

Genotyping *Chlamydia trachomatis* strains among men who have sex with men from a Northern Spain region: a cohort study

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ABSTRACT

Objectives: To determine the prevalence of anorectal *Chlamydia trachomatis* serovars in a group of men who have sex with men (MSM) with high risk sexual behaviour, attendees at a sexually transmitted infection (STI) unit from a region in Northwest Spain.

Design: Retrospective and descriptive study of all swabs obtained from all MSM attendees at an STI unit, from 2007 to 2011. Retrospective ethical approval was granted by the Ethical Regional Committee of Clinical Investigation of the Principality of Asturias.

Setting: The STI clinic in Oviedo, Spain, offers screening and free-of-charge treatment to about 3646 patients per year.

Participants: 303 symptomatic and asymptomatic consecutive and unselected MSM patients (mean age 36.7 and range 21–55 years) were evaluated for anorectal chlamydial infection.

Main outcome measures: *C trachomatis* DNA extraction and detection in all rectal and in 36 urethral swabs. Characterisation of *C trachomatis* genotypes through sequencing of *ompA* gene amplicons and further phylogenetic tree analysis.

Results: We found 40 (13.2%) positive rectal samples. The distribution of genotypes was E (37.5%) followed by G (25%), D (12.5%), J (10%) and L2b (5%). 25 (62.5%, 95% CI 46.2 to 78.7) of the chlamydia-infected MSM showed clinical manifestations while 15 (37.5%, 95% CI 21.25 to 53.75) reported no symptoms. Concurrent infection with other STIs was documented in 27 (67.5%, 95% CI 51.7 to 83.2) patients. The most frequently reported clinical symptom was anal ulcer (7 cases, 17.5%; 95% CI 4.47 to 30.52). E genotype was mostly detected in asymptomatic patients. There were non-E genotypes detected in 21 (84%, 95% CI 63.9 to 95.5) of 25 symptomatic patients ($p < 0.001$).

Conclusions: The first two confirmed cases of lymphogranuloma venereum (LGV) in MSM in Asturias are reported, probably indicating the increase of this infection. The Spanish *C trachomatis* laboratory-based surveillance system may underlie an underestimated number of chlamydial infections. Whenever mild and atypical symptoms exist, laboratory evaluation would contribute to the early implementation of appropriate therapy and prevent LGV dissemination.

ARTICLE SUMMARY

Article focus

- Typing of *Chlamydia trachomatis* strains provide important epidemiological information about the serovars circulating in the community, which facilitates contact tracing and contributes to improvement in the implementation of control measures.
- In Europe, there have been cases of anorectal *C trachomatis* infections with few or no symptoms, which could suggest that the number of reported cases may underestimate the real prevalence.
- *C trachomatis* lymphogranuloma venereum (LGV) infections have increased in the last decade. However, in Northwest Spain, only sporadic cases have been reported among men who have sex with men (MSM) and there are no cases in our region.

Key messages

- MSM with high-risk sexual behaviour should be tested for *C trachomatis* and if found positive, especially in the rectum, LGV should be ruled out.
- It is highly recommended to distinguish *C trachomatis* infection by invasive L serovars in order to ensure appropriate therapy and prevent the spread of LGV in Europe.
- Surveillance programmes, including preventive strategies, are needed to control the onward transmission of *C trachomatis* infection.

Strengths and limitations of this study

- We used nucleic acid amplification test (NAAT) for the detection of *C trachomatis* in rectal swabs, with good analytical validity, which led to a specialisation of the laboratory in the genotyping of *C trachomatis*.
- It was impossible to identify sexual contacts of all the *C trachomatis*-positive patients.

INTRODUCTION

Chlamydia trachomatis is an important human pathogen that causes a spectrum of clinically significant diseases.¹ According to the Centers

for Disease Control and Prevention (CDC), 92 million infections with *C trachomatis* are detected globally each year.² On the basis of disease manifestation, serovars A, B, Ba and C cause trachoma; serovars D–K are associated with urogenital infections and serovars L1–L3 are responsible for lymphogranuloma venereum (LGV).¹ The L2 serovar can be further separated into different genetic variants according to the amino acid differences in the *OmpA* gene.^{3–5} The L2b–L2e genovariants are associated with the development of proctitis in men who have sex with men (MSM).^{3–4} LGV was considered as a sporadic infection in developed countries, occurring endemically only in parts of Africa, Latin America and Asia.⁶ At the end of 2003, there was an outbreak of LGV in MSM in the Netherlands, indicating the emergence of a new epidemic in this high-risk group.⁷ However, few cases of this infection have been reported in the North of Spain, with the exception of Barcelona, where a large number of cases have been notified since 2007.^{8,9}

MATERIALS AND METHODS

Patients and clinical samples

A total of 339 biological samples (303 rectal and 36 urethral Dacron swabs) were tested for *C trachomatis* by the Cobas Taqman CT system according to the manufacturer's instructions (Roche Diagnostic Systems, Branchburg, New Jersey, USA). These specimens were collected between January 2007 and November 2011 from 303 symptomatic and asymptomatic consecutive and unselected MSM (mean age was 36.7 (range 21–55)) attending the STI unit of the Hospital Monte Naranco, Oviedo, Spain, where STI screening and free-of-charge treatment are provided (about 3646 patients per year). Positive *C trachomatis* specimens were kept at -70°C in sucrose-phosphate (2SP) medium until retrospective *ompA* genotyping. All patients had promiscuous behaviour with various sexual partners, and one of them was engaged in prostitution. Three sexual partners include the study group. Three hundred were Spaniards with residence in Spain (297), the Netherlands (2) and the USA (1). Two men were Brazilians and one Moroccan. HIV, hepatitis B virus (HBV), hepatitis C virus, gonorrhoea and syphilis infections are routinely evaluated at the STI clinical unit and were made available to the present evaluation team. Retrospective ethical approval was granted by the Ethical Regional Committee of Clinical Investigation of the Principality of Asturias.

Genotyping

C trachomatis DNA was extracted using the NucliSENS easyMag system (bioMérieux, Marcy l'Etoile, France). To genotype *C trachomatis* specimens, a 990 pb fragment of the *ompA* gene was amplified according to a nested-PCR methodology described earlier.¹⁰ PCR-amplified *OmpA* gene fragments were purified from agarose gels by using the Montage DNA Gel Extraction Kit (Millipore,

Bedford, Massachusetts, USA) and sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) using inner primers (MOMP87-RVS1059).¹⁰

The amplicon sequences were aligned to analogous sequences from reference strains of each genotype by using the ClustalW2 program. The strains were A/Sa1 (accession number M58938), B/TW-5 (M17342), B/IU-1226 (AF063208), C/TW3 (M17343), D/B-120 (X62918), D/IC-Cal8 (X62920), E/Bour (X52557), F/IC-Cal3 (X52080), G/UW57 (AF063199), H/UW4 (X16007), I/UW-12 (AF063200), Ia/IU-4168 (AF063201), J/UW36 (AF063202), Ja/IU-A795 (AF063203), K/UW31 (AF063204), L1/440 (M36533), L2/434 (M14738), L2b/144276 (DQ217607), L3/404 (X55700) and *Chlamydia muridarum* MoPnT (M64171), a murine variant of *C trachomatis*. The alignments were used to obtain phylogenetic trees with the program TreeView V.1.6.6.

Statistical analyses

Statistical analysis of the data was performed using the χ^2 or Fisher's exact test. A p value of <0.05 was considered to be statistically significant. Absolute and relative frequencies and 95% accurate CIs were given.

RESULTS

A total of 40 (13.2%) rectal swabs were positive for *C trachomatis*. To investigate strain variability, *ompA* PCR-positive products were purified and sequenced. The phylogenetic analysis of the *ompA* gene from those 40 *C trachomatis*-positive specimens evidenced genotype E (n=15, 37.5%) as the most prevalent, followed by G (n=10, 25%), D (n=5, 12.5%), J (n=4, 10%) and L2b (n=2, 5%; figure 1). An illegible sequence was obtained in four cases (10%).

The association between genotype and clinical manifestations was analysed. While 25 (62.5%, 95% CI 46.2 to 78.7) patients of the 40 chlamydia-infected MSM showed any of the clinical symptoms described in table 1, no symptoms were present in 15 (37.5%, 95% CI 21.25 to 53.75) patients. The most frequent clinical symptom reported was anal ulcer (7 cases, 17.5%; 95% CI 4.47 to 30.52). It is important to emphasise that non-E genotypes were detected in 21 (84%, 95% CI 63.9 to 95.5) of the 25 patients with symptoms. On the other hand, the asymptomatic participants presented with an exclusively E genotype (p<0.001).

C trachomatis occurred simultaneously to another STI in 27 patients (67.5%, 95% CI 51.7 to 83.2): syphilis (n=10, 25%), human papillomavirus (n=7, 17.5%) and HIV-1 (n=5, 12.5%), herpes simplex virus type 2 (HSV-2; n=3, 7.5%) and gonorrhoea (n=1, 2.5%; table 2).

With regard to the previous STI episodes, only two patients had prior syphilis and HSV-2 infections.

The two patients infected with the L2b strain were detected in 2011. They were Spaniards living in Spain, with no risk for acquisition abroad, and had negative

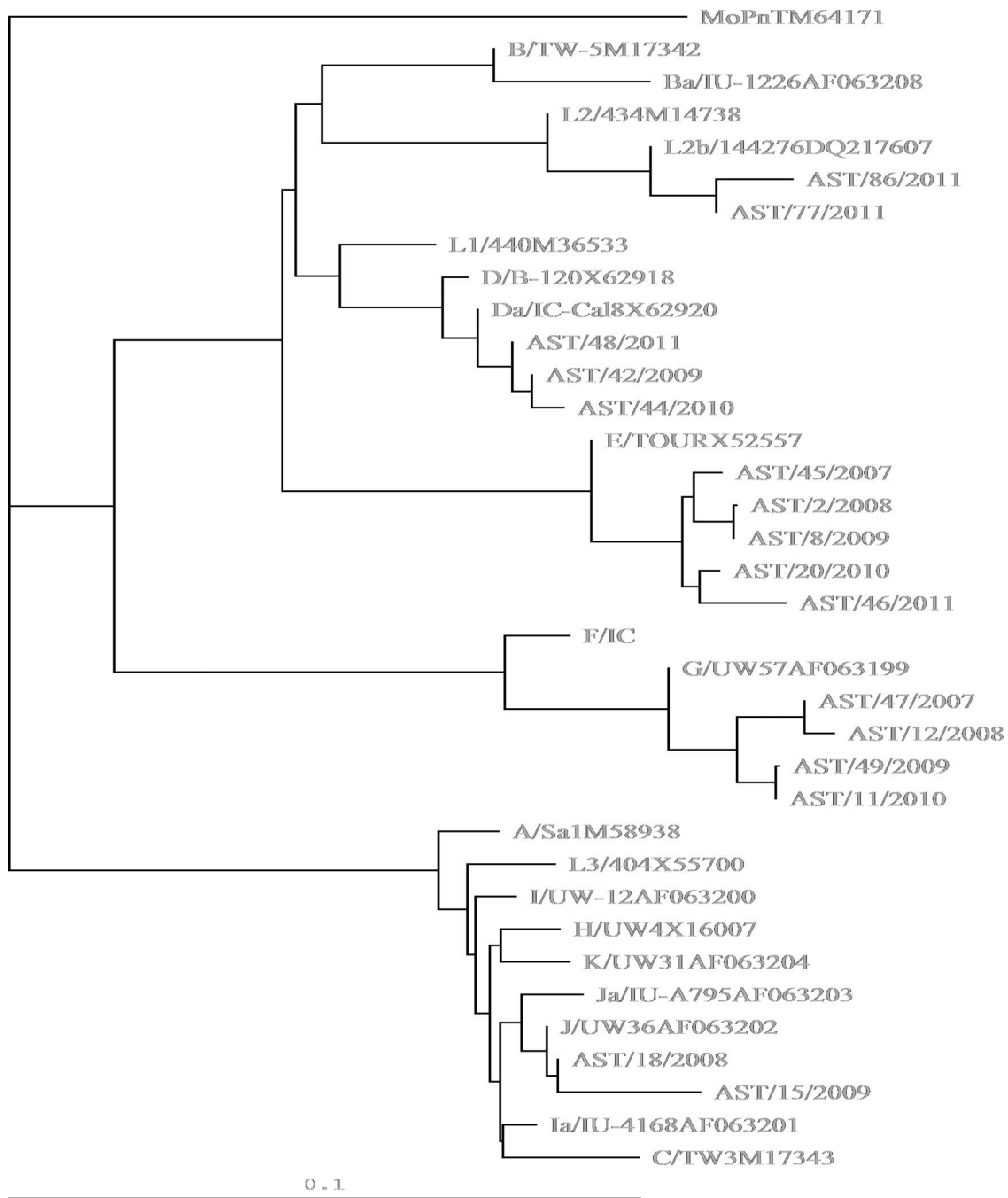


Figure 1 Phylogenetic analysis of OmpA gene sequences of 15 representative *Chlamydia trachomatis* isolates detected in the rectal samples collected from men who have sex with men (MSM) and of representative strains of *C. trachomatis* genotypes. *Chlamydia muridarum* (MoPnT) was used as an outgroup.

HIV infection at the time of diagnosis. Both participated in high-risk sexual behaviour and reported multiple anonymous sexual contacts. While one of the patients evidenced no clear symptoms of LGV (only anal pruritus), the other sought medical attention for anorectal symptoms, presenting rectal pain, mucopurulent and bloody rectal discharge, consistent with a diagnosis of acute haemorrhagic proctitis. Furthermore, this last patient suffered from concurrent venereal warts. Swollen inguinal lymph nodes were not found in either patient.

Rectal and urethral samples were collected from 36 patients, where *C. trachomatis* was detected in both the samples of 2 (5.5%) patients, and in the rectal swab only in 13 (36.1%); no case of only urethral infection was detected.

DISCUSSION

Nowadays, there are different PCR-based test systems facilitating the identification of the *C. trachomatis* genotypes. A real-time multiplex PCR assay simultaneously

Table 1 Clinical manifestations and genotypes

MSM symptomatic	N	Genotype N (%)
Anal fistula	1	D 5 (12.5)
Rectal blood loss	1	
Balanitis	3	
Anal pain	2	E 4 (10)
Unspecific abdominal pain	2	
Perianal erythema	2	G 10 (25)
Anal ulcer	7	
Anal discharge and tenesmus	1	
Unspecific penile pain	1	J 4 (10)
Dysuria and pain	2	
Inguinal pain	1	
Anal pruritus	1	L2b 2 (5)
Proctitis	1	
MSM asymptomatic	15	E 11 (27.5) Untyped 4 (10)

MSM, men who have sex with men.

detects and differentiates LGV from non-LGV strains and provides relatively rapid turnaround time (<2.5 h), being an alternative for the diagnosis of LGV infection. However, it is not capable of replacing the conventional typing system to reveal the variants of all different genotypes or within the same serovar.^{11 12} In fact, direct sequencing constitutes a powerful tool in genotyping procedures. In the present study, the use of this technique to determine *C trachomatis* circulating genotypes showed E, G and D to have the greatest frequency, a similar distribution to that reported in other European countries.¹³ An infection with genotype E was associated with a large number of clinically asymptomatic MSM. In contrast, symptomatic patients presented with a higher proportion of other genotypes, especially G and D, which could be expected, considering the putative biological properties that would facilitate their invasiveness to the rectal mucosa.¹⁴

Diagnosis of LGV, often based on history and clinical presentation, can be difficult as symptoms overlap with those of other STIs such as chancroid, herpes, syphilis and granuloma inguinale.¹⁵ Moreover, the differentiation of *C trachomatis* L genotypes is highly recommended, considering that these invasive strains require

longer antibiotic treatment and that untreated infections may result in chronic granulomatous inflammatory processes (tertiary stage). No LGV genotype was described before June 2011 in the Principality of Asturias and this study reports the two first cases of serovar L2b infection in HIV-uninfected men in this Spanish province.

Although rectal LGV is considered as the principal clinical presentation in Western countries, in this series only one patient exhibited rectal and colorectal gastrointestinal symptoms. None of the LGV-infected participants presented with typical symptoms of lymphogranuloma infection, such as lymphadenopathy.³

A high proportion of asymptomatic anorectal LGV was described in the Netherlands,¹⁶ in contrast with the findings reported by most countries.¹⁷⁻²⁰ No symptoms or unclear clinical signs may result from screening MSM during the presymptomatic phase,²¹ favouring a delay in the diagnosis and misdiagnosis.

In the present study, concurrent infection with other STIs could be documented in a high number of patients, which could evidence a decreasing awareness of MSM about the risk of transmission of STIs. In our series, the most common coinfection was syphilis, followed by condyloma acuminata, HIV-1, genital herpes, gonorrhoea and HBV, in contrast to other studies, where the most frequent coinfection was gonorrhoea.^{22 23}

C trachomatis urethral infection could be detected in a low percentage of patients, as described by others,^{23 24} and without the implications of LGV genotypes; however, considering that it was only possible to obtain samples of 36 patients in both the anus and the urethra, it is likely that there might be undiagnosed asymptomatic urethral infections among the remaining patients. Data on intercourse preferences that could underlie the apparently predominant anorectal involvement were not made available to the present study. However, other hypotheses can be raised, namely those related to particular sexual practices, such as the use of sex toys or fisting,²⁵ or to transient urethritis events that would spontaneously resolve, but during which transmigration of the pathogen occurs from the urethral epithelium to the perirectal lymphatic tissue, leading to a future inflammatory reaction in the anorectal region.²⁶ The detection of the first two confirmed cases of LGV in MSM could indicate a current

Table 2 Concurrent STIs in anorectal *Chlamydia trachomatis*-infected patients

Genotypes	Concomitant STIs (%)					
	Syphilis	HIV-1	Condylomas	HSV-2	Gonorrhoea	HBV
E	6 (15.0)	3 (7.5)	2 (5.0)			
G	2 (5.0)	1 (2.5)	3 (7.5)	3 (7.5)	1 (2.5)	
D	1 (2.5)	1 (2.5)	1 (2.5)			
J	1 (2.5)					
L2b			1 (2.5)			
Untyped						1 (2.5)
Total	10 (25.0)	5 (12.5)	7 (17.5)	3 (7.5)	1 (2.5)	1 (2.5)

HBV, hepatitis B virus; HSV-2, herpes simplex virus type 2; STIs, sexually transmitted infections.

increase of this infection in our region, and evidence the existence of LGV reservoirs, suggesting the need for an attentive surveillance of LGV in Spain where the *C trachomatis* surveillance scheme is conducted through a laboratory-based system (sentinel network composed by 18 laboratories located in 8 regions). Considering that this system does not require clinical reporting, LGV cases may remain undetected. In fact, the valuation of mild and atypical symptoms should imply quick laboratory confirmation that would allow the early implementation of appropriate therapy, contributing to the elimination of LGV reservoirs.

Surveillance programmes comprehending preventive strategies to control the onward transmission of *C trachomatis* infection, namely by the screening of populations at risk such as MSM, are urgently needed and should include the early recognition (through highly sensitive and specific molecular techniques) and treatment of LGV cases.

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