Chlamydia trachomatis serovar distribution and other sexually transmitted coinfections in subjects attending an STD outpatients clinic in Italy

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SUMMARY

We studied the prevalence of *Chlamydia trachomatis* (CT) urogenital infection and the distribution of different genotypes in a non-selected STD population of 1625 patients, evaluating presence of coinfections with other sexually transmitted diseases. Each patient was bled to perform serological tests for syphilis and HIV, then urethral or endocervical swabs were obtained for the detection of CT and *Neisseria gonorrhoeae* by culture. DNA extracted from remnant positive swabs was amplified by *omp1* Nested PCR and products were sequenced.
Total prevalence of CT infection was 6.3% (103/1625), with strong differences between men and women (11.4% vs 3.9%, $P<0.01$). Clinical symptoms and coinfections were much more frequent in men than in women ($P<0.01$). The most common serovar was E (prevalence of 38.8%), followed by G (23.3%), F (13.5%) D/Da (11.6%) and J (4.8%). Serovars distribution was statistically different between men and women ($P=0.042$) and among patients with or without coinfection ($P=0.035$); patients infected by serovar D/Da showed the highest coinfection rate.
This study can be considered a contribution in increasing knowledge on CT serovar distribution in Italy. Further studies are needed to better define molecular epidemiology of CT infection and to investigate its correlation with other STDs.

KEY WORDS: Chlamydia trachomatis, Sexually transmitted infections, Molecular epidemiology

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INTRODUCTION

Chlamydia trachomatis (CT) urogenital infections are the most commonly reported bacterial sexually transmitted diseases (STDs) (Workowski *et al.,* 2006).
Many patients remain asymptomatic and develop persistent infections which can lead to severe reproductive sequelae (Haggerty *et al.,* 2010). Furthermore, CT infection facilitates the transmission of human immunodeficiency virus (HIV) (Fleming *et al.,* 1999) and is often associated with other STDs, such as gonorrhoea, syphilis and condylomas (Donati *et al.,* 2009).
Serotyping of *C. trachomatis* is laborious since it requires multiple passages in cell cultures and the use of a large panel of monoclonal antibodies. Genotyping methods are nowadays commonly used and a variety of molecular methods such as PCR-RFLP, RT-PCR, nested PCR followed by sequencing have been reported recently (Pedersen *et al.*, 2009).
The gene encoding the major outer membrane protein (MOMP), o*mp1*, has been widely used for CT molecular epidemiology, because it contains four spaced variable domains (Bom *et al.*, 2011; Wang *et al.*, 2011). In very recent years this molecular target has been also used to develop high-resolution genotyping strategies based on microarray technologies (Christerson *et al.,* 2011; Ruettger *et al.,* 2011).

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Genotyping of CT strains is important to monitor contact tracing, to enable association with clinical manifestations or pathogenicity and may play a role in developing strategies for vaccine design (Morré *et al.,* 2000; Geisler *et al.,* 2003).
The aim of this study was to investigate CT genotype distribution in a non-selected STD population and to evaluate the presence of STD coinfections.

MATERIALS AND METHODS

In the period between July 2007 and July 2009 we enrolled 1625 consecutive patients attending the STD Outpatients Clinic of St. Orsola University Hospital of Bologna, Italy. Subjects eligible for the study were at least 18 years old, sexually active and met one or more of the following criteria: having STD related symptoms, having sexual contacts with an infected partner or unsafe sexual intercourse with new or multiple sexual partners.
Ethical approval was not required for this study due to its retrospective design and use of data obtained through routine testing.
A blood sample, for serological testing of syphilis and HIV, plus two urethral or endocervical swabs, for detection of *Neisseria gonorrhoeae* (GC) and CT by culture, were obtained from each patient. CT was isolated in LLC-MK2 cells and identified by fluorescein conjugated monoclonal antibodies (Meridian Diagnostics Inc., Cincinnati, USA) (Donati *et al.,* 2009). GC was isolated in Thayer-Martin medium and identified by API NH Assay (bioMérieux, Marcy l'Etolie, France).
Syphilis serology was performed with ARCHITECT® Syphilis TP immunoassay (Abbott Diagnostic, Wiesbaden, Germany). *Treponema pallidum* haemagglutination test (TPHA) and Rapid Plasma Reagin (RPR) card test (Radim, Pomezia, Italy), plus in-house Western Blot were used as confirmatory tests (Marangoni *et al.,* 2008).
HIV serology was performed with ARCHITECT® HIV Ag/Ab Combo assay (Abbott Diagnostic, Wiesbaden, Germany). VIDAS® HIV DUO Quick assay (bioMérieux, Marcy l'Etoile, France) and INNO-LIA™ HIV I/II Score (Innogenetics, Gent, Belgium) were the confirmatory tests.
Diagnosis of genital warts was clinical, made by visual inspection (Centers for Disease Control and Prevention, 2010).
When a positive result was obtained by CT culture, DNA from the corresponding remnant sample was extracted by VERSANT kPCR SP Module (Siemens Healthcare Diagnostics Inc., Tarrytown, USA) and used as a template for *omp1* gene fragment amplification by in-house Nested PCR assay. The primary PCR amplifying a 1222 bp region was performed using following forward and reverse oligonucleotide primers: CT90UF (5'-GGACATCTTGTCTGGCTTTAACT -3') - modified NRO (5'-GTCTCAACTGTAACTGCGTATTT-3'). Each DNA preparation (5 µl) was added to the PCR mixture (final volume 50 µl) containing final concentrations of 50mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 200 µM (each) deoxynucleoside triphosphates (dATP, dTTP, dGTP and dCTP), 20 pmol of each primer and 1.25 U of Taq DNA polymerase (Qiagen, Hilden, Germany). Cycling conditions were as follows: 5 min of denaturation at 95°C and 35 cycles each consisting of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min. A final elongation step of 10 min at 72°C completed the reaction. In the second step PCR, amplifying a 1075 bp fragment, primers SERO 1A (5'-ATGAAAAAACTCTTGAAATCGG-3') – modified NRI (5'-TACCGCAAGATTTTCTAGATTT-3') were used: 1 µl of product from the first PCR step was added to final volume of 50 µl. PCR conditions were as described above except that the annealing temperature was 53°C and 20 cycles were carried out (Lan *et al.*, 1994; Bandea *et al.*, 2001). A negative water control was included in both PCR. The amplified products were visualized after electrophoresis in 1% agarose gel by ethidium bromide staining. Products obtained from Nested-PCR were purified by using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and both strands were sequenced. The nucleotide sequences were compared to *omp1* sequences of known CT strains using the BLAST search tool at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The sequences were assembled into alignments using the following reference strains derived from GenBank: A/Sa1/OT (accession number M58938), B/TW-5 (M17324), B/IU-1226 (AF063208), C/TW3/OT (M17343), D/B-120 (X62918), D/IC-Ca1-8 (X62920), E/Bour (X52557), F/IC-Ca13 (X52080), G/UW57 (AF063199), H/UW4

(X16007), I/UW12 (AF063200), Ia/IU-4168 (AF063201), J/UW36 (AF063202), Ja/IU-A795 (AF063203), K/UW31 (AF063204), L1/440 (M36533), L2/434 (M14738) and L3/404 (X55770). The sequences were manually aligned using BioEdit (version 7.0.0) software.

Student's *t*-test was used to compare quantitative data and categorical data were analyzed with chi-square test. Data were analyzed with SPSS software for Windows 12.0 (SPSS Inc., Chicago, USA); a *P* value of <0.05 was considered statistically significant.

RESULTS

CT was detected in 103/1625 (6.3%) swabs by culture. The prevalence was significantly higher in men (chi-square =33.85; *P*<0.01), with 60 positives out of 525 tested (11.4%), than in women (43/1100; 3.9%). The mean ages (years±SD) for infected men and women were 31.4±8.2 and 26.8±5.5, respectively. Among infected men 42 (70.0%) were Italian, whereas among infected women 22 (51.2%) were Italian and 21 (48.8%) were not (table 1). Clinical symptoms correlated to CT infection were present much more frequently in infected males than in infected females (81.7% vs 44.2%, *P*<0.01). A risk sexual intercourse was reported by 7 (11.7%) men and 12 (27.9%) women, most of them (11/12) prostitutes. Thirty-five out of the 60 infected men (58.3%) had at least one coinfection and 8 of them had more than one. The most common pathogen identified was GC, detected in 27 samples, with a prevalence of 45%. Among women the prevalence of coinfections was significantly lower (chi-square=16.26; *P*<0.01), with a value of 18.6% (8/43); the most common pathogen detected in female coinfections was GC (prevalence =7.0%) (Table 1).

TABLE 1 - *Primary demographic, epidemiological and clinical data and rates of infection with C. trachomatis serovars by sex of patients.*

		Sex		
		Male	*Female*	*P value (χ^2 test)*
Place of birth	Italy	42 (70.0)	22 (51.2)	0.052
	Other country	18 (30.0)	21 (48.8)	
Symptoms	yes	49 (81.7)	19 (44.2)	0.000*
	no	11 (18.3)	24 (55.8)	
N. gonorrhoeae coinfection	yes	27 (45.0)	3 (7.0)	0.000*
	no	33 (55.0)	40 (93.0)	
T. pallidum coinfection	yes	5 (8.3)	2 (4.7)	0.696
	no	55 (91.7)	41 (95.3)	
Anogenital warts	yes	6 (10.0)	3 (7.0)	0.592
	no	54 (90.0)	40 (93.0)	
HIV coinfection	yes	5 (8.3)	0 (0,0)	0.073
	no	55 (91.7)	43 (100)	
C. trachomatis serovar	B	0 (0,0)	2 (4.7)	0.042*
	D/Da	10 (16.7)	2 (4.7)	
	E	24 (40.0)	16 (37.2)	
	F	11 (18.3)	3 (7.0)	
	G	10 (16.7)	14 (32.6)	
	H	0 (0.0)	2 (4.7)	
	I/Ia	2 (3.3)	0 (0.0)	
	J	2 (3.3)	3 (7.0)	
	K	1 (1.7)	1 (2.3)	

TABLE 2 - *Distribution of different serovars among patients with or without coinfections.*

		Presence of coinfection		
		Yes	*No*	*P value (χ^2 test)*
C. trachomatis serovar	B	0 (0.0)	2 (3.3)	0.035*
	D/Da	9 (20.9)	3 (5.0)	
	E	15 (34.8)	25 (41.7)	
	F	8 (18.6)	6 (10.0)	
	G	7 (16.2)	17(28.3)	
	H	2 (4.6)	0 (0.0)	
	I/Ia	1 (2.3)	1 (1.7)	
	J	0 (0.0)	5 (8.3)	
	K	1 (2.3)	1 (1.7)	

*Statistically significant

The most common serovar in our population was E, with a total prevalence of 38.8% (40/103), followed by G (24/103, prevalence 23.3%), F (14/103, prevalence 13.5%), D/Da (12/103, prevalence 11.6%) and J (5/103, prevalence 4.8%): taken together these strains accounted for approximately 90% of infections. Statistically significant differences (P=0.042) in the prevalence of serovars between men and women were detected: in particular, genotypes D/Da and F were much more frequent in males (16.7% vs 2.3% and 18.3% vs 6.9%, respectively), whereas females were more frequently infected by genotypes G and J (32.6% vs 16.7% and 6.9% vs 3.3% respectively). As summarized in table 2, the serovar distribution was statistically different among patients with or without coinfections (P=0.035); genotype D/Da had the highest coinfection rate (75.0%), followed by F (57.1%), E (37.5%), and G (29.2%).

DISCUSSION

Many studies have addressed the epidemiology of *C. trachomatis* genotypes throughout the world, both in selected and unselected male and female populations. The most common genotypes found in unselected populations were E (32-52%), F (14-24%), D (13-19%) and G (3-12%) (Suchland *et al.,* 2003; Gallo Vaulet *et al.,* 2010; Lagergård *et al.,* 2010; Weill *et al.,* 2010). The present findings showed a genotype distribution similar to a previous study conducted in Italy on a selected population of heterosexual men with urethritis, (Donati *et al.*, 2009) and to a similar one conducted in Greece in 2010 (Papadogeorggakis *et al.,* 2010). In both cases the prevalence of serovar G was relatively higher than that previously described in other European countries (Lagergård *et al.,* 2010), whereas the highest prevalence of genotype E was confirmed.

Particular attention has been recently focused on the possible association of specific genotypes with the presence of other STDs in particular to define if there is any difference in the rate of transmission (Papadogeorggakis *et al.,* 2010). Our study suggests that the genotype distribution among patients with or without STD coinfections is not casual.

Some bias could affect our results: first of all, the lack of information regarding uninfected patients and a more exhaustive characterization of infected population (i.e. sexual behaviour). Furthermore, the diagnosis of infection was based on culture, which is far less sensitive than PCR (Jespersen *et al.,* 2005).

On the basis of our findings further studies, possibly conducted by using a NAAT technique, could be useful to shed light on the role of different CT genotypes in affecting other STD pathogens transmission, in particular in specific subgroups of infected population.

COMPETING INTERESTS
None declared.

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