Distribution of *Chlamydia trachomatis* genovars among youths and adults in Brazil

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Despite a high prevalence of sexually transmitted *Chlamydia trachomatis* infections in Brazil and other countries in South America, very little is known about the distribution of *C. trachomatis* genovars. In this study, we genotyped *C. trachomatis* strains from urine or endocervical specimens collected from 163 *C. trachomatis*-positive female and male youths, and female adults, residing in two different regions of Brazil, the city of Goiânia located in the central part of Brazil, and the city of Vitória in the south-east region. *C. trachomatis* strains were genotyped by amplifying and sequencing the *ompA* gene encoding the chlamydial major outer-membrane protein, which is genovar specific. We found nine different *C. trachomatis* genovars: E (39.3 %), F (16.6 %), D (15.9 %), I (8.6 %), J (7.4 %), G (4.9 %), K (3.1 %), H (2.4 %) and B (1.8 %). The distribution of the *C. trachomatis* genovars in the two regions of Brazil was similar, and there was no statistically significant association of serovars with age, gender, number of sexual partners or clinical symptoms. The overall distribution of *C. trachomatis* genovars in Brazil appears similar to that found in other regions of the world, where E, D and F are the most common. This supports the notion that, during the last few decades, the overall distribution of *C. trachomatis* genovars throughout the world has been relatively stable.

Received 24 September 2010

Accepted 22 December 2010

INTRODUCTION

Chlamydia trachomatis urogenital infection is the primary cause of bacterial sexually transmitted diseases (STDs) in the world (WHO, 2001). In Brazil, several studies using highly sensitive diagnostic tests based on nucleic acid amplification technology have showed a prevalence of *C. trachomatis* infections ranging from 5.0 to 19.6 % among youths attending outpatient clinics and gynaecological clinics, and taking part in the Family Health programme (Guimarães *et al.*, 2009; Araújo *et al.*, 2006; Fioravante *et al.*, 2005; Miranda *et al.*, 2004).

Using polyvalent mouse antiserum, *C. trachomatis* isolates historically have been classified into 14 major serovars, A to K and L1, L2 and L3 (Wang *et al.*, 1973). Urogenital tract infections are usually caused by serovars D to K. After it was discovered that serovar specificity resides in the major

outer-membrane protein (MOMP) (Caldwell *et al.*, 1981), immunotyping approaches were replaced by genotyping methods that identify genovar-specific DNA sequence variation markers within the MOMP-encoding gene, *ompA* (referred to in some publications as *omp1*) (Stothard *et al.*, 1998; Yuan *et al.*, 1989; Stephens *et al.*, 1987). New genotyping approaches have recently been developed, such as multilocus variable number tandem repeat and multilocus sequence typing analyses, that increase the epidemiological resolution among the various *C. trachomatis* strains (Pedersen *et al.*, 2008; Klint *et al.*, 2007). However, these approaches have been used within the framework of the current *ompA*-based *C. trachomatis* classification system as there is not yet a standardized nomenclature or classification system based on these novel typing methods (Pedersen *et al.*, 2009).

Although the distribution of *C. trachomatis* genovars in some regions of the world is well known (Taheri Beni *et al.*,

Abbreviation: STD, sexually transmitted disease.

2010; Petrovay *et al.*, 2009; Bandea *et al.*, 2008; Millman *et al.*, 2004; Ngandjio *et al.*, 2003; Jurstrand *et al.*, 2001; Bandea *et al.*, 2001), there is only one published study from Brazil, which reported the distribution of *C. trachomatis* genovars among a small group of nine women attending an STD clinic in the south-east region (Lima *et al.*, 2007). The aims of the present study were to investigate the distribution of *C. trachomatis* genovars in a larger population and their potential associations with socio-demographic characteristics and clinical symptoms.

METHODS

Study population and data collection. The specimen collection, demographic, and sexual and behavioural characteristics of patients included in this study have been described previously (Guimarães *et al.*, 2009; Barcelos *et al.*, 2008; Araújo *et al.*, 2006; Fioravante *et al.*, 2005; Miranda *et al.*, 2004). A summary of these studies is outlined in Table 1. The participants in the study were female and male youths (age range 14–24 years old) and female adults (age range 25–49 years old) residing in Goiânia, in the central region of Brazil, and in Vitória, a city in the south-east region. Most participants were recruited from public health services, except for the male conscripts. The biological samples were collected between 2000 and 2004, and screened for *C. trachomatis* using commercial nucleic acids amplification tests. A total of 187 *C. trachomatis*-positive samples (first catch urine, 82; endocervical swab, 105) were shipped on dry ice to the Centers for Disease Control and Prevention in Atlanta, where they were genotyped. The protocol for this study was approved by the Ethics Committee at the University Hospital, Federal University of Goiás, and the National Council of Research in Brazil, and by the Institutional Review Board of the Centers for Disease Control and Prevention.

C. trachomatis genotyping. *C. trachomatis* genotyping was performed as previously described (Bandea *et al.*, 2001), using the PCR and sequencing primers shown in Table 2. To determine the *C. trachomatis* genovars we used the neighbour-joining phylogenetic program provided in the GeneStudio package (GeneStudio Inc.).

Statistical analysis. Statistical analysis was performed using SPSS 15.0. Association of chlamydial genovars to demographic and behavioural characteristics was assessed with the χ^2 test or Fisher exact test. Statistical significance was established as $P < 0.05$.

RESULTS AND DISCUSSION

This is believed to be the first large molecular epidemiological study on the distribution of urogenital *C.*

trachomatis genovars in Brazil, and their association with age, gender, number of sexual partners and clinical symptoms. From the total of 187 specimens positive for *C. trachomatis* by PCR or LCR (ligase chain reaction) included in this study, we were able to amplify and sequence the *ompA* gene from 163 (87 %) specimens, 91 from endocervical specimens and 72 from urine specimens. Despite at least two amplification attempts with each of two nested primer sets used in the study protocol (Table 2), we were not able to amplify the *ompA* gene from 24 (13 %) specimens. There are several potential reasons for the apparent lower sensitivity of our in-house amplification assays. Possibly, some of the initial diagnostic results were false positives. Another potential explanation is that the target for the commercial diagnostic tests was a multi-copy plasmid (Mahony *et al.*, 1993), whereas the *ompA* gene, which was the target of our assay, is only present at one copy per organism.

We found nine different genovars in our study population: E (39.3 %), F (16.6 %), D (15.9 %), I (8.6 %), J (7.4 %), G (4.9 %), K (3.1 %), H (2.4 %) and B (1.8 %). In the municipality of Goiânia genovar E was the most frequent (36.3 %), followed by genotype D (19.5 %) and F (16.8 %). In Vitória, genovar E also showed higher prevalence (46.0 %) followed by genovar F (16.0 %) and I (10.0 %). Interestingly, three participants from our study population were infected with genovar B, which is usually associated with ocular *C. trachomatis* infections; however, there have been similar reports of ocular genovars found in the urogenital tract (Millman *et al.*, 2004; Darville, 2005).

The only published genotyping study from Brazil was that reported by Lima *et al.* (2007) in which four different *C. trachomatis* genovars (E, D, F and K) were identified among a small group of nine infected female patients between the ages of 15 and 54 attending an STD clinic in south-eastern Brazil. The most prevalent *C. trachomatis* genovars in our Brazilian study population were E, F and D. In Costa Rica, among 806 *C. trachomatis*-positive samples obtained from young women, E (31 %) was the most common genovar, followed by F (21 %) and D (21 %) (Quint *et al.*, 2007). Also, E was the most frequent genovar in the metropolitan area of Buenos Aires, Argentina, where 199 samples positive for *C. trachomatis* infection were genotyped from a population of symptomatic adults and neonates (Gallo Vaulet *et al.*, 2010).

Table 1. Positive *C. trachomatis* subjects and specimen types ($n=187$)

Study	Study population	Specimen	Collection period	No. of samples
Guimarães <i>et al.</i> (2009)	Female youths ($n=427$)	Endocervical swab	July 2003 to January 2004	58
Barcelos <i>et al.</i> (2008)	Female youths and adults ($n=300$)	Urine	August 2003 to March 2004	22
Araújo <i>et al.</i> (2006)	Female youths ($n=296$)	Endocervical swab	March 2000 to March 2001	47
Fioravante <i>et al.</i> (2005)	Male youths – military conscripts ($n=654$)	Urine	September to October 2000	26
Miranda <i>et al.</i> (2004)	Female youths ($n=461$)	Urine	March to June 2003	34

Table 2. *C. trachomatis* primers for *ompA* amplification and sequencing

Name	Sequence
Primary PCR primers	
CT90UF	5'-GGACATCTTGTCTGGCTTAACT-3'
T220DR	5'-GCGCTCAAGTAGACCGATATAGTA-3'
CT662UF	5'-AGCTTAGGAGCTTCTTTCCAATA-3'
CT1180DR	5'-CGGAATTGTGCATTTACGTGAG-3'
Secondary PCR primers	
CT60UF	5'-GTCCCGCCAGAAAAAGATAG-3'
T80DR	5'-CCAGAAACACGGATAGTGTATTATTA-3'
CT725UF	5'-TCTCTGTAATGCATCCGAATTTACTAT-3'
CT1086DR	5'-TCGAATACCGCAAGATTTTCT-3'
Sequencing primers	
CT40F	5'-ATAGCGAGCACAAAGAGAGC-3'
CT419F	5'-TGGGATCGTTTTGATGTATT-3'
CT902F	5'-TCCTTACATTGGAGTTAAATGGTC-3'
CT725F	5'-TCTCTGTAATGCATCCGAATTTACTAT-3'
CT1086R	5'-TCGAATACCGCAAGATTTTCT-3'

Most studies from other regions of the world found a similar distribution of *C. trachomatis* genovars. For example, in a study performed in Sweden with 237

urogenital samples, the most prevalent genotype was E (47.3%), followed by F (17.3%) (Jurstrand *et al.*, 2001). In a larger study ($n=678$) from the same country, the most

Table 3. Distribution of *C. trachomatis* genovars according to socio-demographic characteristics and clinical symptoms

A χ^2 test with three degrees of freedom was applied to compare the relative frequency of four groups (genotypes D, E, F and others) versus socio-demographic characteristics and clinical symptoms. Age group: $\chi^2=1.88$, $P=0.59$; gender: $\chi^2=4.34$, $P=0.23$; city: $\chi^2=3.79$, $P=0.28$; specimens: $\chi^2=7.65$, $P=0.05$; lifetime sexual partners: $\chi^2=4.39$, $P=0.22$; symptoms: $\chi^2=5.52$, $P=0.13$.

Characteristics of patients	No. of samples	No. of isolates of each genotype (%)								
		B	D	E	F	G	H	I	J	K
Age*										
14–19 years	133	3 (2.2)	23 (17.3)	52 (39.1)	23 (17.3)	4 (3.0)	4 (3.0)	13 (9.8)	8 (6.0)	3 (2.2)
≥20 years	25	0	2 (8.0)	10 (40.0)	4 (16.0)	2 (8.0)	0	1 (4.0)	4 (16.0)	2 (8.0)
Gender										
Female	141	3 (2.1)	24 (17.0)	56 (39.7)	25 (17.7)	3 (2.1)	4 (2.8)	12 (8.5)	9 (6.4)	5 (3.5)
Male	22	0	2 (9.1)	8 (36.4)	2 (9.1)	5 (22.7)	0	2 (9.1)	3 (13.6)	0
City										
Goiânia	113	2 (1.8)	22 (19.5)	41 (36.3)	19 (16.8)	7 (6.2)	3 (2.7)	9 (8.0)	9 (8.0)	1 (0.9)
Vitória	50	1 (2.0)	4 (8.0)	23 (46.0)	8 (16.0)	1 (2.0)	1 (2.0)	5 (10.0)	3 (6.0)	4 (8.0)
Specimen										
Endocervical swab	91	2 (2.2)	20 (22.0)	33 (36.3)	17 (18.7)	2 (2.2)	3 (3.3)	7 (7.7)	6 (6.6)	1 (1.1)
Urine	72	1 (1.4)	6 (8.3)	31 (43.0)	10 (13.9)	6 (8.3)	1 (1.4)	7 (9.7)	6 (8.3)	4 (5.5)
No. of sexual partners in life†										
1–3	94	1 (1.1)	17 (18.1)	38 (40.4)	18 (19.1)	2 (2.1)	1 (1.1)	10 (10.6)	5 (5.3)	2 (2.1)
≥4	63	2 (3.2)	8 (12.7)	24 (38.1)	8 (12.7)	4 (6.3)	3 (4.8)	4 (6.3)	7 (11.1)	3 (4.8)
Symptoms‡										
Yes	94	2 (2.1)	17 (18.1)	36 (38.3)	20 (21.3)	2 (2.1)	0	8 (8.5)	6 (6.4)	3 (3.2)
No	63	1 (1.6)	7 (11.1)	28 (44.4)	7 (11.1)	4 (6.3)	4 (6.3)	5 (7.9)	5 (7.9)	2 (3.2)

*There were five participants without this information.

†There were five participants without this information.

‡There were six participants without this information.

frequent genovar was E (39 %), followed by F (21 %), G (11 %), D and K (both 9 %), J (7 %), H (2 %), and B and Ia (both 1 %) (Lysén *et al.*, 2004). A study conducted in Iceland in a population attending an STD clinic ($n=330$) found, in descending order of prevalence, genovars E, D, J, F, K, G, H and I (Jónsdóttir *et al.*, 2003). Among 203 urine samples collected in Australia, the E serovar was also the most prevalent (42.4 %), followed by F (23.6 %) and G (16.3 %) (Mossman *et al.*, 2008); and among 102 samples of endocervical swabs or urine from patients visiting two hospitals in Taiwan, genotype E was the most prevalent (22 %), followed by D (19 %), F (16 %) and J (15 %) (Hsu *et al.*, 2006). However, in a study in Thailand, the prevalence of *C. trachomatis* genovar E was only 9.3 %, with F (25 %) being the most prevalent (Bandeia *et al.*, 2001); and in Hungary, a study conducted among 484 female sex workers identified genotype D as the most prevalent (34.4 %), followed by E (21.9 %) and F (18.8 %) (Petrovay *et al.*, 2009).

Table 3 shows the distribution of *C. trachomatis* genovars according to socio-demographic and clinical symptoms. The distribution *C. trachomatis* genovars was similar among the age groups, and between men and women, and there was no statistically significant association of the *C. trachomatis* genovars with the number of lifetime sexual partners, or with clinical symptoms such as lower abdominal pain, vaginal/urethral discharge or dysuria; however, these comparisons are limited by the low numbers of positives samples in some subgroups and the large diversity of genotypes identified.

The potential association of *C. trachomatis* genovars with specific clinical symptoms, or disease, is not well understood. Van Duynhoven *et al.* (1998) found an association of genovars H and J with urethral discharge and dysuria in men, and an association of genovars F and G with lower abdominal pain in women. A study by Van de Laar *et al.* (1996) indicated that genovars F and G cause milder symptoms and signs of inflammation in men but not in women. Another study conducted by Gao *et al.* (2007) reported an association between genotype G and lower abdominal pain. After controlling for age and race, Geisler *et al.* (2003) found that women who reported abdominal pain and/or dyspareunia were more likely to be infected with genovar F. An association of specific clinical manifestations with genovars was not detected in men. In a study performed with females from an STD high-risk population in Senegal, Sturm-Ramirez *et al.* (2000) reported that there were no visible signs of cervical inflammation in females infected with genovar E compared with those infected with other *C. trachomatis* genovars. Similarly, several other studies have found no statistically significant difference in the distribution of *C. trachomatis* genovars between patients with symptomatic or asymptomatic infections (Lysén *et al.*, 2004; Persson & Osler, 1993; Millman *et al.*, 2004; Batteiger *et al.*, 1989). Hopefully, the new genotyping methods, such as multilocus variable number tandem repeat and multilocus sequence typing,

will lead to a new classification system for *C. trachomatis* strains and to improved algorithms for studying their association with pathological and clinical outcomes (Pedersen *et al.*, 2008, 2009; Klint *et al.*, 2007).

In conclusion, we found that the overall distribution of *C. trachomatis* genovars in Brazil, where E, F and D are the most common genovars, is similar to that found in other regions of the world. This supports the notion that, during the last few decades, the circulation and the overall distribution of *C. trachomatis* genovars throughout the world have been relatively stable.

ACKNOWLEDGEMENTS

A. C. S. M. was supported by a scholarship from the National Council of Technological and Scientific Development (CNPq), Brazil (project number 141821/2006-0). We thank Y. A. R. Lima for technical assistance.

REFERENCES

- Araújo, R. S., Guimarães, E. M., Alves, M. F., Sakurai, E., Domingos, L. T., Fioravante, F. C. & Machado, A. C. (2006). Prevalence and risk factors for *Chlamydia trachomatis* infection in adolescent females and young women in central Brazil. *Eur J Clin Microbiol Infect Dis* 25, 397–400.
- Bandeia, C. I., Kubota, K., Brown, T. M., Kilmarx, P. H., Bhullar, V., Yanpaisarn, S., Chaisilwattana, P., Siriwasin, W. & Black, C. M. (2001). Typing of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp1*). *Sex Transm Infect* 77, 419–422.
- Bandeia, C. I., Debattista, J., Joseph, K., Igietseme, J., Timms, P. & Black, C. M. (2008). *Chlamydia trachomatis* serovars among strains isolated from members of rural indigenous communities and urban populations in Australia. *J Clin Microbiol* 46, 355–356.
- Barcelos, M. R., Vargas, P. R., Baroni, C. & Miranda, A. E. (2008). Genital infections in women attending a primary unit of health: prevalence and risk behaviors. *Rev Bras Ginecol Obstet* 30, 349–354.
- Batteiger, B. E., Lenington, W., Newhall, W. J., Katz, B. P., Morrison, H. T. & Jones, R. B. (1989). Correlation of infecting serovar and local inflammation in genital chlamydial infections. *J Infect Dis* 160, 332–336.
- Caldwell, H. D., Kromhout, J. & Schachter, J. (1981). Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. *Infect Immun* 31, 1161–1176.
- Darville, T. (2005). *Chlamydia trachomatis* infections in neonates and young children. *Semin Pediatr Infect Dis* 16, 235–244.
- Fioravante, F. C., Costa Alves, M. F., Guimarães, E. M., Turchi, M. D., Freitas, H. A. & Domingos, L. T. (2005). Prevalence of *Chlamydia trachomatis* in asymptomatic Brazilian military conscripts. *Sex Transm Dis* 32, 165–169.
- Gallo Vaulet, L., Entrocassi, C., Corominas, A. I. & Rodríguez Fermepein, M. (2010). Distribution study of *Chlamydia trachomatis* genotypes in symptomatic patients in Buenos Aires, Argentina: association between genotype E and neonatal conjunctivitis. *BMC Res Notes* 3, 34.
- Gao, X., Chen, X. S., Yin, Y. P., Zhong, M. Y., Shi, M. Q., Wei, W. H., Chen, Q., Peeling, R. W. & Mabey, D. (2007). Distribution study of *Chlamydia*

- trachomatis* serovars among high-risk women in China performed using PCR-restriction fragment length polymorphism genotyping. *J Clin Microbiol* 45, 1185–1189.
- Geisler, W. M., Suchland, R. J., Whittington, W. L. H. & Stamm, W. E. (2003). The relationship of serovar to clinical manifestations of urogenital *Chlamydia trachomatis* infection. *Sex Transm Dis* 30, 160–165.
- Guimarães, E. M., Guimarães, M. D., Vieira, M. A., Bontempo, N. M., Seixas, M. S., Garcia, M. S., Daud, L. E., Côrtes, R. L. & Alves, M. F. (2009). Lack of utility of risk score and gynecological examination for screening for sexually transmitted infections in sexually active adolescents. *BMC Med* 7, 8.
- Hsu, M. C., Tsai, P. Y., Chen, K. T., Li, L. H., Chiang, C. C., Tsai, J. J., Ke, L. Y., Chen, H. Y. & Li, S. Y. (2006). Genotyping of *Chlamydia trachomatis* from clinical specimens in Taiwan. *J Med Microbiol* 55, 301–308.
- Jónsdóttir, K., Kristjánsson, M., Hjaltalín Olafsson, J. & Steingrímsson, O. (2003). The molecular epidemiology of genital *Chlamydia trachomatis* in the greater Reykjavik area, Iceland. *Sex Transm Dis* 30, 249–256.
- Jurstrand, M., Falk, L., Fredlund, H., Lindberg, M., Olcén, P., Andersson, S., Persson, K., Albert, J. & Bäckman, A. (2001). Characterization of *Chlamydia trachomatis* *omp1* genotypes among sexually transmitted disease patients in Sweden. *J Clin Microbiol* 39, 3915–3919.
- Klint, M., Fuxelius, H. H., Goldkuhl, R. R., Skarin, H., Rutemark, C., Andersson, S. G., Persson, K. & Herrmann, B. (2007). High-resolution genotyping of *Chlamydia trachomatis* strains by multilocus sequence analysis. *J Clin Microbiol* 45, 1410–1414.
- Lima, H. E., Oliveira, M. B., Valente, B. G., Afonso, D. A., Darocha, W. D., Souza, M. C., Alvim, T. C., Barbosa-Stancioli, E. F. & Noronha, F. S. (2007). Genotyping of *Chlamydia trachomatis* from endocervical specimens in Brazil. *Sex Transm Dis* 34, 709–717.
- Lysén, M., Österlund, A., Rubin, C. J., Persson, T., Persson, I. & Herrmann, B. (2004). Characterization of *ompA* genotypes by sequence analysis of DNA from all detected cases of *Chlamydia trachomatis* infections during 1 year of contact tracing in a Swedish county. *J Clin Microbiol* 42, 1641–1647.
- Mahony, J. B., Luinstra, K. E., Sellors, J. W. & Chernesky, M. A. (1993). Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J Clin Microbiol* 31, 1753–1758.
- Millman, K., Black, C. M., Johnson, R. E., Stamm, W. E., Jones, R. B., Hook, E. W., Martin, D. H., Bolan, G., Tavaré, S. & Dean, D. (2004). Population-based genetic and evolutionary analysis of *Chlamydia trachomatis* urogenital strain variation in the United States. *J Bacteriol* 186, 2457–2465.
- Miranda, A. E., Szwarcwald, C. L., Peres, R. L. & Page-Shafer, K. (2004). Prevalence and risk behaviors for chlamydial infection in a population-based study of female adolescents in Brazil. *Sex Transm Dis* 31, 542–546.
- Mossman, D., Beagley, K. W., Landay, A. L., Loewenthal, M., Ooi, C., Timms, P. & Boyle, M. (2008). Genotyping of urogenital *Chlamydia trachomatis* in regional New South Wales, Australia. *Sex Transm Dis* 35, 614–616.
- Ngandjio, A., Clerc, M., Fonkoua, M. C., Thonnon, J., Njock, F., Pouillot, R., Lunel, F., Bebear, C., De Barbeyrac, B. & Bianchi, A. (2003). Screening of volunteer students in Yaounde (Cameroon, Central Africa) for *Chlamydia trachomatis* infection and genotyping of isolated *C. trachomatis* strains. *J Clin Microbiol* 41, 4404–4407.
- Pedersen, L. N., Pødenphant, L. & Møller, J. K. (2008). Highly discriminative genotyping of *Chlamydia trachomatis* using *omp1* and a set of variable number tandem repeats. *Clin Microbiol Infect* 14, 644–652.
- Pedersen, L. N., Herrmann, B. & Møller, J. K. (2009). Typing *Chlamydia trachomatis*: from egg yolk to nanotechnology. *FEMS Immunol Med Microbiol* 55, 120–130.
- Persson, K. & Osler, S. (1993). Lack of evidence of a relationship between genital symptoms, cervicitis and salpingitis and different serovars of *Chlamydia trachomatis*. *Eur J Clin Microbiol Infect Dis* 12, 195–199.
- Petrovay, F., Balla, E., Németh, I. & Gönczöl, E. (2009). Genotyping of *Chlamydia trachomatis* from the endocervical specimens of high-risk women in Hungary. *J Med Microbiol* 58, 760–764.
- Quint, K., Porras, C., Safaeian, M., González, P., Hildesheim, A., Quint, W., Van Doorn, L. J., Silva, S., Melchers, W. & other authors (2007). Evaluation of a novel PCR-based assay for detection and identification of *Chlamydia trachomatis* serovars in cervical specimens. *J Clin Microbiol* 45, 3986–3991.
- Stephens, R. S., Sanchez-Pescador, R., Wagar, E. A., Inouye, C. & Urdea, M. S. (1987). Diversity of *Chlamydia trachomatis* major outer membrane protein genes. *J Bacteriol* 169, 3879–3885.
- Stothard, D. R., Boguslawski, G. & Jones, R. B. (1998). Phylogenetic analysis of the *Chlamydia trachomatis* major outer membrane protein and examination of potential pathogenic determinants. *Infect Immun* 66, 3618–3625.
- Sturm-Ramirez, K., Brumblay, H., Diop, K., Guèye-Ndiaye, A., Sankalé, J. L., Thior, I., N'Doye, I., Hsieh, C. C., Mboup, S. & Kanki, P. J. (2000). Molecular epidemiology of genital *Chlamydia trachomatis* infection in high-risk women in Senegal, West Africa. *J Clin Microbiol* 38, 138–145.
- Taheri Beni, B., Motamedi, H. & Ardakani, M. R. (2010). Genotyping of the prevalent *Chlamydia trachomatis* strains involved in cervical infections in women in Ahvaz, Iran. *J Med Microbiol* 59, 1023–1028.
- Van de Laar, M. J., Van Duynhoven, Y. T., Fennema, J. S., Ossewaarde, J. M., Van den Brule, A. J., Van Doornum, G. J., Coutinho, R. A. & Van den Hoek, J. A. (1996). Differences in clinical manifestations of genital chlamydial infections related to serovars. *Genitourin Med* 72, 261–265.
- Van Duynhoven, Y. T., Ossewaarde, J. M., Derksen-Nawrocki, R. P., Van der Meijden, W. I. & Van de Laar, M. J. (1998). *Chlamydia trachomatis* genotypes: correlation with clinical manifestations of infection and patients' characteristics. *Clin Infect Dis* 26, 314–322.
- Wang, S. P., Kuo, C. C. & Grayston, J. T. (1973). A simplified method for immunological typing of trachoma-inclusion conjunctivitis-lymphogranuloma venereum organisms. *Infect Immun* 7, 356–360.
- WHO (2001). *Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections: Overview and estimates*, pp. 10–14. Geneva: World Health Organization. www.who.int/hiv/pub/sti/who_hiv_aids_2001.02.pdf
- Yuan, Y., Zhang, Y. X., Watkins, N. G. & Caldwell, H. D. (1989). Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. *Infect Immun* 57, 1040–1049.