum* presented with painful anal primary infections. *Shigella* spp. was more common among men with proctitis compared with asymptomatic men (RD, 1.8%; 95% CI, 0.1%–3.5%). Most men with *Shigella* did not report diarrhea. *Mycoplasma genitalium* was more common in men with proctitis (RD, 4.3%; 95% CI, 1.1%–7.5%). Herpes simplex virus (HSV)–1 (RD, 10.1%; 95% CI, 6.8%–13.3%) and HSV-2 (RD, 7.2%; 95% CI, 4.5%–10.0%) were more common with proctitis.

Conclusions. Testing for *T. pallidum*, *Shigella*, and HSV should be considered in MSM presenting with symptomatic proctitis. These data provide support for *M. genitalium* as a significant cause of proctitis. A comprehensive diagnostic evaluation is required for MSM with proctitis.

Keywords. enteric pathogens; *Mycoplasma genitalium*; men who have sex with men; rectal infection; *Shigella*; sexually transmitted infection; syphilis.

Rectal infections with sexually transmitted pathogens are common among men who have sex with men (MSM) and may increase the risk for HIV acquisition through mucosal inflammation [1–4]. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are the most common causes of rectal sexually transmitted infections (STIs) among MSM. Most rectal infections from gonorrhea and chlamydia are asymptomatic; however, a proportion of cases result in symptomatic proctitis [5]. Symptoms from proctitis in MSM include anorectal pain, discharge, bleeding, and tenesmus [5–7]. Proctitis among MSM is a common presentation to STI clinics.

Studies from the 1980s showed that proctitis in MSM is also caused by a range of other sexually acquired pathogens including the *Lymphogranuloma venereum* (LGV) variant of *C. trachomatis*, herpes simplex virus (HSV), and *Treponema pallidum*. Enteric pathogens such as *Shigella* have been shown to cause proctocolitis, with inflammation of the colon and rectum [6, 8–10]. However, many of these earlier studies used older, less sensitive diagnostic methods including dark ground microscopy or serological testing for syphilis and culture for *Shigella*. *Mycoplasma genitalium* has been implicated as a cause of proctitis, but studies have varied in terms of whether it is a causative agent [11]. Greater knowledge of the causative agents of proctitis in MSM may enable improved diagnostic evaluation and prevention of onward spread of potentially transmissible pathogens.

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[a]Co-first authors

[b]Co-last authors

Correspondence: Associate Professor Eric Chow, PhD, Melbourne Sexual Health Centre, 580 Swanston Street, Carlton, VIC 3053, Australia (eric.chow@monash.edu).

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Here, we sought to comprehensively define the sexually acquired rectal and enteric pathogens among MSM presenting with proctitis and compare the prevalence of these pathogens with asymptomatic MSM without proctitis. As *N. gonorrhoeae* and *C. trachomatis* are well-established causes of proctitis in MSM, we were particularly interested in investigating the presence of other "nonclassical" pathogens, including *Treponema pallidum* and *Shigella*, given the increase in these among MSM since the 2010s [12, 13]. Specifically, we hypothesized that the prevalence of sexually transmitted pathogens in MSM with proctitis would be higher than in MSM without proctitis. Further, given recent reports of high rates of bacterial STIs among MSM taking HIV pre-exposure prophylaxis (PrEP) [14–16], we sought to include men with proctitis who were taking HIV PrEP.

The aim of this study was to compare a range of enteric and sexually transmitted pathogens among men with proctitis from routinely stored samples with those of asymptomatic men without proctitis recruited through a previous study from a similar time period.

METHODS

Setting, Data Sources, and Definitions

This study was conducted at the Melbourne Sexual Health Centre, the major public HIV/STI clinic in Melbourne, Australia. The MSHC operates 3 clinics offering HIV/STI testing to MSM: a walk-in STI clinic, an HIV clinic, and a clinic for men taking HIV PrEP. In this study, 2 groups of MSM attending MSHC were identified: (1) men presenting with symptomatic proctitis and (2) asymptomatic men not reporting symptoms of proctitis.

MSM who presented to the MSHC were assessed by a sexual health clinician and asked about anorectal symptoms. Symptomatic men with suspected proctitis had visual examination of the anal and perianal area for signs including anal discharge and ulceration. Proctoscopy was not routinely performed in men with suspected proctitis as proctoscopy can worsen pain, especially in the presence of ulcers. An anorectal swab was inserted 2–3 cm into the anal canal by the clinician for *N. gonorrhoeae* and *C. trachomatis* testing.

The diagnosis of proctitis was a presumptive clinical diagnosis made by the clinician based on clinical criteria including any of the following symptoms and signs: anorectal pain, anal discharge, bleeding, and/or tenesmus. Gram staining of anorectal swab smears was not routine or a criterion for the diagnosis of proctitis. Clinicians followed clinic guidelines on proctitis, which standardized testing, diagnosis, and management of proctitis.

Microbiological Testing

Anorectal swabs were tested for *N. gonorrhoeae* and *C. trachomatis* using the Aptima Combo 2 (AC2) assay (Hologic Panther platform, San Diego, CA, USA). Following AC2 testing for *N. gonorrhoeae* and *C. trachomatis*, anorectal swab samples from MSM diagnosed with proctitis who tested negative for *N. gonorrhoeae* and *C. trachomatis* by the AC2 assay were prospectively stored for future studies (since 2011). An opt-out consent process was in place for storage of these samples for future studies, and ethical approval was granted for this process by the Alfred Hospital Ethics Committee, Melbourne, Australia (Project 331/13). In the present study, stored anorectal samples obtained from MSM diagnosed with proctitis between January 2017 and March 2019 and which had tested negative for *N. gonorrhoeae* and *C. trachomatis* were included. This study period was chosen to compare men with proctitis using stored samples with men without proctitis recruited from a previous study from a similar time period [13].

As a comparison group, asymptomatic MSM not reporting anorectal symptoms were identified from a previous cross-sectional study that recruited MSM at MSHC between November 2018 and February 2019 to determine the prevalence of enteric pathogens among MSM not reporting diarrhea [13]. Written informed consent was obtained from the participants in this previous study to store their samples for future studies, and this was approved by the Alfred Hospital Ethics Committee (Project 271/18). Stored anorectal samples from this previous study were included in the present study after excluding samples from 12 men diagnosed with proctitis. The stored anorectal samples from asymptomatic men in the comparison group were not tested for *N. gonorrhoeae* or *C. trachomatis* by the AC2 assay or excluded based on this.

Separate ethical approval was granted from the Alfred Hospital Ethics Committee (Project 44/19) for future testing of stored samples from men with and without proctitis. All samples were de-identified for this additional testing. Samples from men in both groups were stored at –80°C before testing. DNA extraction of the anorectal samples was performed at the Microbiological Diagnostic Unit Public Health Laboratory, The University of Melbourne, Melbourne, Australia. Genomic DNA was extracted from 400 µL of buffer using the QIASymphony DSP Virus/Pathogen Midi Kit (QIAGEN) Complex 400 protocol according to the manufacturer's instructions. Extracted DNA was tested for 15 viral, bacterial, and protozoal enteric pathogens (*Salmonella* spp., *Shigella* sp., *Campylobacter* spp., *Clostridium difficile toxin B*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, *Shiga toxin 1*, *Shiga toxin 2*, *Plesiomonas shigelloides, Aeromonas* spp., *Vibrio* spp., *Giardia*, *Cryptosporidium*, *Dientamoeba fragilis*, *E. histolytica*, *Blastocystis hominis*, *Cyclospora cayetanensis*) using the AusDiagnostics Faecal Pathogen M 16-well assay (AusDiagnostics Pty Ltd., Sydney, Australia) and for STIs using the PlexPCR VHS for *Treponema pallidum*, HSV-1, HSV-2, and Varicella Zoster Virus and the TV/MG investigational assay (SpeeDx, Sydney, Australia) for *Trichomonas vaginalis* and *M. genitalium* [17]. Confirmation of the detection of *M. genitalium* was performed using the ResistancePlus MG assays (SpeeDx, Sydney, Australia).

Statistical Analysis and Ethical Approval

Demographic characteristics, HIV status, and current PrEP use, obtained through computer-assisted self-interview, were compared between the 2 groups of men using the Mann-Whitney *U* test or the Fisher exact test. The proportions of each pathogen detected were calculated with 95% CIs using exact binomial methods. The risk difference (RD) and 95% CI were used to compare the proportion of each pathogen detected between men with proctitis and asymptomatic men. All statistical analyses were performed in STATA (version 14.2; Stata Corporation, College Station, TX, USA).

RESULTS

Demographic Characteristics

There were 499 men with proctitis and 506 asymptomatic men. Age, HIV status, and PrEP use did not differ between men with proctitis and asymptomatic men (Table 1). Overall, 15.3% (n = 154) of men were HIV-positive, 41.2% (n = 414) were HIV-negative and taking PrEP, and 43.5% (n = 437) were HIV-negative and not taking PrEP.

Detection of Sexually Acquired Pathogens

T. pallidum was more commonly detected among men with proctitis (3.6%; 95% CI, 2.2%–5.6%; 18/499) compared with asymptomatic men (0%; 95% CI, 0%–0.7%; 0/506; $P < .001$), with a risk difference of 3.6% (95% CI, 2.0%–5.2%). The clinical presentation and laboratory results for each of the 18 men with proctitis and *T. pallidum* are detailed in Supplementary Table 1. Seventeen men reported anorectal pain. Ten men had primary anal lesions with external ulceration and *T. pallidum* detected from the anorectal swab. Five men had symptoms of proctitis with anal *T. pallidum* detected but no anal ulcer visible externally. Of the 18 men with proctitis and *T. pallidum* detected, 17 had reactive serological tests for syphilis, 16 of whom had negative serology within 12 months. Most men did not have a

Table 1. Pathogens Among 499 MSM Presenting With Proctitis Compared With 506 Asymptomatic MSM

	Men With Proctitis (n = 499), No. (% [95% CI, %])	Asymptomatic Men (n = 506), No. (% [95% CI, %])	Risk Difference (95% CI), %	*P* Value[a]
Demographic characteristics				
Age, median (IQR), y	31 (26–38)	32 (26–40)	–	.077
HIV status and PrEP use, No. (%)				.074
HIV-positive	79 (15.8)	75 (14.8)	–	
HIV-negative taking PrEP	188 (37.7)	226 (44.7)	–	
HIV-negative not taking PrEP	232 (46.5)	205 (40.5)	–	
Viral				
Adenovirus groups F and G	0 (0 [0 to 0.7])	0 (0 [0 to 0.7])	–	-
Astrovirus	0 (0 [0 to 0.7])	10 (2.0 [1.0 to 3.6])	–2.0 (–3.2 to –0.8)	.001
HSV-1	63 (12.6 [9.8 to 15.9])	13 (2.6 [1.4 to 4.4])	10.1 (6.8 to 13.3)	<.001
HSV-2	44 (8.8 [6.5 to 11.7)	8 (1.6 [0.7 to 3.1])	7.2 (4.5 to 10.0)	<.001
Norovirus genotype G1	2 (0.4 [0.0 to 1.4])	2 (0.4 [0.0 to 1.4])	0.0 (–0.8 to 0.8)	1.000
Norovirus genotype G2	1 (0.2 [0.0 to 1.1])	0 (0 [0 to 0.7])	0.2 (–0.2 to 0.6)	.497
Rotavirus	0 (0 [0 to 0.7])	3 (0.6 [0.1 to 1.7])	–0.6 (–1.3 to 0.1)	.249
Sapovirus	2 (0.4 [0.0 to 1.4])	0 (0 [0 to 0.7])	0.4 (–0.2 to 1.0)	.246
Varicella zoster virus	0 (0 [0 to 0.7])	1 (0.2 [0.0 to 1.1])	–0.2 (–0.6 to 0.2)	1.000
Bacterial				
Aeromonas spp.	0 (0 [0 to 0.7])	3 (0.6 [0.1 to 1.7])	–0.6 (–1.3 to 0.1)	.249
Campylobacter spp.	12 (2.4 [1.2 to 4.2])	13 (2.6 [1.4 to 4.4])	–0.2 (–2.1 to 1.8)	1.000
Clostridium difficile	1 (0.2 [0.0 to 1.1])	0 (0 [0 to 0.7])	0.2 (–0.2 to 0.6)	.497
Mycoplasma genitalium	47 (9.4 [7.0 to 12.3])	26 (5.1 [3.4 to 7.4])	4.3 (1.1 to 7.5)	.010
Salmonella spp.	1 (0.2 [0.0 to 1.1])	2 (0.4 [0.0 to 1.4])	–0.2 (–0.9 to 0.5)	1.000
Shigatoxin 1 & 2	12 (2.4 [1.2 to 4.2])	9 (1.8 [0.8 to 3.3])	0.6 (–1.1 to 2.4)	.518
Shigella spp.	14 (2.8 [1.5 to 4.7])	5 (1.0 [0.3 to 2.3])	1.8 (0.1 to 3.5)	.038
Treponema pallidum	18 (3.6 [2.2 to 5.6])	0 (0 [0 to 0.7])	3.6 (2.0 to 5.2)	<.001
Trichomonas vaginalis	0 (0 [0 to 0.7])	0 (0 [0 to 0.7])	–	-
Yersinia enterocolitica and *pseudotuberculosis*	0 (0 [0 to 0.7])	8 (1.6 [0.7 to 3.1])	–1.6 (–2.7 to –0.5)	.008
Protozoal				
Cryptosporidium hominis & parvum	1 (0.2 [0.0 to 1.1])	0 (0 [0 to 0.7])	0.2 (–0.2 to 0.6)	.497
Entamoeba histolytica	0 (0 [0 to 0.7])	2 (0.4 [0.0 to 1.4])	–0.4 (–0.9 to 0.2)	.500
Giardia spp.	5 (1.0 [0.3 to 2.3])	7 (1.4 [0.6 to 2.8])	–0.4 (–1.7 to 1.0)	.773

Abbreviations: HSV, herpes simplex virus; IQR, interquartile range; MSM, men who have sex with men; PrEP, pre-exposure prophylaxis.

[a]The Fisher exact test was performed to compare proportions between men with proctitis and asymptomatic men. The Mann-Whitney *U* test was performed to compare median age.

concurrent rectal pathogen aside from 3 with HSV and 2 with *M. genitalium*.

M. genitalium was significantly more commonly detected among men with proctitis (9.4%; 95% CI, 7.0%–12.3%; 47/499) compared with asymptomatic men (5.1%; 95% CI, 3.4%–7.4%; 26/506; $P = .010$), with a risk difference of 4.3% (95% CI, 1.1%–7.5%). The clinical presentation and laboratory results for each of the 47 men with proctitis and *M. genitalium* detected by anorectal swab are shown in Supplementary Table 2. Among men with proctitis and *M. genitalium*, the most common symptoms reported were anorectal pain (n = 38, 81%), anal bleeding (n = 18, 38%), anal discharge (n = 13, 28%), and tenesmus (n = 11, 23%).

HSV-1 and HSV-2 were both significantly more commonly detected among men with proctitis compared with asymptomatic men. HSV-1 was detected among 12.6% (95% CI, 9.8%–15.9%; 63/499) of men with proctitis compared with 2.6% (95% CI, 1.4%–4.4%; 13/506) of asymptomatic men ($P < .001$), with a risk difference of 10.1% (95% CI, 6.8%–13.3%). HSV-2 was detected among 8.8% (95% CI, 6.5%–11.7%; 44/499) of men with proctitis compared with 1.6% (95% CI, 0.7%–3.1%; 8/506) of asymptomatic men ($P < .001$), with a risk difference of 7.2% (95% CI, 4.5%–10.0%).

Detection of Enteric Pathogens

Shigella spp. were significantly more commonly detected among men with proctitis (2.8%; 95% CI, 1.5%–4.7%; 14/499) compared with asymptomatic men (1.0%; 95% CI, 0.3%–2.3%; 5/506; $P = .038$), with a risk difference of 1.8% (95% CI, 0.1%–3.5%). The clinical presentation and laboratory results for each of the 14 men with proctitis and *Shigella* detected by anorectal swab are shown in Supplementary Table 3. Most men (n = 12, 86%) reported anorectal pain, but few (n = 3, 21%) reported diarrhea.

A range of other enteric pathogens were detected among men with proctitis, including *Campylobacter* spp. (2.4%; 95% CI, 1.2%–4.2%; 12/499), Shigatoxin 1 and 2 (2.4%; 95% CI, 1.2%–4.2%; 12/499), and *Giardia* spp. (1.0%; 95% CI, 0.3%–2.3%; 5/499); however, these were also detected among asymptomatic men with no significant difference between the 2 groups. *Salmonella* spp. was found in 1 man with proctitis (0.2%; 95% CI, 0.0%–1.1%; 1/499) and 2 asymptomatic men (0.4%; 95% CI, 0.0%–1.4%; 2/506), while *Clostridium difficile* was detected in 1 man with proctitis (0.2%; 95% CI, 0.0%–1.1%; 1/499) but was not detected in asymptomatic men. *Entamoeba histolytica* was detected in 2 asymptomatic men (0.4%; 95% CI, 0.0%–1.4%; 2/506) and no men with proctitis.

DISCUSSION

Here, we describe a comprehensive investigation of the potential etiological agents of infectious proctitis in MSM. We demonstrate that *T. pallidum*, *Shigella*, *M. genitalium*, HSV-1, and HSV-2 are detected more frequently among MSM presenting with symptomatic proctitis compared with asymptomatic MSM. Previous studies indicate that the clinical presentation of proctitis in MSM may vary according to the causative pathogen. For example, rectal gonorrhea characteristically presents with purulent anal discharge, while rectal infection with HSV can cause severe anorectal pain, ulceration, and systemic symptoms [5, 18]. However, in clinical practice, it is often difficult to distinguish clinically between causative pathogens, with laboratory testing needed to confirm the pathogen responsible and to guide management. Our results highlight the fact that infectious proctitis in MSM is caused by a spectrum of sexually acquired and enteric pathogens other than *N. gonorrhoeae* and *C. trachomatis* and that broader testing, particularly for *T. pallidum* and *Shigella,* should be considered in the investigation of MSM with proctitis. Our data also provide support for the role of *M. genitalium* as a cause of proctitis among MSM. As with previous studies [5], we found that HSV-1 and HSV-2 are strongly associated with proctitis. To our knowledge, this is the largest study of infectious proctitis to date and the only study to have tested for such a broad range of potential viral, bacterial, and protozoal pathogens using molecular assays.

While earlier studies found *T. pallidum* to be a less common cause of proctitis among MSM, these studies used dark ground microscopy, which is substantially less sensitive than PCR for *T. pallidum*, or serology, which can be negative during primary syphilis [6, 8–10]. Most of the men with *T. pallidum*–associated proctitis in our study had primary anal infections presenting with anorectal pain. Most did not have other concurrent pathogens that might account for their anorectal symptoms. While primary syphilis often presents as a painless lesion, our study demonstrates that primary anal syphilis can result in painful ulceration. In a previous study, 49% of men with primary anogenital syphilis had painful lesions [19]. In the present study, anal *T. pallidum* was detected in men reporting symptoms of proctitis in the absence of overt anal lesions. It is uncertain whether these men had internal primary ulcers, as proctoscopy was not performed. Painful *T. pallidum*–positive anal ulcers can also be found in MSM with secondary syphilis [20]. In addition to *T. pallidum*, we also demonstrate a higher prevalence of *M. genitalium* in MSM with proctitis. Our data suggest that while rectal *M. genitalium* infection in MSM may be asymptomatic, a subset of men develop symptoms from rectal inflammation [21]. In a previous study of MSM with rectal *M. genitalium*, men with symptomatic proctitis had higher loads of *M. genitalium* compared with men with no rectal symptoms [11], analogous to the higher gonococcal loads observed among MSM with symptomatic gonococcal proctitis, compared with men with asymptomatic rectal gonorrhea [5].

Shigella spp. have re-emerged internationally among MSM, including international dissemination of multidrug-resistant

strains of *Shigella sonnei* and *Shigella flexneri* [12, 13]. *Shigella* spp. were detected more commonly among men with proctitis in our study, likely because *Shigella* spp. can lead to proctocolitis, where mucosal inflammation extends over both the colon and rectum. Most of the men with *Shigella*-associated proctitis did not report diarrhea. This may be because MSM with *Shigella* and diarrhea are more likely to present to health services other than an STI clinic. Our results indicate that testing for *Shigella* spp. should be considered in MSM with proctitis even where diarrhea is absent. While *Shigella* spp. are usually diagnosed using culture from a fecal sample, we detected *Shigella* spp. using PCR from anorectal swabs, as reported in other recent studies [13, 22]. Consideration should be given to reflex retesting using laboratory culture for *Shigella* spp. in the event of a positive PCR for *Shigella* spp. Given increasing resistance among *Shigella* spp., phenotypic antimicrobial susceptibility testing may be necessary to inform the choice of antimicrobial therapy [12]. Several other enteric pathogens were detected among men in this study including *Campylobacter*, Shigatoxin (Stx) 1 and 2–producing *Escherichia. coli*, *Giardia* spp., and *E. histolytica*. Each of these pathogens has been responsible for well-described outbreaks of enteritis or colitis among MSM previously [6, 9, 23–26]. In our study, these pathogens were not more frequently found among men with proctitis. This may be because these enteric pathogens are less likely to cause proctitis than *Shigella*.

There are several limitations to this study. First, proctitis was a presumptive clinical diagnosis made by a sexual health clinician based on clinical findings, before the availability of test results. There is no standard definition for the diagnosis of infectious proctitis. Proctoscopy can be invasive and was not routinely performed. Rectal biopsy is not a standard diagnostic investigation of acute proctitis and was not performed. Second, the prevalence of pathogens among MSM with proctitis may reflect the local prevalence of those pathogens, which will vary between populations. However, we specifically selected similar periods of recruitment for the 2 groups of men in this study to reduce the likelihood of differences between groups arising from different prevalence rates of pathogens over time. Third, the likelihood of detection of rectal pathogens may reflect sexual risk practices including condomless receptive anal sex and, in the case of enteric pathogens, oro-anal sex [13]. We did not ascertain sexual practices of men in the study. Higher rates of rectal STIs and proctitis might be expected among PrEP users and sexually active HIV-positive men [5, 11]. There were no differences in the proportion of men in the 2 groups who were taking PrEP or who were HIV positive. However, we did not match characteristics between men in the 2 groups. Fourth, men who tested positive for *N. gonorrhoeae* and/or *C. trachomatis* were excluded from the proctitis group because these samples were not stored for future testing. In the group of asymptomatic men without proctitis, specimens were not tested for *N. gonorrhoeae* and/or *C. trachomatis* by AC2 assay at the time we tested for the enteric and STI pathogens, and therefore any with chlamydia or gonorrhea were not excluded. Fifth, NAAT detection does not prove causation, although in the absence of other pathogens it is suggestive. Fifth, as proctitis cases who tested positive for *C. trachomatis* were excluded, this would have excluded cases of LGV proctitis. Finally, a proportion of men with proctitis had no pathogen detected. It is uncertain whether these men had an infectious cause. Further research into other possible infectious and noninfectious causes would be of interest.

In summary, our study describes a higher detection of *T. pallidum*, *Shigella*, *M. genitalium*, HSV-1, and HSV-2 in MSM with proctitis and highlights the need for a comprehensive diagnostic evaluation of MSM presenting with proctitis to ensure appropriate clinical and public health management.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. M.Y.C. and D.A.W. have received donated testing assays from SpeeDx for research purposes. All other authors report no conflicts of interest. The Melbourne Sexual Health Centre receives funding from Speedx Pty Ltd to support research on *M. genitalium*. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. E.P.F.C., D.A.W., C.K.F., and M.Y.C. designed and conceived the study. D.L. and S.L. were involved in laboratory testing. E.P.F.C., S.B., and M.Y.C. wrote the first draft of the manuscript. E.P.F.C. performed the statistical analyses. S.B. performed chart review and prepared the tables. D.A.W. and V.D.P. oversaw the laboratory testing. D.A.W. and B.J.H. provided resources for laboratory testing. K.M., R.W., and M.B. were involved in patient recruitment. K.M. and R.W. were involved in database management and sample storage. G.F. managed the stored samples. All authors were involved in data interpretation and contributed to the final version of the manuscript.

Data availability. All relevant data are within the manuscript and its Supplementary Data files.

Patient consent. An opt-out consent process was in place for men with proctitis to store their samples for future studies, and informed consent was

not required for routinely collected samples. Ethics approval was granted for this process by the Alfred Hospital Ethics Committee (Project 331/13). Written informed consent was obtained from asymptomatic men without procitis to store their samples for future studies, and this was approved by the Alfred Hospital Ethics Committee (Project 271/18). Ethical approval was granted from the Alfred Hospital Ethics Committee (Project 44/19) for future testing of stored samples from men with and without proctitis.

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