Original Article

Genotyping of *Chlamydia trachomatis* by *OMP1* Sequencing in Patients with Pelvic Inflammatory Disease

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Abstract

Background: *Chlamydia trachomatis* is one of the most sexually transmitted pathogens. Significant reproductive effects, such as infertility, can result from chlamydial infection, which emerges from the lower vaginal canal. This sexually transmitted micro-organism also contributes significantly to pelvic inflammatory disease (PID), which has been associated with a higher risk of infertility. Additionally, sequence typing was used to distinguish *C. trachomatis* strains and genotyping by using the widely used *ompA* gene polymorphism, a single-locus typing standard. This method, which is frequently employed for genotyping a variety of microorganisms, including the assessment of Chlamydia diversity, is based on the examination of polymorphism of housekeeping genes. Samples from symptomatic *C. trachomatis*-infected patients, were collected for this cross-sectional pilot study. **Objectives:** This work investigates the analyzed sequencing of *C. trachomatis* by using *OMP1* polymerase chain reaction (PCR) sequencing during Chlamydia in women with PID. **Materials and Methods:** The study included 200 endocervical swab samples from patients with PID. Patients' age ranged from 20 to 45 years. *Chlamydia trachomatis* was determined by convention PCR, whereas genotyping was done by *OMP1* gene by PCR sequencing. **Results:** Twenty two (22/200) endocervical swabs were positive for *C. trachomatis* and only 20 were studied by sequencing of C. *trachomatis OMP1* gene. The results also revealed that there are two main clusters of *C. trachomatis* (A and B) the cluster A divided into two subclusters, while cluster B contain two subclusters. **Conclusions:** Genotyping of *C. trachomatis* is important to study the pathogenesis and epidemiology of genital chlamydia infection.

Keywords: Chlamydia, endocervical, *OMP1*, pelvic inflammatory disease, sequences, sexually transmitted infections

Introduction

Chlamydia trachomatis is obligatory intracellular bacterium and is the main causative agent of sexually transmitted infections (STIs). The most common cause of pelvic inflammatory disease (PID) in the globe is *C. trachomatis*.[1]

Taking into account the antigenic differences in the major outer membrane protein (MOMP), *C. trachomatis* strains are categorized into three biovars: the trachoma biovar (serovars A–C); the genital biovar (serovars D–K); and the lymphogranuloma venereum biovar (serovars L1–L3). Although prototype serovar L2 strains are typically employed in investigations on host cell–*C. trachomatis* interactions, genital strains of *C. trachomatis* are responsible for the majority of infections.[2]

In order to better understand how different strains, propagate in epidemiological investigations, *C. trachomatis* genotyping is characterized by methods that can be of assistance. These methods are especially helpful in clinical research when examining transmission from person to person, evaluating the link between genovars, and observing how they respond to treatment.[3]

The MOMP of *C. trachomatis* comprises specific antigens that distinguish chlamydial strains by serovars (based on antigenic cross-reactivity on micro immunofluorescence)

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or genotype (based on nucleotide sequencing of the *omp*A gene). Whether *C. trachomatis* serovars have varied pathogenicity potentials is uncertain. In order to gain a complete understanding of the etiology and epidemiology of genital chlamydial infections, genotyping of *C. trachomatis* strains is essential for monitoring contact tracing.[4]

Materials and Methods

This study is a case–control analysis. Patients admitted to the Gynecology and Obstetrics outpatient clinics of two hospitals in Babylon Province provided clinical samples between February and August 2022. 25 endocervical swabs were taken from healthy individual as control group.

A total of 200 endocervical swab samples obtained from female patients. The ages of the patients spanned from 20 to 45. These women were diagnosed with PID by their gynecologists based on symptoms, signs, an ultrasound of the abdomen and pelvis, and the existence of risk factors all of which met the criteria for PID outlined in the national recommendations for the condition.[5] All 200 endocervical swab samples were collected according to Yeruva *et al*.[6]

DNA extraction and polymerase chain reaction protocol

DNA extraction was performed on endocervical swabs taken from patients. That was done in accordance with the instructions provided by the manufacturer, Geneaid (United States of America)/USA catalogue number (GSK004, GSK100, GSK300). The acquired DNA was processed for *C. trachomatis* molecular identification using one pair of housekeeping OMP (outer membrane protein) gene standard polymerase chain reaction (PCR) primers and stored at 2–8°C for later applications. Table 1 lists the gene sequences and the associated circumstances.

With the aid of agarose gel electrophoresis and ultra violate (UV) light, PCR amplification was verified. A 70 V electric current was allowed to flow for 50 min. The DNA bands were observed using a UV transilluminator at 280 nm, and digital camera was used to photograph the gel.[8]

Amplification of *Chlamydia trachomatis OMP1* gene for using in sequencing

The acquired DNA was processed for the sequences of *C. trachomatis OMP1 gene*, using one pair of OMP1 gene primers by conventional PCR and store at 2–8°C for later applications. Table 2 lists the gene sequences and the associated circumstances.

A 5 µL of the PCR products amplicon were loaded into 2% agarose gels in 1× tris-borate-EDTA (TBE), and run at 100 V in 1× TBE for 40 min.

Gel Extraction Kit from Geneaid (Geneaid Firm, USA) was used to purify all PCR products from positive swab samples. Sample: up to 300 mg of agarose gel, up to 100 L of PCR products. Size of fragment: 70 bp to 20 kb, up to 95% recovery, spin column format 20 min (gel extraction), followed by 10 min of operation (PCR cleanup), volume of elution: 20–50 µL, dry storage should be done between 15 and 25°C, analyzed by sanger sequencing method in Korea (Macrogen Company). The raw data of sequencing were analyzed and aligned by Bioedit.[9]

Statistical analysis

Chlamydia trachomatis frequency distribution was presented with the assistance of percentages and histograms. The chi-square was used for genetic analysis. *P* values under 0.05 are taken into consideration. The software statistical package for the social sciences-19 version was used to conduct the statistical analysis, presenting data as a mean (independent school district).

Ethical approval

The necessary ethical approval was obtained by verbal consent from patients. This work was authorized by the publication ethics committee of the College of Medicine in Babylon Province, Iraq (reference: BMS/0203/016; date: June 18, 2022).

Results

Chlamydia trachomatis molecular identification

All 200 endocervical swab specimens were collected for DNA, and traditional PCR was carried out using these DNA samples to amplify specific housekeeping *OMP* primers in accordance with the sequences and program shown in Table 1. After that, gel electrophoresis revealed that, when compared to an allelic ladder, only 22/200 (11%) of the 200 samples produced the specific 150 bp DNA fragment, as shown in Figure 1.

Table 1: Sequencing of *Chlamydia trachomatis* OMP1 gene primer and polymerase chain reaction conditions

Gene's name	Primer sequences (5′–3′)	Sizes (bp)	Conditions	Reference
Designation genes				
Chlamydia OMP	F-5′-CCTGTGGGGAATCCTGCTGAA-3′	150	94°C, 5 min 1× 94°C, 30 s 56°C, 30 s 35×	[7]
	R-5′-GTCGAAAACAAAGTC ACCATAGTA-3′		72°C, 30 s 72°C, 7 min 1×	

Table 2: The primer sequence and polymerase chain reaction condition with their amplicon size [base pair (bp)] for genotyping

Gene's name	Primer sequence (5′–3′)	Size (bp)	Conditions	Reference
OMP-1	F-5′-GCCGCTTTGAGTTCTGCTTCCTC-3′ R-5′-ATTTACGTGAGCAGCTCTCTCAT-3′	1142	95°C, 5 min 95°C, 1 min 35× 59°C, 1 min 35× 70°C, 1 min 35× 72°C, 5 min	Design in this study

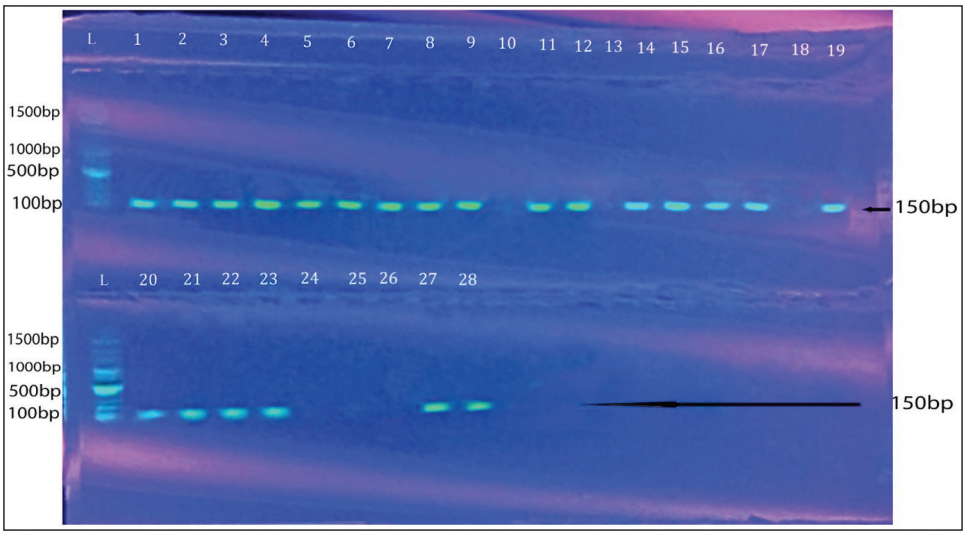

Figure 1: After staining with red safe stain, *OMP* polymerase chain reaction products were electrophoresed at 70 V for 50 min on a 1.5% agarose gel. 1500 bp ladder, L; lane (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 23, 27, and 28). Endocervical swabs from patients with pelvic inflammatory illness tested positive for *Chlamydia trachomatis*. The amplicons are 150 bp in size

Amplified DNA of *Chlamydia trachomatis* by using OPM1 gene polymerase chain reaction for sequencing

DNA was taken from 20 endocervical swab specimens and successfully amplified BY *OMP1* gene PCR with amplicon size 1142 bp as shown in Table 2.

Sequences analysis of the *OMP1* gene from amplified DNA clinical strain of *Chlamydia trachomatis*

When compared using a basic local alignment search tool similarity search, all nucleotide sequences were simple to read and comprehend, and the evolutionary links between clinical isolates could be found.

According to the phylogenetic analysis of the *OMP1* gene, there are two main clusters of *C. trachomatis* (A and B) as shown in Figures 2 and 3.

The A cluster divided was into two subclusters which contain four groups, and these groups contain nine isolates and only two isolates (CT2-CT1) show 100% identical or similarity whereas other isolator was showing close genetic distance between each cluster. While cluster B contain two subcluster which 4 group and these groups contain 11 isolates which have small genetic distance as show in Figure 3.

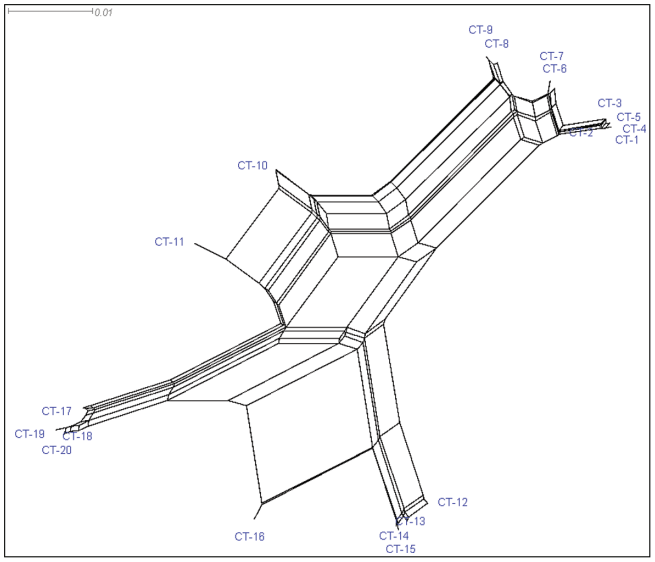

Figure 2: Split tree-decomposition analysis based on *OMP1* gene sequences of 20 *Chlamydia trachomatis* isolates. *Note*: Multi parallelogram formations indicate recombination events

Limited sequence variations were found within genotypes, according to a thorough investigation; nevertheless, some of these sequence variants were only found in one sample,

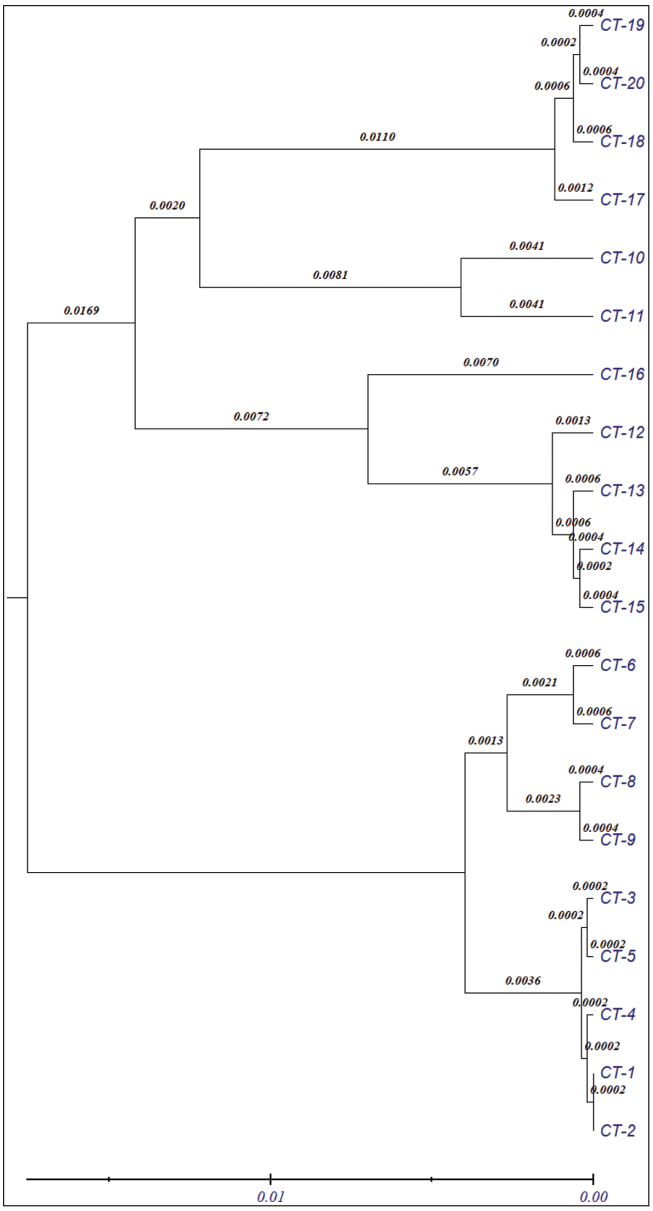

Figure 3: Phylogenetic analysis based on *OMP1* gene sequences of 20 *Chlamydia trachomatis* isolates using unweighted pair group method with arithmetic mean method

whereas others were found in many samples. Sequencing data analysis shows variability in OMP1 sequence as shown in Table 3.

It was found that the most substitution mutation displayed C-T in 18.9612, then G-A in 17.3018 follow by T-G in 14.9856 and T-C in 13.5256.

It suggested that the genetic diversity was found in this study and the most sequence variation was observed in single nucleotide variation or two nucleotide substitutions.

Discussion

The prevalence of *C. trachomatis* infections that lead to PID has been the subject of numerous studies; one of these studies found that 8%–10% of women with chlamydial infection also had PID.[10] A sexually transmitted bacterium is a substantial contributor to PID, which has been related to an elevated risk of infertility and acts as a link between chlamydia infection and infertility.[11]

Table 3: Sequencing data analysis in OMP1 sequence of *Chlamydia trachomatis*

From/To	A	T	C	G
A	–	5.0124	3.5755	14.9859
T	4.8360	–	13.5256	4.1887
C	4.8360	18.9612	–	4.1887
G	17.3018	5.0124	3.5755	–

In other different study, high vaginal swabs were collected from 215 females, of whom 150 were experiencing infertility and miscarriage. The frequency was 84% from swab samples using PCR technique. *Chlamydia trachomatis* infection was found to be significantly linked to both infertility and miscarriage in women.[12]

Currently, the first-line assays for the detection of *C. trachomatis* are nucleic acid amplification tests because of their excellent sensitivity and specificity. When other detection techniques are used in their place, there are more false negative tests, which causes fewer cases to be recorded and, as a result, a lower estimation of the frequency of *C. trachomatis* infection.[13]

Other study showed that the incidence of *C. trachomatis* infection in women's endocervical swabs was 38 (17.43%), and it was found that *C. trachomatis* infection was linked to spontaneous abortion.[14]

The percentage (11%) in this study contrasts with other studies carried out in the Arabic World, which had frequencies of (5.3%) in Qatar[15] and (15%) in Saudi Arabia.[16] In addition, two separate examinations in Iran revealed that the prevalence of *C. trachomatis* was, respectively, 8.3% and 22%.[17]

Because of the varied cultural environments and the screening programs' primary focus on women in the age range under 25 years old, STI, particularly chlamydial illness, were more prevalent among patients in this age group in developed nations.[18]

Young age is the demographic factor associated with chlamydial infection most frequently in women (20 years). Anatomical changes in younger women cervices may result in the squamo-columnar junction, a crucial host target for *C. trachomatis*, to be everted and therefore more exposed. Physiological changes that limit sensitivity to the rising temperatures and lower *C. trachomatis* exposure make the elderly less likely to spread disease than younger people.[19]

Some of nucleotide alteration in amino acid replacement and could thus potentially change the function and antigenicity of the MOMP.

Sequencing of the *ompA* gene may provide discrimination of *C. trachomatis* strain and provided additional information when applied to contact tracing.[20]

Examining *C. trachomatis' ompA* gene and other loci reveals an increase in nucleotide alterations and various phylogenetic histories.[21]

The genomic mutation observed with the *omp* sequence genotypes in *Ch. trachomatis* may be natural consequence of the microbial evolution combined with the high diagnostic selective pressure on *Ch. trachomatis*.[22]

The major outer membrane protein of Chlamydia, encoded by the gene (*ompA*), has four symmetrically spaced variable domains (VDs-IV), which maintain the structural rigidity of the outer membrane and facilitate porin formation to allow solute diffusion through the intracellular reticulate body membrane.[23]

Other found it has been speculated that the *OMP1* genovariant is the result of immunological pressure-selected point mutation and recombination events, however some of the nucleotide substitutions that were found were identical, suggesting that they were evolutionarily neutral.[24]

Other found that the sequencing of 22 *C. trachomatis ompA* performed and mutation were observed and the occurrence of point mutation was observed and single nucleotide variation was observed, nevertheless, two to three nucleotide alterations were found.[25]

Brasiliense *et al*.[26] showed that the phylogenetic analysis of ompA sequence produced a tree that grouped the samples into five clades and the similarity values ranging from 99.7% to 100%; nine nucleotide substitution were observed, and eight of which resulted in amino acid replacement.

The genomic mutation observed with the *omp* sequence genotypes in *C. trachomatis* may be natural consequence of the microbial evolution combined with the high diagnostic selective pressure on *C. trachomatis*.[27,28]

Conclusion

Genotyping of the *ompA g*ene of *C. trachomatis* isolates could be useful for epidemiological characterization of circulating *C. trachomatis* strains in the community and could provide additional information for vaccine development.

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Conflicts of interest

There are no conflicts of interest.

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