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Identification of a dominant *Chlamydia trachomatis* strain in patients attending sexual transmitted infection clinic and female sex workers in Tunisia using a high resolution typing method

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ABSTRACT

Background: The distribution of *Chlamydia trachomatis* genotypes in Tunisia was previously studied using the reverse hybridization method. In this study, we used multilocus sequence typing (MLST) to describe *Chlamydia trachomatis* genetic diversity among heterosexual populations in Tunisia. The obtained sequence types (STs) were compared with those from a heterosexual population from Amsterdam, the Netherlands.
Methods: Clinical Tunisian patients and female sex workers provided 107 *Chlamydia trachomatis* positive samples that were used for MLST. Samples from 256 heterosexuals visiting the Amsterdam STI clinic were included as a reference group. Six highly variable genetic regions including the *ompA* gene were amplified and sequenced. The ST numbers were derived from a *Chlamydia* typing database (http://mlstdb.uu.se) and used to draw minimum spanning trees.
Results: ompA sequencing detected 7 genotypes among the Tunisian populations of which genotype E was the most prevalent (66.3%). This genotype E resolved into 23 different STs and among these the ST3 was predominant (53.5%). MLST displayed 43 STs, of which 28 (65%) were new in the database. Minimum spanning tree analysis of all Tunisian samples identified 4 clusters of which one formed a clonal cluster with samples presenting the most prevalent ST3. When comparing samples from the Tunisian and Dutch populations in one minimum spanning tree, there was little overlap between the *Chlamydia trachomatis* samples.
Conclusion: The CT-hrMLST scheme allowed us to identify that the Tunisian distribution was dominated by one genotype E (ST3) strain which is also highly prevalent in many other countries worldwide.

1. Introduction

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted infection worldwide. While studied extensively in Western and high prevalence countries, little is known about the epidemiology of *Chlamydia trachomatis* in low prevalence regions, such as the Middle East and North Africa (MENA). For the purpose of an epidemiologically relevant classification, the MENA population has been divided into different risk classes. The first class includes groups that are at the highest risk of HIV infection, being drug users (IDUs), men who have sex with men (MSM), and female sex workers (FSWs). The second class includes the bridging populations, such as clients of sex workers, who experience an intermediate risk of HIV infection and provide links between FSW, MSM and the general population. The third class is the general population which experiences the lowest risk of HIV infection and includes most of the population in any community (Abu-Raddad et al., 2010). It is thought that the *Chlamydia* prevalence among the general population is low, but a higher prevalence is expected in the FSW and MSM risk groups. The existence of high risk groups for *Chlamydia* infections may be of concern, as transmission to the general population may occur and therefore affect the population as a whole. Tunisia is one of the few countries in this region in which *Chlamydia trachomatis* has been studied. Data on the prevalence of *Chlamydia trachomatis* infection for low risk heterosexual clinical patients was around 4% (Gharsallah et al., 2012b). However, a prevalence of 73% was found for high risk heterosexual female sex workers and

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rates were also very high for patients with arthritis (59%) (Znazen et al., 2010; Siala et al., 2009).

To reveal whether transmission occurs between populations, epidemiology can be combined with typing of pathogen strains. Initially serotyping was used for the classification of *Chlamydia trachomatis*, based on the antigenicity of the major outer membrane protein (MOMP), which is encoded by the *ompA* gene (Yuan et al., 1989; Grayston and Wang, 1975). Using its nucleotide sequence polymorphism, *Chlamydia trachomatis* could be classified in at least 15 main genotypes, by the use of different genotyping techniques such as reverse hybridization methods (Stothard, 2001). Although *ompA* genotyping has been widely used in epidemiological studies, it was reported to have limited resolution for transmission patterns (Fredlund et al., 2004; Pedersen et al., 2009; Gharsallah et al., 2012b; Klint et al., 2007; Christerson et al., 2012; Bom et al., 2013a). Recently, strain typing techniques with a higher resolution were developed and validated such as multilocus sequence typing (MLST) systems (Klint et al., 2007; Dean et al., 2009; Bom et al., 2011). They rely on PCR amplification and DNA sequencing of several genomic loci of *Chlamydia trachomatis* and allow a better understanding of the genetic population structure and the spread of *Chlamydia trachomatis* among populations.

In a previous study, *Chlamydia trachomatis* genotypes for Tunisian samples were determined using an in house reverse hybridization method (RHM) targeting the VS1–VS2 region of the *Chlamydia trachomatis ompA* gene (Gharsallah et al., 2012a). Using this method, a high percentage of *Chlamydia trachomatis* genotype E and mixed infections were observed in clinical patients and in female sex workers (Gharsallah et al., 2012b). In order to determine the difference between these strains and especially within genotype E strains, a high-resolution genotyping method of *Chlamydia trachomatis* was used: the CT-hrMLST method (Bom et al., 2011). The CT-hrMLST results from Tunisia were compared to a set of samples obtained from men and women attending the Amsterdam clinic of sexually transmitted diseases (Bom et al., 2013a, 2013b).

2. Material and methods

2.1. Chlamydia trachomatis samples

In this study, samples from three populations were included. The first group consisted of high risk heterosexual female sex workers (FSW) contributing 135 *Chlamydia trachomatis* positive samples that were collected in August/September 2007 (Znazen et al., 2010). The second group were low risk heterosexual clinical patients (CP) that attended the University hospital in Sfax between February 2000 and June 2011 (Gharsallah et al., 2012b). This panel included 174 *Chlamydia trachomatis* positive samples by Cobas Amplicor testing. Clinical characteristics were collected for the Tunisian patients. All the subjects provided verbal informed consent, and the study protocol was approved by our ethics committee "Habib Bourguiba University hospital ethics committee" with the given number 17–16. The third panel was a reference group of 256 adult men and women who visited the Amsterdam STI clinic from November 2009 to May 2010 (Bom et al., 2013a, 2013b).

2.2. DNA quantification, amplification and sequencing of MLST regions

Genomic DNA, from Cobas Amplicor CT/NG PCR testing (Roche molecular systems, Mannheim Germany) *Chlamydia trachomatis* positive Tunisian clinical swabs, was extracted using the QIAmp DNA Mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions (Gharsallah et al., 2012b). The selected samples were tested for the presence of a sufficient amount of genomic *Chlamydia trachomatis* DNA with an in-house LGV/non-LGV real-time PCR targeting the *pmpH* gene (Quint et al., 2010). A nested PCR was used for the amplification of the six regions: *ompA*, CT046 (*hctB*), CT058, CT144, CT172, and CT682 (*pbpB*) as described previously, including a detailed summary of the primers per target (Bom et al., 2013a; Bom et al., 2011). The inner PCR was performed using M13-tagged primers in both the forward and reverse sense, which were also used for the sequencing reaction. Sequencing of PCR products was carried out by a DNA sequence service (Academic Medical Center university hospital Amsterdam, the Netherlands) (Bom et al., 2013a).

2.3. Data analysis

The obtained sequences from the forward and reverse primers were assembled and trimmed using BioNumerics 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Sequences were compared with those previously published through a public online database (http://mlstdb.bmc.uu.se) (Bom et al., 2011). Samples of which all alleles were successfully amplified, sequenced, and identified, were included in the study and obtained a full MLST profile. Minimum spanning trees were generated to identify clonal groups or clusters. A cluster was defined as a set of sequence types that did not differ at more than one locus. Clusters containing 5 or more samples were defined as large and small clusters ($n < 5$) and singletons were represented as a residual group for statistical analysis.

2.4. Statistical analysis

Differences between the groups and clusters were tested univariately using the Pearson's χ^2 test, Fisher's exact test, when appropriate. A p-value of ≤0.05 was considered statistically significant. Analyses were performed with SPSS package version 19.0 (SPSS Inc., Chicago, IL, USA). Genetic diversity was calculated using Simpson's index of diversity (D) (Bom et al., 2011).

3. Results

3.1. Study population and samples

During the decade 2001 to 2011, a total of 4067 Tunisian persons were screened by Cobas Amplicor testing for *Chlamydia trachomatis*. Of these persons 309 samples were found positive and 135 samples were derived from heterosexual FSW and 174 from heterosexual CP attending the STI clinics (Supplementary Table 1). The DNA samples were

Table 1
Population characteristics and *Chlamydia trachomatis* strain distribution among 107 Tunisian patients.

	Female sex workers ($n = 22$)		Clinical patients ($n = 85$)		p
	n	(%)	n	(%)	
Gender					0.00
Male	0	(0.0)	59	(69.4)	
Female	22	(100.0)	26	(30.6)	
Age in years[a]					0.00
<25	2	(9.1)	20	(27.4)	
≥25	20	(90.9)	53	(72.6)	
Neisseria gonorrhoeae co-infection[b]					0.11
Positive	4	(18.2)	12	(14.1)	
Negative	17	(77.3)	73	(85.9)	
Genotypes					0.89
D	1	(4.5)	4	(4.7)	
E	15	(68.2)	56	(65.8)	
F	1	(4.5)	6	(7.1)	
G	1	(4.5)	9	(10.6)	
H	3	(13.6)	6	(7.1)	
I	0	(0.0)	1	(1.2)	
K	1	(4.5)	3	(3.5)	

[a] 12 missing data in the clinical patients group.
[b] 1 test not available for *Neisseria gonorrhoeae* co-infection in the female sex workers group.