Genotyping of *Chlamydia trachomatis* from clinical specimens in Taiwan

Min-Chih Hsu,¹ Pei-Yi Tsai,¹ Kow-Tong Chen,² Lan-Hui Li,³
Chien-Chou Chiang,³ Jih-Jin Tsai,⁴ Liang-Yin Ke,⁴ Hour-Young Chen¹
and Shu-Ying Li¹

Correspondence
Shu-Ying Li
syl@cdc.gov.tw

¹Laboratory for Bacteriology and Mycology, Center for Laboratory Research and Diagnostics, Center for Disease Control, Taipei, Taiwan

²Department of Public Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan

³Taipei City Hospital, Branch for Disease Control & Prevention, Taipei, Taiwan

⁴Chung-Ho Memorial Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

This study was conducted to determine the prevalence and distribution of *Chlamydia trachomatis* genotypes in Taiwan. Urine and endocervical-swab samples were collected from two hospitals located in northern and southern Taiwan. The genotypes of a total of 145 samples positive for *C. trachomatis* were analysed by sequencing the *omp1* gene and this was successful in 102 samples. Nine different *C. trachomatis* genotypes were identified. Genotype E was the most prevalent (22 %), followed by D and Da (19 %), F (16 %), J (15 %), K (11 %), G (11 %), H (6 %) and Ba (2 %). There was a geographical difference in the prevalence of genotype H ($P < 0.018$) between northern and southern Taiwan. Sequence mutation analysis by BLAST searching against GenBank reference sequences identified 12 genetic variants from a total of 102 *omp1* gene sequences.

Received 26 July 2005
Accepted 15 November 2005

INTRODUCTION

Chlamydia trachomatis infection is the most prevalent sexually transmitted bacterial disease. It is estimated that 89 million cases occur annually worldwide (Gaydos *et al.*, 2004). Because in 50 % of men and 80 % of women infected individuals are asymptomatic, the actual number of reported cases represents only a fraction of the infected population (Gaydos *et al.*, 2004). Currently 19 human serovars and related variants (A, B/Ba, C, D/Da, E, F, G, Ga, H, I/Ia, J, K, L1, L2, L2a and L3) have been identified by using polyclonal and monoclonal antibodies against the major outer-membrane protein (MOMP) (Grayston & Wang, 1975; Ngandjio *et al.*, 2004; Pedersen *et al.*, 2000; Suchland & Stamm, 1991; Wang *et al.*, 1985; Yuan *et al.*, 1989). Based on the pathogenic potential, serovars A, B, Ba and C are commonly associated with trachoma. Serovars D to K are commonly associated with urogenital infections, such as urethritis, epididymitis, cervicitis, salpingitis, pelvic inflammatory disease and ectopic pregnancy; serovars L1 to L3 are commonly associated with lymphogranuloma venereum (Yuan *et al.*, 1989).

Abbreviation: STD, sexually transmitted disease.

The GenBank/EMBL/DBJ accession numbers for the partial *omp1* sequences from *C. trachomatis* strains determined in this work are DQ155295–DQ155300 and DQ287919–DQ287921.

Furthermore, suggestions have been made that the infections with *C. trachomatis* serovars G, I and D are associated with cervical squamous cell carcinoma, and chronic infections with serotype K in women have been recognized as a cause of infertility (Anttila *et al.*, 2001; Koskela *et al.*, 2000; Marrazzo & Stamm, 1998; Morre *et al.*, 2000).

Serological typing methods have a limitation in that newly emerging types may be missed and culturing of clinical isolate is usually required (Eckert *et al.*, 2000; Suchland & Stamm, 1991). Compared to serotyping, the genotyping methods are more sensitive and specific for *C. trachomatis* serovar identification (Molano *et al.*, 2004). Many studies have shown the feasibility of deducing the serotypes of *C. trachomatis* clinical isolates using PCR-based RFLP or sequencing of the amplified *omp1* gene that encodes the MOMP, the main surface antigen of *C. trachomatis* (Ikehata *et al.*, 2000; Lysén *et al.*, 2004; Ngandjio *et al.*, 2003, 2004; Singh *et al.*, 2003). The *omp1* gene exhibits extensive DNA sequence variation in four discrete regions, termed variable segments (VS1–4) (Stephens *et al.*, 1987; Yuan *et al.*, 1989). Sequence analysis of the variable segments of the *C. trachomatis omp1* genes revealed immunogenic epitopes, which is beneficial for vaccine studies (Peterson *et al.*, 1999; Stothard *et al.*, 1998). Furthermore, by sequencing these four variable segments, subtle sequence differences within the same *C.*

trachomatis genotype can be identified, which provides a valuable and sensitive means for molecular epidemiological analysis to identify high risk groups and track sexual networks (Cabral *et al.*, 2003; Lee *et al.*, 2006). For example, a new genovariant of *C. trachomatis*, L2b, was primarily responsible for outbreaks of lymphogranuloma venereum among high-risk core groups (HIV positive men who have sex with men) in the Netherlands (Spaargaren *et al.*, 2005).

In Taiwan, although the incidence and prevalence of *C. trachomatis* genital infection is rising, only limited information is available (Hsieh *et al.*, 1999; Wu *et al.*, 1992). The aims of the present study were to conduct sequence analysis of the *omp1* gene to determine the genotypes and variants of *C. trachomatis* strains causing genital infections in Taiwan. Such data may give insights into the epidemiology of *C. trachomatis* strains circulating in Taiwan, and contribute to improved control measures in the future.

METHODS

Clinical samples. The study was part of a sexually transmitted disease (STD) surveillance project approved by an STD review committee from the Center for Disease Control, Taiwan, and informed consent was obtained from patients before sample collection. During the period December 2003 to October 2005, 1253 urine and 1151 endocervical-swab samples were collected for detection of *C. trachomatis* from 2360 patients visiting two hospitals for genital chlamydia infection examination. The two hospitals are located in Taipei and Kaohsiung, which are the two largest metropolitan cities located in northern and southern Taiwan, respectively. Among these samples, 476 endocervical-swab samples and 1253 urine samples were from Taipei, and 675 endocervical-swab samples were from Kaohsiung. There were 44 patients with both urine and endocervical-swab specimens in Taipei. Specimens were collected according to the manufacturer's instructions for the COBAS AMPLICOR *C. trachomatis* test (Roche Diagnostic Systems). The criteria used for determining positive and negative samples depended on the absorbance at a wavelength of 660 nm. In this test, negative and positive controls should be included in each run. The absorbance of the negative control should be less than 0.2 and of the positive control should be greater than 2.0 at 660 nm. If the absorbance of the negative control is greater or equal to 0.2 and positive control is less than 2.0 at 660 nm, the entire run is invalid. Properly processed specimens should give positive results with an absorbance greater than or equal to 0.8 at 660 nm. The negative result is below 0.2. Between the absorbance of 0.2 and 0.8, the test should be repeated in duplicate on processed specimen. *C. trachomatis*-positive samples (original urine and endocervical-swabs) were frozen at -70°C for further analysis.

DNA extraction. DNA was extracted using the QIAamp viral RNA minikit (Qiagen) for urine and endocervical-swabs samples

according to the manufacturer's instructions (Lysén *et al.*, 2004). These samples were concentrated by centrifugation for 5 min at 13 000 *g* prior to DNA extraction. The DNA was frozen at -20°C for nested PCR.

PCR. Primary amplification of an approximately 1130 bp fragment encompassing VS1–4 of *omp1* was performed using the outer primer pair NLO/NRO (Ngandjio *et al.*, 2004), and reamplification of a 584 bp fragment spanning VS1–2 was conducted based on the inner primer pair MOMP87/C214 (Table 1) (Stothard *et al.*, 1998). The first PCR step was carried out in a final reaction volume of 25 μl , containing 0.4 μM each NLO/NRO outer primer pair, 5 μl (50 ng) extracted DNA and 12.5 μl $2\times$ PCR master mix (MBI Fermentas). The amplification conditions consisted of initial polymerase activation at 95°C for 5 min; 35 cycles of 94°C for 60 s, 54°C for 60 s and 72°C for 80 s; and a final elongation step at 72°C for 10 min. A negative water control was included in each run. In the second PCR step, 3 μl product from the first PCR step and 0.4 μM each MOMP87/C214 inner primer pair was added to a final volume of 25 μl . The PCR conditions were: 95°C for 5 min; 35 cycles of 94°C for 50 s, 56°C for 50 s and 72°C for 50 s; and a final elongation step at 72°C for 10 min. The amplified products were electrophoresed through a 2% agarose gel and visualized by staining with ethidium bromide.

DNA sequencing. A total of 102 *omp1* fragments successfully amplified from the 145 *C. trachomatis*-positive samples were purified using a QIAquick PCR purification kit (Qiagen), and strands of 584 bp and 1130 bp fragments of the *omp1* segment were sequenced using a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems). The reaction mixtures were loaded onto a 3730 Avant genetic analyser (Applied Biosystems). The primer sets used for sequencing were MOMP87/C214 and NLO/NRO. All the PCR products were sequenced bidirectionally.

BLAST analysis and alignments. The consensus sequences were compared to sequences of known *C. trachomatis* strains by BLAST searching GenBank (www.ncbi.nlm.nih.gov/GenBank). The sequences were assembled into alignments using reference sequences derived from GenBank: B/IU-1226 (AF063208), B/B-16 (AY950630), D/B-120 (X62918), Da/TW-448 (X62921), E/Bour (X52557), F/ICCal3 (X52080), G/UW57 (AF063199), H/Wash (H/UW4) (X16007), J/UW36 (AF063202) and K/UW31 (AF063204). Multiple alignments were performed with BioEdit version 7.0 software. The sequence data of new variants were submitted to NCBI and have the following accession numbers: E3 (DQ155295), E5 (DQ155296), E4 (DQ155297), E2 (DQ155298), G2 (DQ155299), K2 (DQ155300), G3 (DQ287919), H2 (DQ287920) and J2 (DQ287921).

Statistical analysis. We used the chi-square tests of the SPSS version 10.0 software to establish relationships between genotypes and clinical features (specimen type, HIV status, age or sex) as well as geographical parameters.

Table 1. Primers used for the nested PCR of the *omp1* gene

Primer	Strand	Sequence (5'–3')	Position
NLO	Sense	ATGAAAAAACTCTTGAAATCG	1–21
NRO	Antisense	CTCAACTGTAAGTGGTATTT	1108–1128
MOMP87	Sense	TGAACCAAGCCTTATGATCGACGGA	87–111
C214	Antisense	TCTTCGAYTTTAGGTTTAGATTGA	648–671

RESULTS

C. trachomatis prevalence

Of the total 2404 samples, 145 samples (6 %) were positive for *C. trachomatis* in a routine Roche COBAS AMPLICOR PCR test; 79 out of 1253 (6 %) urine samples and 39 out of 476 (8 %) endocervical-swab samples from Taipei, and 27 out of 675 (4 %) endocervical-swab samples from Kaohsiung were *C. trachomatis* positive.

Genotyping

Sequence analysis of the 584 bp and 1130 bp fragments of the *omp1* gene, comprising VS1–2 and VS1–4, was achieved from nested PCR amplified DNA in 102 (70 %) of the total 145 *C. trachomatis*-positive samples. Genotype E was the most prevalent (22 %), followed by D and Da (19 %), F (16 %), J (15 %), G (11 %), K (11 %), H (6 %) and Ba (2 %) (Table 2). Genotype Ba, D, Da and F showed no variation, and were identical to the reference strains used. Of the 102 samples, we observed 12 different *omp1* nucleotide sequences. Multiple sequence alignment of the sequences from all 102 samples showed a total of 17 different variable nucleotide positions, of which 13 resulted in amino acid replacements. All of the mutation positions were rechecked by repeating the nested PCR and then sequencing the fragments bidirectionally.

For genotype E, 4 out of 22 samples (18 %) differed from the reference strains E/Bour. The nucleotide substitutions at positions 258, 321, 572 and 637, changing base pair C to A, T to C, T to C and T to C, respectively, caused the amino acid to change from Asp to Glu, Pro to Pro (silent), Phe to Ser and Phe to Leu, respectively (see Table 3). For genotype G, 2 out of 11 samples (18 %) differed from the reference strain G/UW57. The G2 sample had three nucleotide substitutions at positions 88, 89 and 91 that showed T to C, A to G and G to T variations, respectively, and caused the amino acid to change from Arg to Arg (silent), Met to Val and Met to Val, respectively. The G3 sample had only one mutation at

position 392, which showed a G to A variation, and changed the amino acid from Gly to Ser. For genotype K, compared to K/UW31, two samples (2/11, 18 %) both had two nucleotide substitutions at positions 283 and 293, leading to G to A, and A to G variations, respectively, and causing Gly and Asn, respectively, both to be replaced by Ser.

A high mutation rate (4/6, 67 %) was found for genotype H compared with the reference strain H/Wash (H/UW4). Nucleotide substitutions at positions 440, 675, 682, 778 and 1018 were identified in our genotype H, leading to A to G, T to A, A to G, C to T and C to T mutations, respectively, and causing the amino acid to change from Asn to Ser, Asp to Glu, Thr to Ala, Leu to Phe and Leu to Leu (silent), respectively. In our 15 confirmed genotype J samples, all of them (15/15, 100 %) differed from reference strain J/UW36 at the 369 position. One sample, J2, had another mutation at position 572. After BLAST searching, we found that the position 369 mutation was the same as the sequence of the *C. trachomatis* J/isolate 6858 (AY950622) from China, not the reference strain J/UW36.

DISCUSSION

To our knowledge, this is the first paper on genotype distribution of *C. trachomatis* genital infections in Taiwan. In this study, we found that genotype E was the most common genotype (22 %), followed by genotypes D (19 %), F (16 %), J (15 %), G (11 %), K (11 %), H (6 %) and genotype B (2 %). Genotype B was rarely involved in urogenital infections in Taiwan, which is in accordance with previous findings. Table 4 shows the distribution of *C. trachomatis* genotypes in Asian countries. In Asia, there is little data on the distribution of the *C. trachomatis* genotypes. From our result, E, D and F were the top three genotypes in Taiwan, similar results were found in Japan and Korea. In Thailand, F (25–60 %) was the most prevalent genotype followed by E (9–20 %) and D (7–23 %) (Table 4). Differences in genotype prevalence were found in different study populations. In Thailand, for example, in non-sex workers, F (60 %) was the most predominant genotype, while in sex workers the rate of F genotype decreased to 29 % (Yamazaki *et al.*, 2005).

Other studies have reported the genetic variability of the *omp1* gene sequences as ranging from 10 % to 81 % (Brunham *et al.*, 1996; Dean *et al.*, 1995; Frost *et al.*, 1995; Sturm-Ramirez *et al.*, 2000). We found 12 genetic variants out of a total of 102 *omp1* gene sequences. No variant was identified for genotypes Ba, D, Da and F compared to the reference strains. For the most prevalent genotype E, four samples were diverged (4/22, 18 %) from the reference sequence E/Bour, which was analogous to a prior study reporting on the genetic variants of E strains (11/67, 16 %) (Dean & Millman, 1997). For genotype G, two new variants (2/11, 18 %) were identified with nucleotide mutations at one and three positions, respectively. For genotype K, two new variants (2/11, 18 %) were discovered, each with two nucleotide mutations. The mutation positions in the genotype E, G and

Table 2. Distribution of 584 and 1130 bp sequence fragments in different serovars of *C. trachomatis*

Genotype	No. of cases (%)	No. of cases (%) with:	
		584 bp fragment	1130 bp fragment
B	2 (2)	1 (50)	1 (50)
D	19 (19)	11 (58)	8 (42)
E	22 (22)	14 (64)	8 (36)
F	16 (16)	11 (69)	5 (31)
G	11 (11)	9 (82)	2 (18)
H	6 (6)	4 (67)	2 (33)
J	15 (15)	9 (60)	6 (40)
K	11 (11)	6 (55)	5 (45)
Total	102	65 (64)	37 (36)

Table 3. Nucleotide changes found in the 12 genetic variants out of the 102 clinical *C. trachomatis* specimens compared to reference sequences

The following reference strains were used for comparison with the sequences obtained in this study: B/B-16, identical to Ba; D/B-120, identical to D1; Da/TW-448, identical to D2; E/Bour, identical to E1; F/IC-Cal3, identical to F; G/UW57, identical to G1; H/Wash (H/UW4), identical to H1; J/UW36, identical to J1; K/UW31, identical to K1.

Genotype (no. of cases)	No. of strains with nucleotide changes/total (%) with changes)	Nucleotide change	Position*	Amino acid change
Ba (2)	No variant			
D1 (13)	No variant			
Da2 (6)	No variant			
E1 (18)				
E2 (1)	1/22 (5)	C to A	258	Asp to Glu
E3 (1)	1/22 (5)	T to C	321	Silent
E4 (1)	1/22 (5)	T to C	572	Phe to Ser
E5 (1)	1/22 (5)	T to C	637	Phe to Leu
F (16)	No variant			
G1 (9)				
G2 (1)	1/11 (9)	T to C	88	Silent
	1/11 (9)	A to G	89	Met to Val
	1/11 (9)	G to T	91	Met to Val
G3 (1)	1/11 (9)	G to A	392	Gly to Ser
H1 (2)				
H2 (1)	4/6 (67)	A to G	440	Asn to Ser
	1/6 (17)	C to T	778	Leu to Phe
H3 (2)	4/6 (67)	A to G	440	Asn to Ser
	2/6 (33)	C to T	1018	Silent
H4 (1)	4/6 (67)	A to G	440	Asn to Ser
	1/6 (17)	T to A	675	Asp to Glu
	1/6 (17)	A to G	682	Thr to Ala
J1 (14)	15/15 (100)	C to T	369	Silent
J2 (1)	15/15 (100)	C to T	369	Silent
	1/15 (7)	A to G	572	Asp to Gly
K1 (9)				
K2 (2)	2/11 (18)	G to A	283	Gly to Ser
	2/11 (18)	A to G	293	Asn to Ser

*Position refers to the reference sequence of each serotype and is not homologous between serotypes.

K samples in this study were all new compared with other papers reporting the analysis of mutation sites (Cabral *et al.*, 2003; Jurstrand *et al.*, 2001; Lister *et al.*, 2004b; Lysén *et al.*, 2004; Ngandjio *et al.*, 2003; Pedersen *et al.*, 2000). Four genetic variants (67%) were identified in our genotype H samples. After BLAST searching at the NCBI, we found the mutations at positions 440 and 1018 of H3 were identical to the *C. trachomatis* isolate H/IS 1075 (AF414959) from Iceland, not the reference strain H/Wash (H/UW4). In Sweden, Lysén *et al.* (2004) have reported the same mutation at positions 440 and 1018 of genotype H. The mutation at position 778 in H2 was a new finding. The mutations at positions 440, 675 and 682 of H4 were the same as those reported by Lysén *et al.* (2004).

For genotype J, fifteen sequences of genotype J were confirmed to be identical to the *C. trachomatis* J/isolate 6858 (AY950622) isolated from China in 2005, not the generally used reference strain J/UW36. The J2 genotype sample was found to have one additional mutation at position 572. Compared with the Ja genotype reported from the United States and Jv genotypes reported from the Netherlands (Dean *et al.*, 2000; Morre *et al.*, 1998), our genotype J was quite different. Our J1 and J2 genotypes exhibited only two mutations at position 369 and 572, and both were new compared to the reported Ja and Jv genotypes. The two mutation sites were in the conserved segment 2 and 3. Compared with the reference strain J/UW36, mutation at residue 369 of our genotype J changed C to T, and resulted

Table 4. Distribution of genotypes of *C. trachomatis* in Asia in recent years

Typing method	Sample no. for typing	Genotype distribution	Country	Reference
RFLP	207	E (24 %), D (19 %), G (18 %), F (11 %), H (7 %), I (7 %), K (4 %)	Japan	Ikehata <i>et al.</i> (2000)
RFLP	47	D (32 %), E (17 %), F (17 %), G (15 %), K (9 %), H (6 %), I (2 %)	Japan	Yamazaki <i>et al.</i> (2005)
RFLP, dot-ELISA	28	E>D>F>H	Korea	Choi <i>et al.</i> (2001)
Sequencing	40	E (45 %), F (20 %), G (15 %), D (5 %), H (5 %), J (2.5 %)	Korea	Lee <i>et al.</i> (2006)
Sequencing	102	E (22 %), D (19 %), F (16 %), J (15 %), G (11 %), K (11 %), H (6 %), B (2 %)	Taiwan	This study
Sequencing	45	F (25 %), D (23 %), H (12 %), K (12 %), E (9 %), Ia (7 %), B (7 %), Ja (5 %), G (2 %)	Thailand	Bandea <i>et al.</i> (2001)
RFLP	56	F (29 %), E (20 %), K (18 %), D (14 %), G (7 %), H (5 %), J (5 %), I (2 %), Ba (2 %)	Thailand	Yamazaki <i>et al.</i> (2005)
RFLP	15	F (60 %), E (13 %), H (13 %), D (7 %), K (7 %)	Thailand	Yamazaki <i>et al.</i> (2005)

in a silent substitution, which has been described by others (Jurstrand *et al.*, 2001; Ngandjio *et al.*, 2003). Interestingly, there was significantly higher mutation rate at position 369 of genotype J in Taiwan (15/15, 100 %) compared to that in Sweden (4/10, 40 %, $p < 0.001$) (Jurstrand *et al.*, 2001) and in Cameroon (1/3, 33 %, $P < 0.02$) (Ngandjio *et al.*, 2003), as analysed by Fisher's exact test.

For genotype K, there were two variants (K2), both with the same mutation at positions 283 and 293. After BLAST searching, we found that the sequence of K2 was more similar to the *C. trachomatis* K/isolate 4011 (AY950621) from China than to the reference strain K/UW31. The mutation at position 283 A of K2 was new for both K/isolate 4011 (AY950621), and the reference strain K/UW31 (283:G). The mutation at position 293 G of K2 was identical to K/isolate 4011 (AY950621) but different from that of the reference strain K/UW31 (293:A). A study by Parish *et al.* (2003) reported that the incidence of STDs is increasing rapidly in China. We found the mutants of genotype J and K identified in our research related to specific *C. trachomatis* isolates, J/isolate 6858 (AY950622) and K/isolate 4011 (AY950621) reported from China. Based on the geographical proximity of Taiwan and China, and frequent travel between the two nations such molecular data could have implications for the surveillance of *C. trachomatis* transmission.

Out of 145 *C. trachomatis*-positive tests from the COBAS routine analysis, the DNA of 43/145 (30 %) did not amplify using the *omp1* nested PCR. This was high compared to the failure rate of 1 to 24 % in similar studies (Jonsdottir *et al.*, 2003; Jurstrand *et al.*, 2001; Sturm-Ramirez *et al.*, 2000). The high failure rate may be due to a lower sensitivity of the *omp1* nested PCR because only a single-copy of the gene target, *omp1*, is present. In contrast, the COBAS AMPLICOR assay exhibited a higher sensitivity for targeting cryptic plasmid that is present as approximately 10 copies (Lister *et al.*, 2004a; Mahony *et al.*, 1993). Other studies also reported that positive genital samples identified using the

COBAS or ligase chain reaction (LCR) assay could not be amplified by *omp1* PCR (Pedersen *et al.*, 2000; Vincelette *et al.*, 1999). Further analysis showed that DNA in 47 out of 79 (60 %) urine samples and 55 out of 66 (83 %) endocervical-swab samples could be amplified by the *omp1* nested PCR, indicating a superior sensitivity of endocervical-swab specimens compared to urine specimens. A possible explanation is that urine might dilute the exfoliated urethral epithelial cells infected by *C. trachomatis* and cause the high failure rate of urine samples amplified by *omp1* PCR. Furthermore, most women with chlamydia are infected at the cervix, a site remote from the female urethra. Hence, female urine testing may not adequately detect cervical infections (Wiesenfeld *et al.*, 1996). A study of human papillomavirus reported that β -globin DNA was amplifiable from 87 % of the cervical-swab specimens and 73 % of the urine specimens ($P = 0.01$) (Brinkman *et al.*, 2002). Other studies reported that phosphate in urine is a powerful inhibitor of the LCR reaction, and DNA degradation is enhanced in DNase-rich body fluids like urine (Schachter *et al.*, 2003; Woegerbauer *et al.*, 2002). All these findings mentioned above could explain why the sequencing rate was lower in urine specimens than in endocervical-swab specimens.

In our study, all of our sequences were unambiguous and we did not find any mixed infections in one single urine or endocervical-swab specimen. There were six *C. trachomatis*-positive patients with dual urine and endocervical-swab specimens. Among these patients, two showed dual infections of different genotypes in two kinds of specimens (urine/endocervical swab; K/G and E/H). The remaining four patients had identical genotypes in both specimens. Mixed infections have been reported in a single endocervical specimen, confirmed by RFLP (Ikehata *et al.*, 2000) and different genotypes have also been found in urine and endocervical-swab specimens, respectively (Jurstrand *et al.*, 2001). The dual infections by different genotypes in the same patient may be the result of different infection episodes, an immunity difference in different body sites or the lack of

Table 5. Distribution of genotypes and mutations in samples from Taipei and Kaohsiung hospitals

Genotype	Taipei		Kaohsiung		Total no. of samples (%)
	No. of samples (%)	No. of mutations (%)	No. of samples (%)	No. of mutations (%)	
B	0 (0)	0	2 (11)	0	2 (2)
D	18 (22)	0	1 (5)	0	19 (19)
E	20 (24)	4/20 (20)	2 (11)	0	22 (22)
F	13 (17)	0	3 (15)	0	16 (16)
G	9 (11)	2/9 (22)	2 (11)	0	11 (11)
H	2 (2)	2/2 (100)	4 (21)	2/4 (50)	6 (6)
J	10 (12)	1/10 (10)	5 (26)	0	15 (15)
K	11 (13)	2/11 (18)	0	0	11 (11)
Total	83	11/82 (13)	19	2/19 (11)	102

cross-protection between genotypes. No multiple peaks were found and the data were rechecked twice. Although the possibility could not be ruled out that differences in the nucleotide sequences (mainly primer preference), as well as in molar ratio of the individual genotypes, may lead to the different PCR performances (Banda *et al.*, 2001), and some genotypes may be easier to amplify using PCR than others. This may perhaps cause mixed infections to be overlooked and also lead to genotype prevalence bias (Eley & Khalili, 1993; Pedersen *et al.*, 2000).

In this study, no correlation between specimen type, HIV status, age or sex and genotype was found (data not shown). After further analysis, we found different genotype distributions in Taipei and Kaohsiung. Types E (24 %) and D (23 %) were predominant in Taipei. However, types J (26 %) and H (21 %) were more prevalent in Kaohsiung (Table 5). The percentage of genotype H in the two geographical regions showed significant differences ($P < 0.018$), and no genotype K was found in Kaohsiung. More specimens should be gathered, especially from Kaohsiung, to further confirm such differences. The mutation rate of genotype H (4/6, 67 %) was the highest amongst the eight genotypes (Table 5), especially in Taipei where the two H samples were both mutant (2/2, 100 %) compared with Kaohsiung where the rate was lower (2/4, 50 %). For genotypes J, K, E and G, the percentage of mutants was from 10 to 22 %.

In conclusion, we conducted what is believed to be the first investigation into the prevalence and distribution of genotypes of *C. trachomatis* in Taiwan. Seven of the eight genotypes usually associated with urogenital infections were identified in our samples. Genotypes E, D and F dominated in our Taiwanese clinical material. Sequencing the *omp1* gene enables analysis of sequence variations within each genotype and provides discrimination between *C. trachomatis* strains, which is helpful in tracing the sexual transmission pathway, which ranges from individual contact to international transportation. The data obtained in this study could have implications for the control and vaccine development of *C. trachomatis* in Taiwan.

ACKNOWLEDGEMENTS

This work was supported in part by grant DOH94-DC-2011 from the Center for Disease Control, Department of Health, Taiwan, and a pathogenic genomic databases project grant (93-0324-19-F-00-00-35) from the National Science Council, Executive Yuan, Taiwan.

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