II. LITERATURE REVIEWS

1. Taxonomy (1, 2)

The chlamydiae were originally classified as protozoa and subsequently viruses, but it became clear later that the chlamydiae had all the requisite properties of bacteria. At present, chlamydiae are placed in an order of their own, Chlamydiales, with one family of Chlamydiaceae and a single genus, *Chlamydia.* The genus, *Chlamydia*, consists of four species namely, *C. trachomatis*, *C. psittaci*, *C. pneumoniae* and *C. pecorum.*

Chlamydia trachomatis is an important human pathogen responsible for significant diseases worldwide. It includes blinding trachoma, severe genitourinary diseases, neonatal pneumonitis and ophthalmitis. Differentiation of *C. trachomatis* from the other species is based on its two distinct biological properties. *C. trachomatis* has the ability to produce glycogen in the inclusions and it is rather susceptible to sulfonamides.

C. trachomatis has been divided further into three biovars; trachoma, lymphogranuloma venereum (LGV) and murine biovar. The nucleotide sequence homology studies of the genomic DNA show that the trachoma and LGV biovars are essentially similar, while the murine biovar is distantly related. However, the trachoma and LGV biovars can be distinguished by their clinical features and disease process. The LGV biovar proliferates in lymphnodes and readily causes a more systemic infections, while the trachoma is limited to the mucosal surfaces. The trachoma biovar currently consists of 14 serovars designated by the letter A through K including Ba, Da and Ia serovars. The LGV biovar consists of 4 serovars, L1, L2, L2a and L3. A, B and C serovars are found predominantly in ocular trachoma, whereas, D-K serovars are

common in genital infections. The murine biovar that consists of one strain, MoPn, causes mouse pneumonitis, but does not infect human.

2. Morphology and composition (1, 2)

The chlamydiae possess cell walls and membranes are similar to those of gram negative bacteria. The outer membrane contains lipopolysaccharide, but lack of peptidoglycan in the cell wall. The lack of peptidoglycan is shown by the inability to detect muramic acid and antibodies directed against it. The chlamydiae also contain DNA, RNA and ribosomes. They synthesize their own proteins, nucleic acids and lipids. They are non motile, lacking in flagella and pili, but possess the cylindrical surface projections.

The chlamydiae have two morphologically distinct forms: the elementary body (EB) and the reticulate body (RB). The EB is a small dense spherical body ranging from 0.2 to 0.4 μm in diameter. It is an infectious form, responsible for attaching itself to the target host cell and promoting its entry. The RB is an intracellular metabolically active form that divides by binary fission. It is approximately 0.6 to 1.0 μm in diameter. The RBs can synthesize DNA, RNA and proteins, but they cannot generate ATP. As a consequence of their inadequate energy generation, chlamydiae are the obligate intracellular parasite of eucaryotic cells for energy.

The chlamydial celll wall consists of subunits approximately 20 nm in diameter that are arranged in a regular geometrical pattern. The outer membrane contains approximately 60% of a highly antigenic transmembrane protein called the major outer membrane protein (MOMP). Its surface antigenic components are responsible for the serovar, serogroup and species-specificity. This protein represents about 30 % by weight of the whole organism. The MOMP has a molecular weight that ranges from 38 to 43 kDa. It is a cystein-rich protein with a disulfide linkage. In order

to maintain the structural rigidity, it also links to other proteins of approximately 15 and 60 kDa.

It is presumed that the surface components of chlamydiae moderate numbers of essential biological events such as attachment, inducing phagocytosis, inhibition of phagolysosome fusion, infectivity, toxicity, and host immune responses, which contribute to immunity and pathogenesis.

3. Developmental cycle (3)

It is the developmental cycle of the chlamydiae that sets them apart from all other bacteria. The generation time of the chlamydiae in the host cells is about 48-72 hours. The cycle consists of 4 steps, as follows.

3.1 Attachment

The initial event in the infectious process begins with attachment of the EB to the microvilli of a susceptible columnar epithelial cell. The EB travels down the microvilli and localizes in the indentations of the plasma membrane that resembles the coated pits.

3.2 Cell entry

The EB is internalized to the host cell by endocytosis. The inclusion membrane does not fuse with lysosomes. It is bound and protected by the endosome membrane throughout its development.

3.3 Intracellular growth

During approximately 8 hours after cell entry, the EB changes into RB and loses infectivity. The cell wall loses its rigidity and becomes spheroplast-like. At this stage, DNA, RNA and proteins are synthesized. The RBs increase in number and size.

They are later divided by binary fission. Only the RBs are seen in the vacuole after 18-24 hours of infection.

During 24-48 hours of infection, the immature RBs condense to become infectious EBs. The RNA production decreases, while the production of DNA and glycogen increase.

3.4 Release of EBs

About 48-72 hours after infection, the infectivity of chlamydiae reaches its maximum. Glycogen degrades and eventually becomes undetectable. The inclusions are filled with mature EBs, which are later released by the destruction of the host cell.

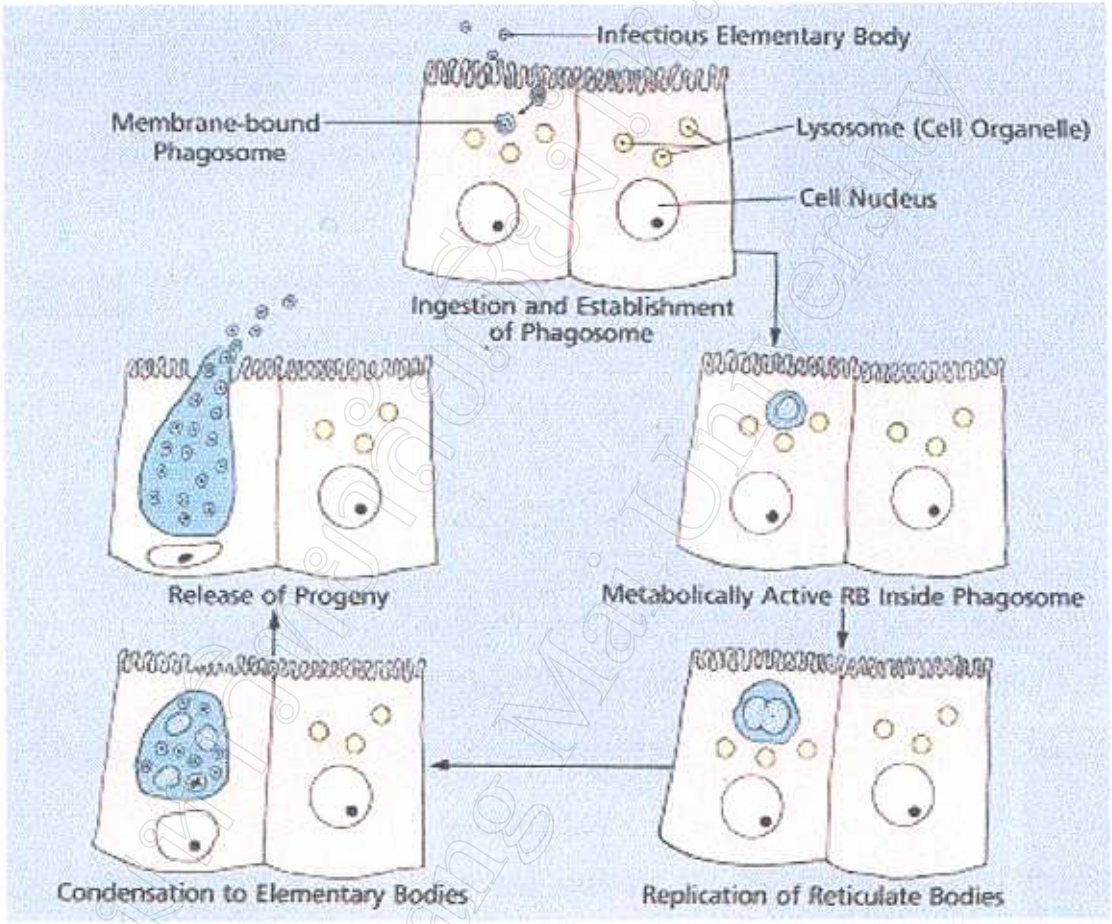

Figure 1. The developmental cycle of *Chlamydia trachomatis*

4. Pathogenesis (1, 2)

The pathogenesis of chlamydial infection has not been well explained. For LGV, it is known as a systemic infection involving the lymphoid tissue. The non-LGV is a parasite of squamocolumnar and columnar epithelial cells. It causes cell damage and prevail because it is an obligate intracellular parasite that kills host cells at the end stage of its growth cycle. Scarring of the affected mucous membranes is a common pathologic conclusion of chlamydial infection. This is what ultimately leads to blindness in trachoma and infertility and ectopic pregnancy after acute salpingitis.

The disease process and clinical manifestation of chlamydial infection probably represent the combined effects of tissue damage from chlamydial replication, inflammatory responses to chlamydiae antigens and necrotic materials from destroyed host cells. Both circulating antibodies and cell-mediated immunity are the immune response to chlamydial infection. There is now evidence that chlamydial diseases result in part from hypersensitivity or immunopathology. The putative sensitizing antigen has been identified as the HSP 60 (Heat shock protein). It may be synthesized by chlamydiae in cells where replication has been actively suppressed by the action of gamma interferon, thus, ongoing sensitization may occurred. These HSPs are similar to the heat-shock protein of other organisms. The HSP 60 contains antigenic sites that are shared with mycobacteria, *Escherichai coli* and even humans cells. Therefore, it is possible that some of the chlamydial diseases could be due to an autoimmune reaction or other infections, which might sensitize a person to worse diseases following their exposure to chlamydiae.

5. *Chlamydia trachomatis* infection

C. trachomatis can cause disease of eyes and genital tract. These diseases are distributed worldwide. However, the incidence of a specific disease may be more

prevalent within certain populations in different geographical and/or socio-economic conditions.

5.1 Ocular infections

Trachoma is a chronic keratoconjunctivitis caused by *C. trachomatis* serotype A, B, Ba and C. It is transmitted from eye to eye in endemic areas (4). Serotypes D through K have been isolated from the eyes of trachoma patients in non-endemic areas. Trachoma occurs as a sporadic eye disease dependent on genital tract to eye transmission was also reported (5).

Inclusion conjunctivitis is a disease of the newborn that derives from the passage through the infected maternal birth canal. It is caused by *C. trachomatis* serotype D through K and characterized by a sticky exudate and conjunctivitis.

5.2 Genital tract infection

The genital tract infection of *C. trachomatis* is divided into two types: (I) urogenital infections caused by oculogenital serotypes D through K and (II) Lymphogranuloma venereum (LGV) caused by serotypes L1-L3.

Chlamydial urogenital infections are caused mostly by serotypes D through K. The infection in women includes chronic cervicitis, urethritis, salpingitis and pelvic inflammatory diseases. Postpartum fever in infected mothers is also common. In addition, maternal cervical infection is associated with an increased rate of premature deliveries and perinatal morbidity. The infection in men is mostly non gonococcal urethritis (NGU), which usually results in a scant mucoid discharge. However, approximately 35-50 % of men with NGU was found with an absence of mucoid or frankly purulent discharge (6, 7).

LGV is caused by *C. trachomatis* serotypes L1, L2, L2a and L3. The LGV serotypes are more invasive than other serotypes of *C. trachomatis*. The

preferential site for multiplication of LGV is the regional lymph nodes, while serotypes of the trachoma group prefer squamo-columnar epithelial cells. Characteristic symptoms of LGV comprise enlarged matled inguinal and femoral lymph nodes, which are moderately painful and firm, and may fluctuate.

5.3 Respiratory tract infection

C. trachomatis serotypes D through K can cause neonatal pneumonia. The infection frequently occurs at the age of 4 to 16 weeks. The disease has prominent respiratory symptoms such as wheezing, coughing, and a lack of systemic findings of fever or toxicity. Chlamydial neonatal conjunctivitis often precedes the onset of pneumonia (7).

6. Laboratory diagnosis (1, 3)

The laboratory diagnosis of chlamydial infections can be accomplished by several methods, as follows.

6.1 Direct staining methods

C. trachomatis developes compact and clearly defined, glycogen-containing intracytoplasmic inclusions in the infected cells. The cells scraped from infected tissues can be examined directly under the microscope after Giemsa or iodine staining. The test is rapid and simple, but low in sensitivity.

6.2 Culture technique

Cell culture is a standard method for the detection and identification of *C. trachomatis*. Cell lines commonly used in the cultivation of chlamydiae are McCoy, Hela229, BHK-21, L929 and Buffalo green monkey kidney cells. After incubating the

culture for 48 to 72 hours, chlamydial inclusions are seen by using Giemsa, iodine or fluorescent antibody staining.

6.3 Antigen detection methods

Direct fluorescent antibody (DFA) staining and enzyme immunoassay (EIA) are methods used for the direct detection of *C. trachomatis* from genital, conjunctival and urine samples. These techniques are more sensitive than direct staining methods. DFA staining requires considerable experience and thoughtful interpretation (8). It is not suitable for processing a large number of specimens, and provides quantitative results. Meanwhile, EIA is commonly used for a large number of samples because it can be applied to an automatic system. It requires minimal training and also provides quantitative results.

6.4 Serological diagnosis

The microimmunofluorescence (Micro- IF) and complement fixation (CF) tests are the serological tests and commonly used for the detection of *C. trachomatis* antibodies. The CF test using a genus-specific antigen is applied for the diagnosis of infections caused by *C. psittaci*, *C. pneumoniae* and the LGV strains of *C. trachomatis*.

The Micro-IF test has been used widely in the diagnosis of occular and genital infections caused by *C. trachomatis*. This method is more sensitive than the CF test (9). In primary infection, the early antibody formation is the IgM, which persists for approximately 1 month before being replaced by the IgG. Reinfection with new serotypes results in increasing of IgM titres to the newly infected serotype, and elevates the level of IgG to the previous serotype.

6.5 Direct nucleic acid detection

The nucleic acid hybridization technique has been developed for the detection of chlamydial nucleic acid from the clinical specimens and cultured cells. It is the technique that couples the target nucleotides (DNA or RNA) with the detecting nucleotide probes. The hybridization occurs when the target and nucleotide probe are complementary. The nucleotide probes can be labeled with radioisotopic or non-radioisotopic material.

6.6 Polymerase chain reaction (PCR)

The polymerase chain reaction is a very sensitive molecular technique for the detection of a small number of nucleic acids. The PCR is an *in vitro* method used for nucleic acid amplification, by which a particular segment of DNA can be specifically amplified. It involves the binding of two oligonucleotide primers then the DNA fragment was amplified in repeated cycles. Each cycle comprises heat denaturation of the target DNA, annealing the primers to their complementary sequence and, finally, extending the annealed primers by the reaction of the Taq DNA polymerase. The reaction results in an exponential accumulation of the amplified DNA fragments of approximately 2^{n}, where "n" is the number of amplification cycles (10, 11).

The PCR is found to be a highly sensitive and specific technique for the detection of *C. trachomatis* in clinical samples (12, 13). In addition to sensitivity and specificity, the PCR is also simple. It becomes a method of choice for the detection of *C. trachomatis* in both research and routine laboratories.

6.7 Ligase chain reaction (LCR)

The ligase chain reaction (LCR) is another molecular technique. It is a method of using two complementary pairs of probes, when the correct template is

available. Both probes hybridize next to each other and are then ligated together by the action of the ligase enzyme. These ligated probes, plus the original template, serve as the template for the next cycle of hybridization and ligation. As subsequent cycles are performed, the amplification proceeds exponentially. The LCR has proven to be a fast and sensitive amplification procedure that is equivalent to the of PCR (14). The LCR assay becomes widely used for the diagnosis of chlamydial genitourinary infections. According to results from many investigators, the LCR technique is also highly effective for the detection of *C.trachomatis* in both urine and cervical samples (15, 16, 17).

7. Antigens and serotypes

Chlamydial antigens have been classified into three major groups: genus, species and type specific antigens (7).

7.1 Genus specific antigen

All members of the genus share a heat stable (100 °C for 30 mins), common antigen, referred to as the chlamydial group or genus specific antigen. It is a lipopolysaccharide (LPS) in nature with an acidic polysaccharide as an antigenic determinant. The immunodominant group, 2- keto- 3- deoxyoctanoic acid, is similar, but not identical to the LPS of *Salmonella* spp.(18). It is a complement fixing antigen with a molecular weight of 10^6 daltons.

7.2 Species specific antigen

The species specific antigen is shared by all 18 serotypes of *C. trachomatis*. It is shown as a protein with subunits that have a molecular weight of 1.55×10^5 daltons. Its antigenicity is destroyed by heating at 56 ° C for 30 minutes or by treating

with proteinase. As it appears to be highly immunogenic and common to all *C. trachomatis* strains, it becomes suitable as a candidate antigen for serological tests.

7.3 Serotype specific antigens

Serotrype specific antigens are observed only in certain isolates of *C. trachomatis*, and they are used for the serologically classification of chlamydial isolates. The monoclonal antibodies that neutralize infectivity of the organisms bind serotype-specific epitopes. These antigenic determinants would also be important for eliciting protective immunity against chlamydial infection. Up until now, 18 distinct serotypes of *C. trachomatis* have been identified (19).

The chlamydial antigens that confer to type, group and species specificities of the organism, are resident in the major outer membrane protein (MOMP) (20, 21). The serotype-specific epitopes have been mapped to VD1 and VD2, while the serogroup- and species-specific determinants have been mapped to VD4. The locations of VD1 to VD4 on the MOMP amino acid sequence are positioned at 64 to 83, 139 to 160, 224 to 237 and 288 to 317, respectively.

8. Genetics (1, 22, 23)

Chlamydiae have a small circular chromosome consisting of 1,045 kilobase pairs of nucleotide. It is approximately one fourth the size of an *E. coli* chromosome. The DNA of *C. trachomatis* strains has a G plus C content of approximately 44- 45 %. The DNA homology within chlamydial species has been analyzed and, so far, there is no interspecies homology. Thus, the restriction endonuclease analysis may provide the basis for molecular fingerprinting characterization among those chlamydial species. The sequences of the 16s RNA genes of *C. psittaci* and *C. trachomatis* have also been determined, and there is only a 5 % difference between them. These sequences are similar to those observed in other eubacteria.

And analysis of the chlamydia genome resulted in the identification of 894 likely protein-coding sequences. A similar study revealed as inferred functional assignment of 604 (68%) encoded proteins, of which 35 (4%) were similar to the hypothetical proteins deposited in other bacteria. The remaining 255 (28%) predicted proteins were not similar to other sequences deposited in the GenBank.

The MOMP genes of various *C. trachomatis* serovars have been cloned and sequenced which has revealed that they contain four variable domain regions designated to VD1, VD2, VD3 and VD4, and they are separated by a five constant domains (CDs) sequence. Currently the amino acid sequences of the MOMP of all serovars are also well characterized. They contain approximately 370-375 amino acids. The molecular weights fall between 39.5 and 40.5 kDa. The MOMP exhibits a high amino acid sequence heterogeneity that is mainly localized in these four VDs. They are exposed on the surface of the EBs and react with human immune sera. It is likely that these variable sequences are responsible for subgroup- or serovar-specific antigenic reactivity. Thus, the MOMP gene is likely to be useful and critical for the designation of genotyping methods as well as vaccine development.

Most strains of chlamydiae also have a 7.4 kilobase pair plasmid. The plasmid gene products appear relatively late in the developmental cycle, during the multiplication of RBs and transformation of RBs to EBs. The functions of these plasmid gene products are not known. However, they are not required for chlamydial growth or the development of diseases, since some *C. trachomatis* strains are isolated from the patients who lack plasmid.

9. Genotyping of *C. trachomatis*

Serotyping *C. trachomatis* is based on the differences in antigenic epitopes on the MOMP. The first method for serotyping *C. trachomatis* was the microimmunofluorescence (Micro- IF) test (24, 25). At the beginning, immune serum

obtained from mice, which had been immunized with the *C. trachomatis* isolates, was used to react with the elementary body (EB). Since MOMP specific monoclonal antibodies became available, mice immune sera are now replaced. To date, 18 serotypes of *C. trachomatis* have been identified by using type specific monoclonal antibodies. They are serotype A, B, Ba, C, D, Da, E, F, G, H, I, Ia, J, K, L1, L2, L2a, L3 (19, 25, 27). However, *C. trachomatis* serotyping is limited by those monoclonal antibodies that can react only to known antigens. Thus, the new serovariants that occur by mutation cannot be identified. Moreover, this technique needs to cultivate the organisms in tissue culture, which is tedious and time consuming.

As molecular biology techniques are explored, *C. trachomatis* genotyping becomes easier. *C. trachomatis* genotyping using the restriction fragment length polymorphism (RFLP) technique has been studied by many investigators (28, 29, 30, 31). This method is based upon the differential restriction endonuclease digestion of the MOMP gene. Frost *et al.* (28) and Rodriguez *et al.* (31) used the PCR to amplify the MOMP gene of the reference strains of *C. trachomatis*, which was then digested with one to four restriction endonucleases (RE) to obtain the RFLP patterns. This technique is capable of distinguishing at least 13 different serotypes of *C. trachomatis*.

However, amplification of a large DNA fragment is difficult in giving a high yield of a PCR product that is enough for subsequent digestion by the restriction enzymes. In addition, the large fragment of DNA usually gives a more complex banding pattern after RFLP, which makes it difficult to identify. To overcome these problems, an attempt to amplify and type is made by using only the VD4 sequence of the MOMP gene. The VD4 is the largest variable domain and confers to group specific reactivity. A small fragment of amplified product gives a smaller number of less complex DNA banding patterns in RFLP, thus, enabling easier differentiation .

The restriction pattern of the VD4-MOMP gene, which was deduced from the nucleotide sequence of the reference strains of 18 *C.trachomatis* serotypes was

shown by Leechanachai *et al.* (32). The nucleotide sequences of *C. trachomatis* reference strains were downloaded from the GenBank (National Center Biotechnology Information, NCBI). The Nest 2 and Nest 4 primers, which derived from the conserved sequence within the MOMP gene, was used to amplify the VD4 sequence. The sequence bracketed by these primers was analyzed for the restriction sites of all different restriction endonucleases by using the DNASIS version 2.1 computer program. The number and size of the RE fragments, generated by each enzyme, were determined in order to find an appropriate restriction treatment of the fragment, which could, in turn, give a different electrophoresis profile for each serotype. According to the results of the computer analysis, the Nest 2-Nest 4 amplified DNA were digested separately with 4 restriction endonucleases : *Alu*I, *Hind*III, *Dde*I and *Eco*RII. The projected digested fragment size of all *C. trachomatis* genotypes were tabulated (Table 1). At least 10 of 18 serotypes were identified as A/I/L3, H/Ia/J, C, E, L2/L2a, D/Da/L1, B/Ba, F, G and K. If necessary, serotypes A/I/L3 could be separated further by using *Hpa*II and *Mac*III, while serotypes D/Da and L1 were divided by *Bsm*AI. The VD4-RFLP was, however, successfully identified most of the common genital serotypes of *C. trachomatis*.

Table 1. The fragment size of PCR-RFLP in the VD4-MOMP gene of 18 *C. trachomatis* serotypes, generated from the GenBank database and analyzed by computer program DNASIS version 2.1 (32).

Group	Serotype of *C. trachomatis*	*Alu*I	*Hind*III	*Dde*I	*Eco*RII
B - complex	B	142	267	238	348
	Ba	142	267	238	348
	D	145	267	348	348
	Da	145	267	348	348
	L1	142	267	348	348
	E	142	348	348	348
	L2	142	348	237	348
	L2a	142	348	237	348
Intermediate	F	160	270	351	351
	G	160	260	350	350
C – complex	K	173	350	242	350
	C	330	350	241	348
	A	348	348	237	348
	I	350	350	240	350
	L3	351	351	241	351
	H	351	351	240	146
	Ia	351	351	240	146
	J	350	350	240	146

Direct nucleotide sequencing is another method for genotyping of *C. trachomatis*. The sequencing of the PCR-amplified MOMP DNA fragment is determined. Comparison of the MOMP gene sequence among 15 serotypes were performed by Yaun *et al.* (21). These serotypes were A/Har-13 (VR571B), Ba/AP-2 (VR347), B/TW-5, C/TW-3(VR578), D/UW-3(VR885), E/BOUR(VR348B), F/IC-Cal-3(VR346), G/UW-57(VR878), H/UW-4(VR879), I/UW-12(VR880), J/UW-36 (VR886), K/UW-31(VR887), L1-440(VR901B), L2-434(VR902B) and L3-404 (VR903B). In addition, the clinically isolated strains: Da/TW-448, Ia/UW-202 and L2a/TW-396, were sequenced by Dean et al. (33).

On the basis of amino acid sequence comparisons for all four VDs, the *C. trachomatis* serotypes can be separated into three groups: B complex group which are serotypes B, Ba, D, Da, E, L1, L2 and L2a; intermediate group which are serotypes G and F ; C complex group which are serotypes A, C, H, I, Ia, J, K and L3. It is found that the MOMP gene is much more diverse at DNA level than that expected from serologic typing (27, 29, 34, 35). Thus, the nucleotide sequencing method will be useful for the identification of chlamydial genotypes and evolution of variants or new types.

10. Genetic variation of the MOMP gene

The MOMP gene of *C. trachomatis* frequently exhibits DNA sequence variation, mainly in the VD regions. Three new chlamydial serotypes, Da, Ia, and L2a, were detected and compared to their prototype by Dean *et al.* (33). They reported that the serotype Da differed from the D prototype with one nucleotide substitution and one amino acid transition in the VD4, while the serotype L2a varied from the L2 serotype in the VD2 with one nucleotide substitution and one amino acid transtion. The Ia differed from the I prototype in VD1 and VD2 with one nucleotide substitution and

one amino acid transition, and in the VD4, four nucleotide and amino acid substitutions were observed.

Yang *et al.* (29) reported the nucleotide sequences of VD1, VD2 and VD4 of the MOMP gene of *C. trachomatis* from 49 positive clinical samples. Thirty samples (61%) had VD sequences identical to the prototype and 15 (31%) had a sequence that varied from the prototype. Eleven of 15 (73%) nucleotide sequence variations had two or fewer nucleotide substitutions in one or two VDs, which represented point mutation, while the other 4 (27%) had a large number of nucleotide changes that represented genetic recombination. This variant exhibited the nucleotide sequences of some VDs that were mostly closed to one genotype, while the other VDs resembled other genotypes.

Brunham *et al.* (34) determined the nucleotide sequence of *C. trachomatis* MOMP genes that were isolated from patients with a sexually transmitted disease (STD), who were at a high risk and exposed frequently to STD pathogens. It was found that 63% had a variant VD sequence, while 32% possessed the prototype. Among the variant VD types, 48% had a point mutation and 52% displayed a genetic recombination. These recombinations could be a result of co-infection with multiple *C. trachomatis* genotypes. In addition, Lampe *et al.*(36) reported that genetic recombination was a potential genetic mechanism for MOMP sequence polymorphism. Nevertheless, in both the genetic recombination of VDs between two distinct genotypes and accumulated point mutation with nucleotide substitution, deletion or insertion on prototype sequence could be the genetic mechanism for generating MOMP sequence polymorphism. This polymorphism could have aided the organisms in establishing infection or persistence in persons who had neutralizing antibodies to non-variant MOMP epitopes.

Dean *et al.* (37) found the mix sequences of serovar B and D in specimens from a population in Tunisia where trachoma was endemic. Furthermore, Morre *et al.*

(38) reported the new variant of J serovar, which designated Jv. The nucleotide sequences of Jv, which differed from the J prototype, were found in VD1, VD2, VD3 and VD4. In the same study, it was found that Ba variant, which contained the nucleotide in VD3 differed from the Ba prototype. Although most variations of the MOMP gene occur in 4 VDs, some studies reported the variation outside variable segments of the MOMP gene. Frost *et al.* (39) found two sites that changed amino acid in the constant region upstream of VD1 in the urogenital Ba sample. Dean *et al.* (40) revealed that identical E immunotypes displayed sequence diversities within both constant and variable regions of the MOMP gene. Thus, nucleotide sequencing for the whole MOMP gene is still needed.

In this study, the nucleotide sequence of *C. trachomatis* was determined by using the cycle sequencing technique with the BigDye ™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Applied Biosystems, USA.) The method was based on the dideoxynucleotide chain terminator technique of Sanger *et al.* (41). As the 2', 3' dideoxyribonucleoside triphosphate (ddNTPs) lacked a hydroxyl residue at the 3' position of deoxyribose, the formation of a phosphodiester bond with the succeeding dNTP was prevented. The extension of the growing DNA chain was therefore terminated. Since each of the four ddNTP in this kit was tagged with four different fluorescent dyes: A- dyeT, C- dyeT, G- dyeT and T- dyeT, they were able to incorporated into a DNA extension product by Ampli Tag DNA polymerase, FS enzyme. Thus, the growing chain was terminated simultaneously and labeled with the dye that corresponded to that base. As *Ampli Taq* DNA polymerase, FS enzyme was a member of the *Taq* F667Y family, in which phenylalanine was substituted by a tyrosine at position 667, it could incorporate chain termination ddNTPs more efficiency than the *Taq* DNA polymerase. This enzyme provides rapid nucleotide incorporation and had no 5' to 3' nuclease activity. Additional, this kit was suitable for

performing cycle sequencing reaction on a single or double stranded DNA template or PCR product (42).

After sequencing, the ABI 310 automated DNA sequencer was used to detect fluorescence from four difference dye that were used to identify the A, G, C, T extension reactions. Each dye emitted light at a different wavelength when excite by a laser. Thus, all four colors in the singer reaction could be detected and distinguished in a capillary electrophoresis. The direct on-line monitoring of the gel capillary electrophoresis and direct data entry were controlled by computer program ABI 310 data collection version 3.10 and ABI 310 DNA sequencing version 2.2 (Perkin Elmer, Applied Biosystem, USA).