Sexually transmitted rectal infections in a cohort of ‘men having sex with men’

Claudio Foschi,1,* Valeria Gaspari,2 Paola Sgubbi,2 Melissa Salvo,1 Antonietta D’Antuono2 and Antonella Marangoni1

Abstract
Purpose. We assessed the prevalence and predictors of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium rectal infections in a population of ‘men having sex with men’ (MSM).

Methodology. From January to November 2017, 165 MSM attending a STI outpatients clinic in Bologna (Italy) and reporting unsafe anal intercourses were enrolled. An ano-rectal swab was collected from each patient: chlamydial and gonococcal infections were diagnosed by a commercial NAAT, whereas an in-house quantitative PCR was used for M. genitalium detection. In addition, 131 urine samples and 84 pharyngeal swabs underwent testing for C. trachomatis and N. gonorrhoeae. A molecular C. trachomatis typing, a serological screening for anti-Chlamydia IgG and IgA, as well as the assessment of HIV, HCV and syphilis infections, were performed.

Results/Key findings. The prevalence of C. trachomatis, N. gonorrhoeae and M. genitalium rectal infections was 27.2, 25.4 and 4.8 %, respectively. Globally, 63.1 % of cases were asymptomatic and up to 80 % of chlamydial and gonococcal infections would have been missed if the rectal site had not been tested. All the patients with rectal M. genitalium carriage were asymptomatic and characterized by low bacterial loads (<2500 DNA copies/reaction). Lymphogranuloma venereum (LGV) prevalence was 12.1 % with a considerable proportion of asymptomatic infections (35 %). The presence of symptoms, age >30, HIV-positivity and elevated levels of anti-Chlamydia antibodies were the most significant predictors of LGV.

Conclusions. Sexually transmitted rectal infections are frequent and often asymptomatic among MSM. LGV prevalence is high in our country and there is increasing evidence of symptomless cases.

INTRODUCTION
Rectal infections among ‘men having sex with other men’ (MSM) are very common and are caused by different sexually transmitted pathogens [1, 2].

Chlamydia trachomatis and Neisseria gonorrhoeae represent the most common agents of bacterial rectal infections in the MSM population and they are associated with the increased risk of human immunodeficiency virus (HIV) infection transmission and acquisition [3–6].

Besides C. trachomatis and N. gonorrhoeae infections, there is increasing evidence of the contribution of Mycoplasma genitalium as a possible cause of bacterial proctitis, particularly in HIV-positive MSM [7–10].

The rectal site can be an important reservoir for the spread of sexually transmitted infections (STIs) that can lead to several sequelae and complications when left untreated [11, 12]. Signs and symptoms, especially when L1–L3 C. trachomatis serovars (lymphogranuloma venereum – LGV) are present, include rectal bleeding, pain, muco-purulent discharge and tenesmus [13, 14].

Over the last decade, nucleic acid amplification techniques (NAATs) have become the reference methods for the diagnosis of rectal STIs, considering their excellent performance in terms of specificity and sensitivity [15, 16], and at present the international guidelines recommend at least one annual screening for rectal C. trachomatis and N. gonorrhoeae infections in the MSM population [2, 17].

On the contrary, the need for rectal screening for M. genitalium infection is still unclear and under debate [10, 18].

The aim of this study was to assess the prevalence and predictors of rectal infections due to C. trachomatis,
N. gonorrhoeae and M. genitalium in a cohort of MSM patients, attending a STI outpatient clinic in the north of Italy.

**METHODS**

**Study design**

From January to November 2017, all consecutive MSM patients attending the STI Outpatients Clinic of St. Orsola-Malpighi University Hospital in Bologna (Italy) and reporting unsafe anal intercourse were considered eligible for the study. Exclusion criteria were the following: being younger than 18 years, and having used enemas in the last 3 days or any antibiotics in the last month.

After a preliminary interview, a clinical visit was carried out for each patient. Personal data and information about urogenital and ano-rectal symptoms were recorded. The external genitalia, perianal skin and anal mucosa were evaluated for the presence of lesions (e.g. ulcers, condylomas, lymph nodes).

Afterwards, an ano-rectal swab (E-Swab, Copan, Brescia, Italy) for the molecular detection of C. trachomatis, N. gonorrhoeae and M. genitalium was collected from each patient. Moreover, based on patients' sexual behaviour and symptoms and according to clinician discretion, urine samples and pharyngeal swabs for C. trachomatis/N. gonorrhoeae detection were also collected. To avoid biases related to antimicrobial treatment, only samples collected at the first visit were considered, excluding specimens obtained during the follow-up period.

When available, clinical and microbiological data about previously known STIs (HIV, HCV, syphilis, C. trachomatis and N. gonorrhoeae genital and extra-genital infections) were recorded from each patient. Since M. genitalium testing was introduced in this study only for research purposes, no information about previous M. genitalium infections was available.

For HIV, HCV and syphilis infections, patients were managed following the regular STI evaluation of the Clinic. In particular: (i) a serological screening for HIV and HCV was performed for all subjects previously negative or for those who had never previously had it performed; (ii) patients with known HIV and/or HCV infections were monitored only with RNA viral loads; and (iii) syphilis serology was performed in both naïve patients and previously positive subjects, in order to correctly classify the disease stage and to exclude possible re-infection.

Syphilis diagnosis was performed by following a reverse algorithm. In particular, Architect Syphilis TP chemiluminescent immunoassay (Abbott GmbH and Co. KG, Wiesbaden, Germany) was used as a screening test, and positive results were confirmed by both a treponemal (TPHA; Randox, UK) and a non-treponemal test (RPR; Randox, UK). In accordance with the clinical and laboratory criteria proposed by Larsen [19], *Treponema pallidum* infection was classified as follows: early syphilis (primary, secondary or early latent infection) or late latent syphilis.

HIV serology was performed with Architect HIV Ag/Ab Combo assay (Abbott) as a screening test, whereas VIDAS HIV DUO Quick assay (bioMérieux, Marcy l’Etoile, France) and INNO-LIA HIV I/II Score (Innogenetics, Gent, Belgium) were used as confirmatory tests.

HCV infection was assessed evaluating serological markers (total anti-HCV antibodies) with chemiluminescent assay (Architect anti-HCV assay; Abbott). HCV-RNA and HIV-RNA viral loads were performed with real-time PCR assays (COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test and COBAS AmpliPrep/COBAS TaqMan HIV-1 Quantitative Test; Roche Molecular Systems, Pleasanton, CA).

Serological screening for anti-Chlamydia antibodies was performed in all the patients by immune-enzymatic assays (Chlamydia IgG and Chlamydia IgA; Virion/Serion GmbH, Würzburg, Germany). The level of antibodies was expressed as arbitrary units (AU)/ml.

A case of 'bacterial rectal infection' was defined when a rectal positivity for at least one of the bacterial pathogens tested (C. trachomatis, N. gonorrhoeae, M. genitalium) was found, irrespective of the presence of signs and symptoms.

The Ethical Committee of St. Orsola-Malpighi Hospital approved the study protocol (78/2017/U/Tess), and all subjects gave written informed consent to the work.

**Study population**

During the study period, 165 MSM patients were enrolled. The mean age was 35.5±9.7 years (±standard deviation, SD) (min–max 18–64 years). Of the 165 subjects, 53 (32.1%) complained of various ano-rectal symptoms, including rectal bleeding, tenesmus, ano-rectal discharge, diarrhoea and ano-rectal pain.

A total of 165 rectal swabs, 131 urine samples and 84 pharyngeal swabs were collected. In particular, 25 (15.1%) patients provided only a rectal swab, 56 (33.9%) a rectal swab and a urine sample, 9 (5.4%) a rectal and a pharyngeal swab, whereas the remaining 75 (45.4%) provided the three different types of sample (extra-genital swabs and urine).

Globally in the cohort of MSM enrolled, 62 patients (37.5%) were HIV-positive (17 with detectable viral load) and 8 (4.8%) tested positive for anti-HCV antibodies. All but one HCV-positive patient showed a contemporary positivity for HIV. Ten of the 62 HIV-positive patients (16.1%) and 3 of the 8 HCV-positive subjects (37.5%) received the diagnosis at the time of enrolment.

Moreover, 17 cases of early syphilis (17/165; 10.3%) (5 primary, 12 secondary and 3 early latent), 6 of late latent syphilis (17/165; 3.6%) and 47 cases (47/165; 28.5%) of past *T. pallidum* infection were detected in the study population. All primary syphilitic lesions were found in the genital area. All cases of early and late latent syphilis were diagnosed at
the time of enrolment and no cases of syphilis re-infection were found.

Eleven out of the 17 patients (64.7%) with early syphilis and 4 out of the 6 (66.6%) with a late latent infection were HIV-positive.

For all patients enrolled, data regarding previous chlamydial/gonococcal infections were available: 43 subjects (43/165; 26.0%) reported previous genital and/or extra-genital C. trachomatis and/or N. gonorrhoeae infections and, in particular, 26 patients (26/165; 15.7%) suffered from previous C. trachomatis and/or N. gonorrhoeae rectal infections.

**Diagnosis of chlamydial/gonococcal infections and C. trachomatis genotyping**

Urine specimens and ano-rectal and pharyngeal swabs were processed by Versant CT/GC DNA 1.0 Assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), a commercial real-time PCR test simultaneously detecting the presence of C. trachomatis and/or N. gonorrhoeae DNA [20]. An estimate of the load of C. trachomatis and N. gonorrhoeae NAAT-positive specimens was obtained using the cycle threshold (Ct) values. As previously reported [20, 21], Ct values are inversely proportional to the DNA load and can be used for a semi-quantitative estimate of bacterial load.

In case of a C. trachomatis positive result by Versant CT/GC, the corresponding remnant DNA was collected from the extraction microplate and used for C. trachomatis molecular genotyping. Molecular genotyping was based on an omp1 gene semi-nested PCR, followed by RFLP analysis, as previously described [22, 23]. Briefly, the first product of 1033 base pairs (bp) was amplified using the following paired primers: SERO1A (5'-ATGAAAAAACCTCTGAAAA TCGG-3') and SERO2A (5'-TTTCTGATCTTCACTT TTGTT-3'). In the second PCR step, amplifying a 978 bp fragment, primers SERO2A and PCTM3 (5'-TCC TTGCAAAGCTCTGCGTTGGGGAATCCT-3') were used. After the PCR step, the amplified product was digested with Alul, Ddel and/or HinfI as restriction enzymes (Promega, Madison, WI, USA) and visualized after electrophoresis run in ethidium bromide-stained 12% polyacrylamide gel. C. trachomatis serovar identification was carried out by analysis of the specific restriction pattern.

**Mycoplasma genitalium real-time qPCR**

Starting from the remaining DNA eluate of Versant PCR plate, each sample was tested against M. genitalium with a quantitative PCR (qPCR) assay, targeting the mg219 gene. This assay for detecting M. genitalium in clinical samples proved to be reproducible, highly specific and sensitive and applicable to a wide range of specimen types [24].

After the production of a standard curve using serial dilutions of individual plasmids containing the qPCR amplicon, a single-plex TaqMan real-time qPCR assay was used, as described previously [24, 25]. Briefly, the qPCR assays were carried out using the following reaction mix composition: 1× Platinum Quantitative PCR SuperMix-UDG (Thermo Fisher Scientific, Waltham, MA, USA), and final working concentrations of reagents of 0.4 mM forward and reverse primer, 0.1 mM TaqMan probe, 0.2 mg BSA ml⁻¹ (Promega) and 4 mM MgCl₂ (Thermo Fisher Scientific). Final reaction volumes of 10 µl, comprising 2 µl of nucleic acid extract and 8 µl of reaction mix, were used. Thermal cycling reactions were performed using an ABI Prism 7300 System (Thermo Fisher Scientific) with the following cycling conditions: 95°C for 5 min, and 45 cycles of 95°C for 15 s and 59°C for 45 s. Any reaction which failed to produce a cycling threshold (Ct) value after 45 cycles was recorded as negative. Results were expressed as DNA copies/reaction.

**Statistical analysis**

All the statistical analyses were performed using GraphPad Prism software (GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

In order to evaluate statistically significant differences among the subgroups of subjects, t-test and ANOVA were used to compare quantitative data, whereas categorical data were analysed with chi-square or Fisher’s exact test. A value of P<0.05 was considered statistically significant.

**RESULTS**

**Prevalence of bacterial rectal infections**

Globally, in the cohort of MSM analysed, a total of 45 cases of gonorrhoea (45/165; 27.2%), 42 of chlamydial infection (42/165; 25.4%) and 8 of M. genitalium (8/165; 4.8%) were detected in the rectal site.

Overall, 73 patients were found to be positive (73/165; 44.2%); in particular, 23 subjects were positive only for N. gonorrhoeae (23/165; 13.9%), 22 only for C. trachomatis (22/165; 13.3%) and 6 for M. genitalium single rectal infection (6/165; 3.6%), whereas 20 showed a chlamydial/gonococcal co-infection (20/165; 12.1%) and 2 were characterized by contemporary positivity for N. gonorrhoeae and M. genitalium (2/165; 1.2%). Table 1 shows the prevalence and predictors of rectal infections stratified by aetiological agent.

It is worth emphasizing that, globally, more than half of all bacterial rectal infections (46/73; 63.1%) were completely asymptomatic.

Subjects with rectal carriage of M. genitalium were significantly younger compared to negative patients (P=0.02), and all cases were found to be asymptomatic and characterized by the presence of low bacterial DNA (mean±SD: 369±819 M. genitalium copies/reaction; min–max: 5–2365 copies/reaction).

The presence of ano-rectal symptoms, with particular reference to rectal discharge, was significantly associated with C. trachomatis single infections compared to the other causative agents (P=0.03). Moreover, we found that the presence
of an early syphilis infection was related to the rectal detection of *C. trachomatis–N. gonorrhoeae* co-infection (*P*=0.04).

Considering *C. trachomatis* rectal infections, we noted that patients with rectal symptoms were characterized by significantly higher bacterial loads compared to symptomless subjects (mean Ct values±SD: 20.9±3.0 vs 28.6±5.0; *P*=0.0002).

On the contrary, no association between Ct values and the presence of symptoms in the case of *N. gonorrhoeae* rectal infections was found (mean Ct values±SD: 22.1±6.7 vs 25.6±7.8; *P*=0.3). Similarly, no correlation between rectal symptoms and HIV positivity or detectable HIV viral load was noticed (*P*=0.62 and 1.0, respectively).

Regarding patients providing urine samples in addition to a rectal swab, only 10 of the 50 MSM (20 %) with a bacterial infection were positive for *C. trachomatis* and/or *N. gonorrhoeae* at the urogenital site. In particular, a total of 27 gonococcal (81.8 %) and 27 chlamydial infections (79.4 %) would have been missed if the rectal site had not been tested. In the group of patients with no rectal infections (92/165), 3 cases of urethral *C. trachomatis*, 1 case of genital gonorrhoea and 5 pharyngeal *N. gonorrhoeae* infections were found.

Globally, we detected 16 *N. gonorrhoeae* (19.0 %) and 2 *C. trachomatis* (2.4 %) pharyngeal infections. Most of these (72.2 %) were associated with a contemporary rectal positivity, whereas only in 25 % of cases were a genital and pharyngeal infection found concurrently.

### Table 1. Epidemiological and clinical characteristics of MSM patients with bacterial rectal infections, stratified by the type of pathogens detected

<table>
<thead>
<tr>
<th></th>
<th><em>N. gonorrhoeae</em> infection N=23 (13.9 %)</th>
<th><em>C. trachomatis</em> infection N=22 (13.3 %)</th>
<th><em>M. genitalium</em> infection N=6 (3.6 %)</th>
<th><em>C. trachomatis–N. gonorrhoeae</em> co-infection N=20 (12.1 %)</th>
<th><em>N. gonorrhoeae–M. genitalium</em> co-infection N=2 (1.2 %)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years±SD)</td>
<td>37.2±10.2</td>
<td>35.0±9.4</td>
<td>35.0±8.8</td>
<td>25.5±5.2</td>
<td>31.8±8.1</td>
<td>33.0±2.8</td>
</tr>
<tr>
<td>Ano-rectal symptoms</td>
<td>At least one symptom</td>
<td></td>
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<tr>
<td></td>
<td>26 (28.2 %)</td>
<td></td>
<td>13 (59.1 %)</td>
<td>0 (0.0 %)</td>
<td>6 (30.0 %)</td>
<td>0 (0.0 %)</td>
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<tr>
<td></td>
<td>Rectal bleeding</td>
<td>2 (2.1 %)</td>
<td>1 (4.5 %)</td>
<td>0 (0.0 %)</td>
<td>1 (5.0 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td></td>
<td>Rectal discharge</td>
<td>9 (9.8 %)</td>
<td>4 (17.3 %)</td>
<td>0 (0.0 %)</td>
<td>2 (10.0 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td></td>
<td>Tenesmus</td>
<td>3 (3.2 %)</td>
<td>0 (0.0 %)</td>
<td>0 (0.0 %)</td>
<td>1 (5.0 %)</td>
<td>0 (0.0 %)</td>
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<tr>
<td></td>
<td>Diarrhoea</td>
<td>6 (6.5 %)</td>
<td>2 (8.7 %)</td>
<td>1 (4.5 %)</td>
<td>0 (0.0 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td></td>
<td>Ano-rectal pain</td>
<td>9 (9.8 %)</td>
<td>2 (8.7 %)</td>
<td>2 (9.1 %)</td>
<td>0 (0.0 %)</td>
<td>4 (20.0 %)</td>
</tr>
<tr>
<td>HIV positivity*</td>
<td>30 (32.6 %)</td>
<td>10 (43.5 %)</td>
<td>11 (50.0 %)</td>
<td>2 (33.3 %)</td>
<td>8 (40.0 %)</td>
<td>1 (50.0 %)</td>
</tr>
<tr>
<td>Negative viral load</td>
<td>24 (26.1 %)</td>
<td>9 (39.1 %)</td>
<td>5 (22.7 %)</td>
<td>1 (16.6 %)</td>
<td>6 (30.0 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td>Detectable viral load</td>
<td>6 (6.5 %)</td>
<td>1 (4.3 %)</td>
<td>6 (27.3 %)</td>
<td>1 (16.6 %)</td>
<td>2 (10.0 %)</td>
<td>1 (50.0 %)</td>
</tr>
<tr>
<td>HCV positivity*</td>
<td>6 (6.5 %)</td>
<td>0 (0 %)</td>
<td>2 (9.1 %)</td>
<td>0 (0.0 %)</td>
<td>0 (0.0 %)</td>
<td>0 (0.0 %)</td>
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<tr>
<td>Syphilis*</td>
<td>Early</td>
<td>7 (7.6 %)</td>
<td>1 (4.3 %)</td>
<td>3 (13.6 %)</td>
<td>0 (0.0 %)</td>
<td>6 (30.0 %)</td>
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<tr>
<td></td>
<td>Late latent</td>
<td>3 (3.3 %)</td>
<td>0 (0.0 %)</td>
<td>1 (4.5 %)</td>
<td>1 (16.6 %)</td>
<td>1 (5.0 %)</td>
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<td></td>
<td>Past infection</td>
<td>23 (25.0 %)</td>
<td>11 (47.8 %)</td>
<td>4 (18.2 %)</td>
<td>1 (16.6 %)</td>
<td>7 (35.0 %)</td>
</tr>
<tr>
<td>Previous infections</td>
<td><em>N. gonorrhoeae</em></td>
<td>7 (7.6 %)</td>
<td>6 (26.1 %)</td>
<td>3 (13.6 %)</td>
<td>0 (0.0 %)</td>
<td>2 (10.0 %)</td>
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<td></td>
<td><em>C. trachomatis</em></td>
<td>8 (8.7 %)</td>
<td>4 (17.4 %)</td>
<td>1 (4.5 %)</td>
<td>1 (16.6 %)</td>
<td>0 (0.0 %)</td>
</tr>
<tr>
<td></td>
<td><em>M. genitalium</em></td>
<td>22 (23.9 %)</td>
<td>9 (39.1 %)</td>
<td>6 (27.3 %)</td>
<td>1 (16.6 %)</td>
<td>4 (20.0 %)</td>
</tr>
</tbody>
</table>

*For HIV, HCV and syphilis, both the infections detected at the time of the enrolment and cases previously diagnosed were taken into account.

†A significant difference was found between the mean age of patients with *M. genitalium* rectal infection and those with no infection.
symptomatic and complained especially about the presence of tenesmus and rectal discharge (P<0.05).

Moreover, LGV cases were significantly associated with HIV positivity (30/92 vs 14/20; 32.6 vs 70 %; P=0.004), the presence of early syphilis stage (7/92 vs 5/20; 7.6 vs 25 %; P=0.03) and elevated levels of anti-Chlamydia IgG (mean antibody level±SD: 104.5±108.2 vs 182.7±70.9 AU ml⁻¹; P=0.02) and IgA (26.7±28.4 vs 57.2±34.0 AU ml⁻¹; P=0.003).

It follows that the presence of ano-rectal symptoms, age of 30 or more, association with HIV positivity and the presence of higher levels of anti-Chlamydia antibodies were the most significant predictors of LGV compared to non-L. Chlamydia trachomatis cases (Table 2).

Although not statistically significant (P=0.06), LGV infections were characterized by higher bacterial loads compared to rectal infections caused by non-L. Chlamydia trachomatis serovars (mean Ct values±SD: 22.8±4.4 vs 26.1±6.0).

DISCUSSION

Unsafe anal intercourse is very common among MSM, leading to the creation of a high-risk network for STI transmission and acquisition. In this scenario, bacterial rectal infections are frequent and often characterized by the absence of clinical symptoms [26, 27]. Adequate management in terms of correct diagnosis and appropriate treatment is crucial to the avoidance of complications and prevention of further transmission [11, 28].

Many studies have investigated the characteristics of rectal infections due to C. trachomatis and N. gonorrhoeae [3, 5], whereas many aspects of the role of M. genitalium remain to be elucidated [10, 18].

In this study, we assessed the prevalence and predictors of rectal infections caused by C. trachomatis (comprising genotypes D–L), N. gonorrhoeae and M. genitalium in a selected population of MSM, attending a STI outpatient clinic in the north of Italy.

Our data confirmed the extremely high prevalence of C. trachomatis and N. gonorrhoeae rectal infections among MSM (both >25 %) and the frequent detection of these infections in asymptomatic subjects. As described previously, the prevalence of these infections can exceed 15–20 % [4, 26, 29] and the rate of asymptomatic cases can be very significant [17, 26]. In this context, we found that the presence of symptoms in subjects suffering from C. trachomatis or N. gonorrhoeae rectal infection was not influenced by HIV status, but rather, at least for C. trachomatis, it might be related to higher bacterial loads.

Although it has been shown that N. gonorrhoeae loads among men with symptomatic proctitis are strikingly high [30], we found no association between rectal symptoms and gonococcal DNA levels, suggesting that other factors probably contribute to the onset of symptoms (e.g. age, local factors, individual susceptibility, co-infections). Nevertheless, we are fully aware that the methodology used (i.e. a semi-quantitative estimation of bacterial loads by PCR Ct values) could have affected our results, and more accurate methods for accurate bacterial quantification are needed to confirm our hypothesis.

All the aspects mentioned above, combined with the high rate of co-infection, underline the importance of a duplex chlamydial/gonococcal rectal screening in the MSM population on the basis of individual risk and sexual behaviour and not according to clinical symptoms [2, 28]. In addition, we noted that the majority of chlamydial/gonococcal rectal infections (about 80 %) would not have been identified and probably would have remained untreated if screening only the urethral site. As suggested by other authors, massive efforts are needed to facilitate the implementation of rectal C. trachomatis and N. gonorrhoeae screening recommendations for MSM [4, 17].

When considering M. genitalium rectal infections, the overall prevalence found in our population (4.8 %) was consistent with previous reports, showing a detection rate between 1.6 and 12 % in MSM subjects [7–10, 31].

<table>
<thead>
<tr>
<th>Table 2. Predictors of LGV rectal infection compared to non-L Chlamydia trachomatis serovar infection</th>
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<tbody>
<tr>
<td><strong>LGV infection N=20</strong> (12.1 %)</td>
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<tr>
<td>Mean age (years±SD)</td>
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<tr>
<td>Ano-rectal symptoms</td>
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<td>Contemporary gonococcal rectal infection</td>
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<td>Anti-Chlamydia antibodies AU/ml</td>
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<td>IgG (mean±SD)</td>
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<td>IgA (mean±SD)</td>
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*For HIV, HCV and syphilis, both the infections detected at the time of the enrolment and cases previously diagnosed were taken into account.
Interestingly, all patients with *M. genitalium* rectal carriage denied the presence of any rectal symptoms. As previously demonstrated by Bissessor *et al.* [10], the presence of symptoms could be associated with higher *M. genitalium* rectal loads compared to non-symptomatic cases (10 744 vs 60 000 copies of organism/swab). The detection of low *M. genitalium* DNA copies (<2500 copies of organism/reaction) could explain the absence of clinical symptoms in our setting.

Moreover, contrary to several studies [7, 9, 10], no correlation between *M. genitalium* rectal carriage and HIV positivity was found. This aspect could reflect the specific dynamics of transmission and risk networks found in the present study, as suggested, for example, by the detection of *M. genitalium* in younger patients.

To the best of our knowledge, this is the first report on *M. genitalium* rectal infections in Italy and our data suggest that rectal *M. genitalium* screening among MSM, based on individual risk, should be carefully evaluated on the basis of the cost–benefit ratio. Indeed, if on the one hand a screening programme for *M. genitalium* would be useful to prevent potential complications (e.g. reduction of HIV transmission and acquisition) and to break the chain of transmission, on the other hand the low prevalence of the infection might represent a deterrent [32].

As a major limitation of this work, we have no data on the presence and prevalence of *M. genitalium* infection at pharyngeal and urethral sites since, at present, no diagnostic routine testing is performed for *M. genitalium* molecular detection in our laboratory. It is desirable that this problem can be overcome shortly and that *M. genitalium* testing is implemented in the diagnostic routine workflow. It would then be possible to evaluate the efficacy of different screening programmes and the prevalence of *M. genitalium* infections at various anatomical sites.

Other interesting aspects emerged from *C. trachomatis* molecular typing. Initially we found that L2 was the most common serovar at the rectal site, with a marked prevalence of LGV infection (12.1%). Compared to a previous study conducted between 2012 and 2013 in the same setting [14], the total prevalence of LGV infection remained high and constant, indicating that LGV is still endemic in both Italy and other European countries [33–35].

Interestingly, in the present study we found a considerable proportion of asymptomatic LGV infections (35%; 7/20), in agreement with recent observations [36, 37] but in contrast to the data reported by Foschi *et al.* in 2014 [14]. It is possible to hypothesize that changes in screening practice, selection pressure after treatment of patients with symptomatic LGV or an adaptation of specific LGV strains to the rectal site may have contributed to this phenomenon.

Second, considering that *C. trachomatis* typing tests are generally restricted to research laboratories or public health settings, we looked for predictors capable of differentiating L serovars from non-L serovars. Our data suggest that, in the absence of a confirmatory *C. trachomatis* test, a LGV antimicrobial regimen should be considered in all HIV-positive MSM, especially in the presence of symptoms.

Moreover, our results indicate that elevated anti-*Chlamydia* antibodies can be of assistance in the diagnostic pathway for LGV diagnosis, as previously indicated [14]. Nevertheless, we are fully aware that serological tests are not recommended for LGV diagnosis and that high levels of anti-*Chlamydia* antibodies can persist even after LGV adequate treatment and recovery [2, 38].

Since these features concur with what has previously been reported [14, 39–41], we hypothesize that the epidemiology and pathobiology of LGV infection have changed little over recent years, because of the creation of ‘high-risk core groups’, where L *C. trachomatis* serovars find an appropriate niche to establish and disseminate.

Related to that, our data also underline the existence of an alarming ‘STI burden’ among the MSM population, as indicated by the significant prevalence of HIV and syphilis infections, the high rate of co-infections and the widespread history of previous STIs. In this context, specific prevention and surveillance programmes are urgently needed to meet the STI challenge [42].

In conclusion, the major findings of this study are the followings: (i) since bacterial rectal infections are extremely frequent and often asymptomatic among MSM, rectal screening based on a history of unsafe anal intercourse is crucial for correct management; (ii) considering its low prevalence, careful evaluation of the cost–benefit ratio for *M. genitalium* rectal screening among MSM should be carried out; and (iii) the prevalence of LGV infection is high and constant in our country, but there is increasing evidence of asymptomatic cases.

Since we have no data on antibiotic resistance profiles among the pathogens investigated, future perspectives of this work will include the detection of markers associated with *N. gonorrhoeae* antimicrobial resistance, as well as implementation of the molecular detection of macrolide resistance for *M. genitalium*.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
The Ethical Committee of St. Orsola-Malpighi Hospital approved the study protocol (78/2017/U/Tess), and all the subjects gave written informed consent to the work.
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