

V. DISCUSSION

Differentiation of chlamydial serotypes in clinical isolates is necessary for epidemiological studies in order to establish a regional serotype prevalence and prevention of infections. Immunotyping with a panel of serovar-specific monoclonal antibodies is the referential standard method for *C. trachomatis*. However, this technique is limited by the commercial availability of serovar-specific monoclonal antibodies known and, as yet, undiscovered variants. Furthermore, in serotyping, *C. trachomatis* needs to grow in a cell culture, and several culture passages have to be performed in some strains. To avoid these limitations, molecular biology techniques such as PCR-RFLP and nucleotide sequencing have been introduced by many investigators. As the MOMP gene of *C. trachomatis* encodes several surface antigens, including group and type-specific determinants, analysis of this gene will probably prove fruitful for epidemiological studies. The RFLP technique cannot detect the type of variants if those variations do not occur in the recognition site of the restriction endonuclease used. Nevertheless, the application of RFLP analysis is still useful in studying the type distribution of *C. trachomatis* in different geographies. This is because it does not require sophisticated equipment or anything as expensive as the direct nucleotide sequencing technique.

The RFLP analysis performed in this study was based on the amplification of the VD4 region, the largest VD of the MOMP gene, which was subsequently digested with 4 restriction endonucleases. This technique identified at least 10 of 15 *C. trachomatis* serotypes that included all predominant genital serotypes currently identified. From 34 *C. trachomatis* positive samples, genotype F and D/Da/L1 were predominantly identified in 9 (26.5%) and 8 (23.5%) respectively. These serotypes accounted for 50% of the total isolates. The overall prevalence of *C. trachomatis* type

observed in this study does not much differ from those reported in other countries. Genotype E, D and F were the major genital serotypes detected in many countries (4, 47, 48, 49, 50), especially genotype E, which was found to be the most predominant, and accounted for almost 50% of these genital genotypes. Therefore, it was surprising to find from this study that instead of E, the genotype F and D were the most prevalent. Indeed, genotype E was detected in only 14.7% of the isolates, which ranked it at the 4th. This might suggest a transmission advantage of F and D over other genotypes in this area. Since all except 3 samples collected for this study were from asymptomatic individuals, the association between a specific serotype and disease symptom could not be evaluated at present.

Generally, the RFLP technique could not differentiate closely related or variant organisms, especially those that have an identical nucleotide sequence in an appropriated endonuclease recognition site. Since the *C. trachomatis* genotype D/Da/L1 and H/Ia/J have identical VD4 sequences, they could not be differentiated into individual types by this technique. However, after nucleotide sequencing of all VDs, all samples previously identified as a group of genotypes, D/Da/L1 and H/Ia/J, were clearly classified into individual genotypes. For example, all 8 D/Da/L1 genotypes were later classified as genotype D on the basis of their differences in VD2 sequences. However, the VD4 sequence from 7 of 8 D genotype was different from the prototype, but similar to the D variant reported by Veeraseatakul (44), Poole *et al.* (20), Sayada *et al.* (43) and Lampe *et al.* (45). In France, this kind of D variant has been detected increasingly to 73% of the D prototype (43). Furthermore, Veeraseatakul (44) reported that 21 samples of D serotype found in Chiang Mai were all sequence variants. Similarly, 1 from 4 H/Ia/J genotype was later identified as genotype J, and the rest were H after nucleotide sequencing. However, 2 of them were H variants according to their differences in VD1, VD2 and VD3. A similar H variant was also observed by Lampe *et al.* (45). In this case, the variation was found only in

the VD1 region. The F variant was rather rare in this area (1 of 9 isolates), as compared to the K variant (all 6 isolates). This observation agreed with that reported by Veeraseatakul (44), which none of the K prototypes were detected in her study. However, when the prototype strain K/UW-31/CX was resequenced by Poole *et al.* (20) and Stothard *et al.* (46), it differed from those originally sequenced by Yuan *et al.* (21). Nevertheless, it was identical to the variants reported later by several investigators including the ones in this study. It has been discussed controversially that the K strain sequence reported earlier by Yuan *et al.* might actually be a variant one. If so, the K variants found in this study, as well as in others, might be non-variants. Three from five E samples were E variant serotypes, based on the sequence variation observed in the VD1. However, they were different from the E variants reported by Sayada *et al.* (43) and Yang *et al.* (29), as they observed the variation that occurred in VD4. Two of the genotype G variants, identified in this population, were similar to the Ga reported by Morre *et al.* (38) and G' reported by Poole *et al.* (20). However, Morre and Poole did not find the nucleotide variation in VD 2 or the pre-region of VD1, as in the G variants of this study.

Among 34 *C.trachomatis* samples, 21 (61.8%) had a nucleotide sequence that was different from their prototype, and only 13 (38.2%) were prototypes. A high number of *C. trachomatis* variants found in this study were similar to those reported from Kenya, by Brunham *et al.*(34), where as 63% were variant sequences. Veeraseatakul (44) also found *C. trachomatis* variants at 48% in the STD high risk group residing in Chiang Mai. The high prevalence of variant type found in these populations correlated to the high risk of STD. Individuals in a high risk group had probably experienced repeated infection, which might have necessitated a boost to antibody titers at the mucosal infection site, ultimately selecting a variant in those populations (27, 51). Since the MOMP is the major surface antigen of *C.trachomatis*, the gene encoding those proteins frequently exhibits a DNA sequence variation. The

sequence variation usually results in amino acid transition, which might confer some antigenic changes. Lampe *et al.* (51) reported that the MOMP variants could escape neutralization by both serotype specific monoclonal antibodies and human immune sera. This is a likely result of the immune selected mechanism. It has been accepted that the host immune selection pressure plays an important role in the adaptability of organisms, perhaps in an effort to evade the host immune surveillance. The genetic mechanisms for diversification of the MOMP gene are unclear. Stephen *et al.* (52) suggested that the clustered base substitutions or genetic recombination in closely related serotypes, and insertions or deletions in distantly related serotypes, probably accounted for the mechanism of the MOMP diversification. In this study, all 21 variant serotypes were a mutational drift with one nucleotide substitution form. The mix of infection that is usually observed in the high risk group (34, 37) was not found in this study. Therefore, it was suggested that a vaccine strategy, based on a MOMP epitope from an individual *C. trachomatis* serovar, could fail if a patient is infected with a variant having a single amino acid change within the immunizing MOMP epitopes. Further studies are needed to identify conserved MOMP epitopes that exhibit little sequence variation and induce cross-serovar neutralizing antibodies. It is also important to determine how frequently changes in MOMP VDs occur over time in clinical isolates in a community and whether or not those variants could lead to the changes in the virulence of the organism.

From this study, the type distribution of *C. trachomatis* in Chiang Mai and surrounding areas was presented. It also provided information about the mutation in the MOMP gene of *C. trachomatis* that circulated in the population. This information could be useful for prospective molecular epidemiological studies and also vaccine development.