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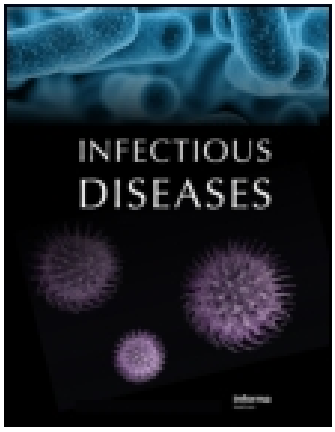

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ORIGINAL ARTICLE

Chlamydia trachomatis genovars causing urogenital infections in Santiago, Chile

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Abstract

Background: Chlamydia trachomatis is a common sexually transmitted infection in Chile, but little is known about the genovar distribution in genital infections. Thus, the objective of this study was to determine the distribution of *C. trachomatis* genovars in such cases. *Methods:* A total of 522 urogenital specimens, 403 from women and 119 from men, were analyzed for *C. trachomatis* by nested polymerase chain reaction (PCR) targeting of the *ompA* gene. Positive specimens were genotyped by DNA sequencing of the amplicons. *Results:* Sixty-two (11.9%) specimens were positive. Of these, 43 (69.4%) were collected from men and 19 (30.6%) from women ($p < 0.0001$). Eight genovars were identified in men and seven in women. Genovar E was the most common in both men and women, followed by genovar Da in men, and F in women. Together these three genovars accounted for 84% of infections. Genovar D was the third most common genovar ($n = 4$). Genovar G was detected in two samples, and sequences of genovars Ba, H, and Ja were each found in single samples. One sample (1.6%) contained mixed sequences. No association was found between gender and specific genovars. Fifty-six (92%) sequences were identical to those reported for the respective reference genovars and the other two have been described in several regions. *Conclusions:* Our findings add to the results of most studies, which indicate that genovars E, F, and D/Da are the most frequent. No association was found between gender and specific genovars. Despite the heterogeneous population of genovars, most *ompA* sequences were conserved.

Keywords: *Chlamydia, genovars, urogenital infections*

Introduction

Genital infections caused by *Chlamydia trachomatis* are associated with a wide spectrum of diseases and long-term complications: cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy, infertility, urethritis, and epididymitis [1–3]. *C. trachomatis* can be classified into 19 serovars or genovars, based on the immunoreactivity of its major outer membrane protein (MOMP/OmpA), which is encoded by the *ompA* gene, or on *ompA* polymorphism, respectively. Serovars A to C plus Ba are usually associated with endemic trachoma, and serovars L1 to L3 are the etiological agents of lymphogranuloma venereum [1]. Serovars D to K plus Da, Ia, and Ja, have a worldwide distribution and are a common cause of urogenital and neonatal infections [2,3]. Sequence analysis has demonstrated that the *ompA* gene contains five regions of conserved DNA sequence (CS1–CS5) that are interspaced with four variable sequence regions (VS1–VS4) [4,5]. OmpA has structural and functional participation in the outer membrane and is the main target of immune response [6,7]. The extent of variability exhibited by MOMP is probably driven by the host immune pressure as well as functional and structural constraints [8,9]. OmpA or its gene, *ompA*, is the most widely used target for typing of *C. trachomatis*. In addition to the fact that MOMP is a major candidate

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for inclusion in a vaccine [10], there are several reasons for *Chlamydia* typing [11]. It has provided epidemiological information for understanding the geographical or temporal dynamics of circulation of strains [12–14], for the surveillance of specific clones or strains [15,16], and for tracing of contact networks [17]. Typing has also proven to be useful to distinguish whether infections are persistent or new in cases of recurrences [18]. However, the degree of epidemiological resolution that is achieved by *ompA* genotyping is limited, especially in non-selected populations. Recent studies based on multilocus sequence typing (MLST) or multilocus variable number tandem repeat assay (MLVA) have demonstrated a greater power for strain discrimination, but this approach is still too complex to be implemented in all centers [19]. *C. trachomatis* is a prevalent sexually transmitted bacterial infection in Chile, but the molecular epidemiology of the microorganism in urogenital infections is unknown [20,21]. The objective of this study was to determine the distribution of *C. trachomatis* genovars in urogenital specimens.

Materials and methods

Study population and specimen collection

Consecutive sexually active women were enrolled during 2003–2005 at two public clinics and one private outpatient clinic in Santiago, Chile. The women were not pregnant and had not received antimicrobial agents within the past 30 days. After obtaining informed consent, endocervical specimens were collected using sterile Dacron swabs, and placed in 1.5 ml of 2 sucrose phosphate (2SP). The specimens were transported to the laboratory on ice. In parallel, 122 anonymous male urogenital samples were processed as a free quality control service offered to a clinical laboratory during the course of the study.

Polymerase chain reaction (PCR)

DNA was extracted with the Wizard SV genomic kit (Promega, WI, USA). The extracted DNA was eluted in 70 µl of H_2O, and processed within 72 h by an in-house nested PCR targeting *ompA*, as described previously [20]. Primary amplifications were performed with primers SERO1A and SERO2A, obtaining 1076 bp of the *omp1* gene [12]. Nested PCR reactions were performed with primer pairs OMP1-OMP6AS and 6S-VD4 [12]. The resulting amplicons, 458 bp and 390 bp long, respectively, included VS1–VS4.

OmpA sequence analysis

All cervical, urethral or urine specimens that were positive by PCR were subjected to *omp1* genotyping, performed as described previously [22]. Amplicons were purified using the Qiaquick^R PCR purification kit (Qiagen, Hilden, Germany) and sequenced in each direction, applying the Big Dye Sequencing Terminator kit (Applied Biosystems, CA, USA) in an automated DNA sequencer, either ABI 377 or ABI 310 (Applied Biosystems). Individual consensus sequences of the clinical specimens were analyzed by comparison with the *ompA* nucleotide sequences of known *C. trachomatis* strains by BLAST searching at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The sequences were assembled into alignments using the freeware sequence tool BioEdit (version 7.0) software. Reference sequences used for comparison were derived from GenBank: Ba/Apache-2 (AFO63194); B/IU 1226 (AF063208); D/IC-Cal8 (DQ064285); D/B-120 (X62918); D/UW-3 (DQ064284; Da/TW-448 (X62921); E/Bour (X52557); F/IC-CAL3 (DQ064287); G-UW57 (DQ064299); H/UW4 (X16007); J/UW/36/Cx (AF063202); and Ja/IU-37538 (AF202458). Nucleotide sequences of the new variants, 2117 and 1717, have been deposited in GenBank under accession numbers KF615762 and KF615763, respectively.

Statistical analysis

The association of chlamydial genovars with gender was estimated by the Fisher's exact test. Statistical significance was established at $p < 0.05$.

Ethics

The study was approved by the Committee of Ethics of the Clinical Hospital of the University of Chile, Santiago, Chile, and patients gave their informed consent.

Results

A total of 403 samples from women and 119 from men were adequate for PCR analysis and were processed. The mean (± SD) ages of the women and men were 33.2 (± 8.65) and 34.2 (± 11.52) years, respectively ($p = 0.190$).

Sixty-two of 522 specimens (11.9%) were positive for *C. trachomatis*. Of these, 43 (69.4%) were collected from men and 19 (30.6%) from women ($p < 0.0001$).

Eight genovars were identified in men and seven in women: genovars Ba, D, Da, E, F, G, H, and J. Table I shows the distribution of *ompA* sequences according to gender. Genovar E was the most common in both men and women, followed by genovar Da in men, and F in women. Together these three genovars accounted for 52 (84%) of the infections. Genovar D, with four positive sequences was the third most common genovar. Genovar G was detected in two samples, and sequences of genovars Ba, H, and J were each found in single samples. The specimen from one man contained a mix of sequences corresponding to genovars D and E. No association was found between gender and specific genovars.

Fifty-six samples (92%) had *ompA* sequences identical to those reported for the respective reference strains. The other five sequences (8%) were variants but three of them had been reported before. The single Ba sequence detected in this study is a variant of reference strain Ba/Apache-2. The *ompA* sequence was identical to that of strain B/IU-1226, which was first described as genotype B/b by Frost et al. [23] and subsequently as strain B/IU-1226 by Stothard et al. [24]. With regard to genovars D and Da, six sequences of genovar Da and four sequences of genovar D were identified. The sequences of both genovars were identical to that of their prototype genovars: strain D/IC-Cal-8 and D/B-120, respectively. For genovar E, 38 sequences were identical to the reference strain E/Bour published by Peterson et al. [25] and 2 (5%) differed in 1 base. Sequence 1717 had the transition C266T that resulted in the amino acid substitution Ala→Gly, while sequence 2117 presented the transition A307G, resulting in the amino acid change Thr→Ala. Both non-synonymous mutations presented in the VS1 of *ompA*. To confirm the nucleotide changes in the *ompA* of the E variants, the samples were reamplified and subsequently sequenced. Since these mutations have not been described before, they seem to be restricted to Chile. Five of six sequences of genovar F were identical to reference strain F/IC-CAL3. The sixth sequence was similar to strain CL-1-2003 (FJ156357), previously identified in a case of neonatal pneumonia in Chile [22]. Strain CL-1-2003 exhibits two synonymous mutations in comparison with prototype strain F/IC-CAL3, one in CS3 and one in CS5. With regard to genovar G, the sequence of one sample was identical to that of reference strain G-UW57, while the second sequence had the transversion G487A, which substitutes glycine for serine at the residue 163 of VS2, and that has been reported in several regions [17,26,27]. The single *ompA* sequence of genovar J differed in 12 nucleotides compared with the reference strain J/UW/36/Cx, being identical to strain Ja/IU-37538 reported previously by Stothard et al. [24] in the USA. No variation was detected for the single sequence of genovar H compared to its reference strain.

Table I. *C. trachomatis* genovars in 522 urogenital samples from adults.

Genovar	No. (%) of persons infected with genovar			*p* value
	Men	Women	Total: *n* (%)	
Ba	1 (2.3)	0	1 (1.6)	
D	3 (7)	1 (5.3)	4 (6.5)	1
Da	6 (14)	0	6 (9.7)	0.17
E	29 (67.4)	11 (57.8)	40 (64.5)	0.57
F	2 (4.7)	4 (21)	6 (9.7)	0.06
G	1 (2.3)	1 (5.3)	2 (3.2)	0.57
H	0	1 (5.3)	1 (1.6)	
Ja	0	1 (5.3)	1 (1.6)	
Mixed D + E	1 (2.3)	0	1 (1.6)	
Total	43 (100)	19 (100)	62 (100)	

Discussion

Overall, eight distinct genovars were observed in this cohort. Genovars E, D/Da, and F were the most prevalent, accounting for most chlamydial infections. This distribution is similar to that reported in most regions [12–14,26–30]. By contrast, the geographical distribution of the less frequent genovars comprising the serogroup C of *C. trachomatis* (genovars A, C, H, I, Ia, J, Ja, and K) is quite heterogeneous. For example, genovar H is usually reported in 1–2% of urogenital specimens [12,14,17] but two groups reported this genovar in 13–19% of specimens [27,28]. Genovar Ia is very uncommon, but in the USA it constitutes 14–16% of the reported genovars [14,27]. Interestingly, Suchland et al. followed the temporal dynamics of chlamydial genovars for 9 years and showed that most common genovars had a stable distribution over time, while minor genovars such as I and K showed large fluctuations [13]. The single sequence of genovar Ja in this study has the same genotype as strain Ja/IU-37538, described by Dean et al. [18]. This strain has been previously identified from three cases of neonatal pneumonia, suggesting that although infrequent it is the only Ja genotype circulating in Santiago [22]. Four genovars of serogroup B were identified: genovars Ba, D, Da, and E. The single sequence of genovar Ba was identical to strain B/IU 1226. Within Latin America, genovars B/Ba have been found in 1.8% of urogenital infections in Brazil [31] and 1.2% in Costa Rica [32], whereas in Argentina they were absent from a large sample of patients [26].

Two genotypes of genovar D/Da, with a comparable frequency, were found in this study. One of them corresponds to genovar D, and the other to genovar Da. The same was observed in a previous study confirming that genovars D and Da circulate with apparently similar success [22]. As described by Sayada et al. [33], 10 point mutations distinguish prototype D strain D/UW-3 from prototype Da strain D/IC-Cal8. Furthermore, three point mutations at positions 636, 939, and 997, distinguish reference strain Da/TW-448 from prototype Da strains such as D/IC-Cal8. With respect to genovar E, both genetic variants present non-synonymous mutations in VS1, which is surface exposed and likely under the immune pressure of the host. Interestingly, these mutations have not been described before and are probably restricted to Chile, suggesting that mutations do not provide an adaptative advantage to the variants. A similar mutation pattern is exhibited by the F variant (intermediate serogroup), with two silent mutations presenting in CS3 and CS5.

Nunes et al. [9] analyzed the *ompA* mutational trends of *C. trachomatis* in 795 clinical specimens from Portugal, showing genetic facts that help to understand the ecological success of genotypes E and F with respect to other genotypes. They found that genovars E and F show a significant lower mutational rate than other genovars, and that the *ompA* mutations exhibited by some variants provide a better structural and functional fitness to OmpA. Thus, the ecological success of genovars E and F may be the result of a better fitness in their interaction with the host immune system and structural and functional constraints in MOMP.

In conclusion, our findings add to the results of most studies, which indicate that genovars E, F, and D/Da are the most frequent. No association was found between gender and specific genovars. Despite the heterogeneous population of genovars, most *ompA* sequences were conserved.

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