

I. INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacteria that causes a wide spectrum of human diseases. It affects millions of people worldwide. The most notable diseases are blinding trachoma and genitourinary tract infections that are the important sexually transmitted diseases in men and women. Infants are also at risk for chlamydial eye infection and pneumonia if they pass through an infected cervix. *C. trachomatis* has a unique biphasic growth cycle, which facilitates its survival in two discontinuous habitats.

Several methods for the detection of *C. trachomatis* infection have been established and the gold standard has been the cell culture technique. After cultivation, *C. trachomatis* is detected by staining with iodine or fluorescein conjugated with a monoclonal antibody to demonstrate the intracytoplasmic inclusions. The techniques for detecting *C. trachomatis* antigens are a direct fluorescent antibody (DFA) and enzyme immunoassay (EIA), using monoclonal antibodies directed against the major outer membrane protein (MOMP). Direct detection of *C. trachomatis* nucleic acid using a specific probe has also been developed. All these methods can detect organisms directly from specimens. However, they are not sensitive for specimens that contain only a few chlamydial organisms. Moreover, the viability of organisms in specimens is critical for the culture technique.

The highly sensitive amplification methods for the detection of chlamydia have been developed recently. They are polymerase chain reaction (PCR) and ligase chain reaction (LCR). These two methods can detect even the smallest amount of specific nucleic acids from clinical specimens by using repeated amplification steps of a respective target sequence.

C. trachomatis has been characterized serologically into 18 serotypes that are defined by polyvalent antisera and monoclonal antibodies. Differentiation of *C. trachomatis* serotypes has been necessary in epidemiologic studies to establish the regional prevalence of organisms. However, the serotyping of this organism is limited, due to the commercially unavailable panel of antisera for serotyping and the poor growth of *C. trachomatis* in the culture system. Moreover, the monoclonal antibodies can react only with known serotype specific MOMP antigens. Hence, the new variant serovar cannot be detected.

Genotyping of *C. trachomatis* using molecular techniques has been developed in order to solve those problems. The restriction fragment length polymorphism (RFLP) and nucleotide sequencing techniques, based on the sequence variation analysis of the MOMP gene, are now widely used. The MOMP gene encoding proteins are responsible for group and type specific antigens. They consist of four variable domains (VDs) that are separated by five constant regions.

The PCR based RFLP is the method that combines the amplification and differential digestion of restriction endonucleases. The PCR-RFLP of the MOMP sequence has proved to correlate well with the serotypes performed by the immunological assay. However, PCR-RFLP of the whole MOMP gene frequently shows the complex banding patterns of the digested fragments, which make it difficult for interpreting of the result. The PCR- RFLP of a short sequence, especially the VD4 region, gives a less complex patterns and is also useful in differentiating genital *C. trachomatis* genotypes. At least 10 characteristic patterns of *C. trachomatis* have been differentiated successfully. However, the RFLP method does not recover the nucleotide sequence polymorphism of the MOMP gene, since the nucleotides that change only in the recognition sequence can be detected as an altered RFLP pattern. This can cause a number of variants to be underestimated.

The nucleotide sequencing of the MOMP gene has been recently applied to molecular epidemiological study. Based on data from the nucleotide sequence homology of all 4 VDs, 18 serotypes of *C. trachomatis* have been separated into 3 groups, B- complex (B, Ba, D, Da, E, L1, L2 and L2a), intermediate group G and F, and C- complex (A, C, H, I, Ia, J, K and L3). Interestingly, the detailed nucleotide sequence analysis shows an increasing number of MOMP sequence variations from clinical isolates. They have then been characterized as possible new *C. trachomatis* variants by many investigators. The sequence variations are found mostly in VD1, VD2, and VD4, while the VD3 sequence of most serotypes is rather conserved. The genetic mechanism for MOMP diversification can occur either by point mutation or genetic recombination. The sequence variation may produce new variants that could evade an immune neutralization, which would result in infection becoming established or more persistent in patients. Therefore, examination of the *C. trachomatis* type distribution, and new type variants in a particular area, are valuable for the accession of epidemiological data, which will facilitate the development of a vaccine.

In this study, the nucleotide sequences of the MOMP gene of *C. trachomatis* isolated, from clinical samples collected in Chiang Mai and surrounding area, were determined. The MOMP gene of *C. trachomatis* was amplified by PCR and sequenced by BigDye™ Terminator Cycle Sequencing (Perkin- Elmer, Applied Biosystems, USA). The nucleotide sequence was resolved by a 310 automated DNA sequencer (Perkin- Elmer, Applied Biosystems, USA). This sequence was aligned with a reference sequence serotype by using the Autoassembler™ 1.4.0 computer program (Perkin-Elmer, Applied Biosystem, USA).

Additionally, the amplification of the VD4-MOMP gene of *C. trachomatis* was performed by PCR, and the amplified 350 bp. fragment was genotyped by using RFLP with the 4 restriction endonucleases: *AluI*, *HindIII*, *DdeI*

and *Eco*RII. The RFLP patterns were analyzed by comparing with the reference genotypes.

Finally, *C. trachomatis* isolates were classified into specific genotypes and the nucleotide sequence variation in the MOMP gene was characterized. These data will be useful for prospective molecular epidemiological studies and to determine the antigenic variation of the organisms, which would also greatly enhance vaccine development.

Aims of the study

1. To study the nucleotide sequence variations of the *C. trachomatis* whole MOMP gene that were detected in Chiang Mai and the surrounding area.
2. To study the genotype distribution of *C. trachomatis*.
3. To compare *C. trachomatis* genotyping by the PCR based RFLP of the VD4-MOMP gene and nucleotide sequencing of the whole MOMP gene.