Original
articleTyping of *Chlamydia trachomatis* strains from urine samples by amplification and sequencing the major outer membrane protein gene (*omp1*)

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Objectives: To develop a novel protocol for the extraction, amplification, and sequencing of *Chlamydia trachomatis* MOMP gene (*omp1*) from urine, a non-invasive source, and apply it to an epidemiological study on the distribution of *C trachomatis* strains in a population of pregnant women in Thailand.

Methods: The *C trachomatis* DNA was extracted from culture stocks and urine using a slightly modified commercially available kit, the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, IN, USA). The PCR and sequencing primers used for the amplification and sequencing of the *omp1* were designed based on the nucleotide sequence of multiple *C trachomatis* strains found in GenBank. The protocol for the extraction, amplification, and sequencing was tested on laboratory culture stocks of reference strains of all *C trachomatis* serovars and on urine samples collected in a cross sectional study designed to assess the prevalence of *C trachomatis* infections in the cities of Bangkok and Chiang Rai, Thailand.

Results: The *omp1* gene was successfully amplified and sequenced from 18 laboratory *C trachomatis* reference strains and from 45 *C trachomatis* positive urine clinical samples collected from asymptomatic pregnant women. Among clinical samples, we found nine different *C trachomatis* genotypes: F (11, 25%), D (10, 22.6%), H (5, 11.7%), K (5, 11.7%), E (4, 9.3%), Ia (3, 7%), B (3, 7%), Ja (2, 4.5%), and G (1, 2.3%). One specimen generated an *omp1* DNA sequence pattern indicating the presence of a mixed infection with at least two different serovars.

Conclusions: Urine is a convenient and reliable source for genotyping *C trachomatis* strains. A clear advantage of urine over traditional samples, such as cervical swabs, is that urine is a non-invasive source which makes collection easier and thus facilitates the enrolment of patients in clinical and epidemiological studies. In addition to typing, urine is increasingly used for diagnosis of *C trachomatis* infection by several commercially available nucleic acid amplification assays which represents a distinct advantage for collecting, transport, storage, and laboratory handling of samples. (*Sex Transm Inf* 2001;77:419-422)

Keywords: genotype; *Chlamydia trachomatis*; urine

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Accepted for publication 22 May 2001

Introduction

Chlamydia trachomatis infections are the leading cause of bacterial sexually transmitted diseases and the main cause of preventable blindness in the world.¹ *C trachomatis* strains have been conventionally classified into serotypes or serovars by immunotyping using polyclonal or monoclonal antibodies specific for the major outer membrane protein (MOMP). Fifteen prototypic serovars labelled A to K and L₁, L₂, and L₃ were initially recognised by polyclonal antibodies, and additional immunovariants (Ba, Da, Ia, etc), which in some publications are referred to as distinct serovars, have been identified by monoclonal antibodies.²⁻⁴ Serovars A, B, and C have been found usually associated with trachoma, D to K with urogenital infections, and L₁, L₂, and L₃ with lymphogranuloma venereum (LGV), a systemic disease. The urogenital serovars are found all regions of the world, whereas the LGV and trachoma serovars are found primarily in tropical and subtropical regions.¹

Typing of *C trachomatis* strains is an important goal in field of epidemiology as well as clinical and basic research on *C trachomatis* infections. The temporal and geographical distribution of *C*

trachomatis strains throughout the world has significant implications for our understanding of the epidemiology of this infectious agent and for vaccine development.⁵⁻⁸ Differential pathogenicity and transmission rates of *C trachomatis* strains are also important topics in both clinical and basic research.⁹⁻¹³ The traditional immunotyping methods for the identification of *C trachomatis* strains require collection of invasive samples, such as cervical swabs, and culture isolation and growth of the organisms. The immunotyping methods are currently being replaced by genotyping methods using restriction fragment length polymorphism (RFLP) or DNA sequence analyses of the major outer membrane protein (MOMP) gene (*omp1*).¹⁰⁻²³ Compared with immunotyping, the genotyping methods, particularly *omp1* sequencing, are more sensitive and precise in revealing *C trachomatis* variants within a serovar as well as potential recombinants among serovars.²⁴ An additional advantage of the genotyping methods is that they have the potential to be used with non-invasive samples such as urine, which is also the source of choice for current commercially available nucleic acid amplification diagnostic assays (NAA). In this paper we report the development of a protocol for the extraction, amplification

Key messages

- The objective of the study was to develop a novel protocol for the extraction, amplification, and sequencing of *C trachomatis* MOMP gene (*omp1*) from the urine, a non-invasive source, and apply it to an epidemiological study regarding the distribution of *C trachomatis* strains in a population of pregnant women in Thailand.
- The *omp1* gene was successfully amplified and sequenced from 18 laboratory *C trachomatis* reference strains and from 45 *C trachomatis* positive urine clinical samples collected from asymptomatic pregnant women.
- A clear advantage of urine over traditional samples, such as cervical swabs, is that urine is a non-invasive source which makes collection easier and thus facilitates the enrolment of patients in clinical and epidemiological studies. In addition to typing, urine is increasingly used for diagnosis of *C trachomatis* infection by several commercially available nucleic and amplification assays which represents a distinct advantage for collecting, transport, storage, and laboratory handling of samples.

and sequencing of *C trachomatis* MOMP gene from urine samples collected from a population of pregnant women in Thailand.

Materials and methods

First catch urine (30 ml) was collected in 1996 from pregnant women (n= 1021) enrolled in a cross sectional study designed to assess the prevalence of *C trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, and HIV infections in the cities of Bangkok (Siriraj and Rajavithi Hospitals) and Chiang Rai (Chiang Rai Hospital), Thailand. The demographic and clinical characteristics of the study population have been previously described.²⁵ The protocol for this study was reviewed and approved by institutional review boards at the Centers for Disease Control and Prevention (CDC) and the hospitals in Thailand. The urine samples were shipped on dry ice to the CDC, Atlanta, where they were screened for *C trachomatis* and *N gonorrhoeae* by using a commercial polymerase chain reaction (PCR) assay (Amplicor CT/NG, Roche Molecular Systems, IN, USA) according to the manufacturer's instructions.

The Amplicor positive urine samples were stored at -20°C. The nested PCR amplification of the *omp1* gene was performed as follows: 200 µl of urine was centrifuged at 1000 g for 15 minutes in a microcentrifuge; the supernatant was discarded and the pellet was resuspended in 200 µl of buffer (10 mM TRIS-HCl, pH 7.5, 0.05% Triton X), 400 µl of binding buffer, and 40 µl of proteinase K from the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, IN, USA) and incubated at 72°C for 60 minutes. The

DNA was further purified using the High Pure PCR Template Preparation Kit and suspended in 200 µl elution buffer according to the manufacturer's instructions. Ten µl of the prepared DNA template, which represented the equivalent of 10 µl of urine, was used in the 100 µl primary PCR reaction (High Fidelity Expand Kit, Roche) using first PCR primers CT90UF and CT220DR located upstream and downstream, respectively, of the *omp1* coding region (table 1). The thermocycler profile was: 94°C for 30 seconds, 52°C for 1 minute, and 72°C for 1 minute for 40 cycles. Two µl of the primary PCR were used for secondary PCR which was prepared and run using the same reagents and conditions as the primary PCR, except for primers, which were CT60UF and CT80DR. The laboratory culture stocks of 18 reference *C trachomatis* strains (A to K, L₁-L₃, Da, Ja, and Ia) were prepared and amplified using the same protocol.

The amplified DNA was purified using the Qiagen Qiaquick PCR Purification Kit (Qiagen, Santa Clarita, CA, USA) as instructed by the manufacturer. The *omp1* was sequenced using the Big Dye Sequencing Terminator Kit and ABI Prism 377 automated sequencing system (PE-Applied Biosystems, Foster City, CA, USA) and five sequencing primers: CT40F, CT160F, CT419F, CT662F, and CT902F (table 1).

The *omp1* sequences were edited, aligned, and analysed using the software Wisconsin Package Version 10.0 (GCG, Madison, WI, USA).

Results

The entire coding region of *omp1* from 18 laboratory reference strains representing all of the serovars and some immunovariants of *C trachomatis* was successfully amplified and sequenced using the protocol and the primers described above in the methods section. One round of PCR was sufficient to generate enough DNA from culture stocks to perform multiple rounds of sequencing (data not shown).

All 45 *C trachomatis* positive urine samples that were available for this genotyping study were also successfully amplified. However, for consistent results and to obtain enough amplified DNA to perform multiple rounds of sequencing reactions, a nested PCR procedure was necessary. Presumably, the need for a nested PCR procedure was the result of a low number of *C trachomatis* organisms in some urine samples or, potentially, because of PCR inhibitors present in urine.

Except for one sample that generated a DNA sequence pattern indicating a mixed infection with at least two different strains, all of the remaining 44 sequences showed the presence of a single or a predominant strain. The sequencing procedure used in this study can not detect mixed infections when the molar ratio of the amplified mixed strains is higher than five to one (data not shown). Among the 44 Thai sequences analysed by sequence comparison and phylogenetic analysis, nine different serovars were identified: F (11, 25%), D

Table 1 PCR and sequencing primers used for amplifying and sequencing the *omp1* gene of *C trachomatis* strains

First PCR primers:	
CT90UF	5'-GGACATCTGTCTGGCTTTAACT-3'
CT220DR	5'-GCGCTCAAGTAGACCGATATAGTA-3'
Secondary PCR primers:	
CT60UF	5'-GTCCCGCCAGAAAAAGATAG-3'
CT80DR	5'-CCAGAAACACGGATAGTGTATTATTA-3'
Sequencing primers:	
CT40F	5'-ATAGCGAGCACAAAGAGAGC-3'
CT160F	5'-ACCACTTGGTGTGACGCTATCCAG-3'
CT419F	5'-TGGGATCGTTTTGATGTATT-3'
CT662F	5'-ACGTTAGGAGCTTCTTTCCAATA-3'
CT902F	5'-TCCTTACATTGGAGTTAAATGGTC-3'

(10, 22.6%), H (5, 11.7%), K (5, 11.7%), E (4, 9.3%), Ia (3, 7%), B (3, 7%), Ja (2, 4.5%), and G (1, 2.3%).

Discussion

We have developed a protocol for genotyping *C trachomatis* strains from urine samples and applied it to a preliminary epidemiological study of the distribution of *C trachomatis* serovars among pregnant women in Thailand. Firstly, we used this protocol to amplify and sequence laboratory strains representing all *C trachomatis* serovars. Then we successfully extracted, amplified, and sequenced the *omp1* gene from 45 urine samples collected from pregnant women in Thailand. Overall, the distribution of *C trachomatis* serovars among pregnant women in Thailand appears to be similar to that observed in non-pregnant women living in Western countries, in which E, D, and F are the predominant serovars. In Thailand, we found that F (25%) and D (22.6%) were the most common serovars, followed by more or less equal distributions of serovars H (11.7%), K (11.7%), and E (9.3%). A similar distribution of *C trachomatis* serovars among asymptomatic pregnant women in the same region of the world was recently reported from Japan.²⁶ In this study the *C trachomatis* serovars were identified from cervical samples by RFLP and partial *omp1* sequencing.

The use of urine samples for typing of *C trachomatis* strains presents distinct advantages compared with other samples, such as cervical swabs, particularly when conducting large epidemiological investigations. Urine is a non-invasive source which makes collection easier and thus facilitates the enrolment of patients. It also enables easier access to asymptomatic people and those who are not seeking clinical care. In this regard it should be particularly useful for certain epidemiological applications, such as partner transmission studies, in which the convenience of sample collection is key to successful enrolment of participants. Urine samples are also relatively stable at refrigerated temperatures and if necessary can be collected in relatively large volumes that permit repeat testing when required.

Another significant advantage in using urine for typing *C trachomatis* strains is that it can also be used for the diagnosis of *C trachomatis* infection by several commercially available NAA.²⁷ Collecting only one sample for both diagnostic testing and for genotyping helps with enrolment of patients and with collecting, transport, storage, and laboratory handling of samples. Also, the NAA diagnostic tests, which are semiautomated and highly sensitive, can be used as a screening tool for identifying *C trachomatis* positive samples for typing.

Interest in genotyping *C trachomatis* directly from urine has been recently highlighted by Morre *et al* who outlined the advantages of using this source for epidemiological studies and successfully used urine in their PCR based RFLP genotyping protocol.²⁸ They showed that, as expected, the same *C trachomatis* strains are present in urine and cervical samples collected from the same patient. Their success

rate of amplifying a portion of the *omp1* gene from *C trachomatis* positive urine samples from females and males was 94% (n=81). In our study, by using samples from pregnant women we were able to amplify the entire *omp1* gene from all 45 urine samples available for this study.

In summary, urine represents a convenient and reliable source for genotyping *C trachomatis* strains. As a non-invasive source, it can be easily collected from symptomatic and asymptomatic patients enrolled in epidemiological and clinical studies designed to address *C trachomatis* pathogenicity, transmission, and vaccine development.

We thank Ms Azita Eslamiyan, Ms Nancy Young, Ms Supaporn Korattana, Mr Jirasak Laosakitiboran, and Dr Prayoon Yuentrakul, for their assistance in performing this research; Dr Nathan Shaffer for his assistance and critical review of the manuscript; and Dr David Martin for critical review of the manuscript.

Contributors: CIB, PHK, and CMB were responsible for study design and supervision, data analysis, and preparation of the manuscript; SY, PC, and WS were responsible for subjects' recruitment and preparation of manuscript; CIB, KK, TMB, and VB were the laboratory based scientists who, in addition to data analysis and manuscript preparation, were responsible for laboratory testing.

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