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Original Article

Distribution of *Chlamydia trachomatis* Serovars among Female Prostitutes and Non-Prostitutes in Thailand, and Non-Prostitutes in Japan during the Mid-90s

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SUMMARY: The distribution of *Chlamydia trachomatis* serovars in Thailand and Japan during the same period of the mid-90s was determined. Seventy-one *C. trachomatis* specimens isolated from female patients who visited the Venereal Diseases Center at Bangkok, Thailand in 1994 were used in this study. Of these, 56 patients were prostitutes. Forty-seven specimens obtained from female non-prostitutes who attended the Department of Obstetrics and Gynecology, Saitama Medical School, Japan during the period from 1993 to 1995 were also used in this study. DNA was extracted from these specimens and typing of *C. trachomatis* serovars was performed by the polymerase chain reaction-restriction fragment length polymorphism method. The identified serovars among prostitutes of Thailand (n = 56) /non-prostitutes of Thailand (n = 15) /non-prostitutes of Japan (n = 47) were as follows: Ba 1/0/2, D 8/1/15, E 11/2/8, F 16/9/8, G 4/0/7, H 3/2/3, I 1/0/1, J 3/0/0, and K 10/1/4. Serovar F was most prevalent (35.2%) in both prostitutes and non-prostitutes from Thailand, followed by serovar E (18.3%). On the other hand, serovar D was the most frequent serovar in non-prostitutes in Japan (31.9%) followed by serovars F (17.0%) and E (17.0%). A difference in the distribution of *C. trachomatis* serovars of Thailand and Japan was noted.

INTRODUCTION

Chlamydia trachomatis is a ubiquitous pathogen worldwide and causes ocular, urogenital and respiratory infections in humans. *C. trachomatis* is classified into 18 serovars (1), the distributions of which have been reported in several countries (2-8). However, there are only a few reports on *C. trachomatis* serovar distribution in Thailand (9,10) and Japan (11-13). There is no data on *C. trachomatis* serovars before 1995 in Thailand. The number of Thai people visiting Japan is increasing each year. Statistics of the Immigration Bureau of Japan show that the numbers of Thai people staying in Japan has increased 6.2-fold from 1985 to 1995, indicating a growing relationship between the two countries.

The objectives of this study were to identify the distribution of *C. trachomatis* serovars in Thailand and Japan during the same period of the mid-90s and to clarify a possible relationship.

MATERIALS AND METHODS

Seventy-one female patients aged from 17 to 36 years old who visited the Venereal Diseases Center at Bangkok, Thailand from April to June, 1994, were included in this study. Of these, 56 patients were prostitutes. An endocervical swab was taken using a sterile cotton swab and placed into 2SP (sucrose phosphate) solution. Cell cultures were performed using McCoy cells and inclusions of *C. trachomatis* were identified by staining with iodine. Cells with positive inclusions were harvested with a sterile spatula and were frozen in 2SP at −70°C. Cell culture was done at the Venereal Diseases Center, Thailand, and the frozen specimens were transported in dry ice to the National Institute of Infectious Diseases, Tokyo, Japan, for serotyping.

Forty-seven specimens obtained from female non-prostitutes who attended the Department of Obstetrics and Gynecology, Saitama Medical School, Saitama, Japan during the period from 1993 to 1995 were also included in this study. Most were pregnant women. Endocervical swabs were taken and were placed in IDEIA Chlamydia transport medium (DakoCytomation Co., Ltd., Kyoto, Japan) for antigen detection according to the manufacturer's instructions. After all procedures were finished, the residual fluids of transport medium with positive antigen were kept at −30°C. Symptoms of each patient were not recorded. Informed consent was obtained from all patients in Thailand and Japan.

DNA was extracted either by heating at 100°C for 15 min in the case of Thai samples or using a commercially available kit, Sepa Gene® (Sanko Junyaku Co., Ltd., Tokyo, Japan), in the case of Japanese samples. Typing of *C. trachomatis* serovars in DNA extracts was then performed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method reported by Yoshida et al. (11). Briefly, a specific part of the *omp* 1 gene was amplified by two sets of primers and the amplified product was digested by 5 endonucleases (*Alu*I, *Eco*RV, *Hind*III, *Taq*I and *Hha*I). Digested samples were then electrophoresed on 4% agarose gel and stained with an ethidium bromide. The serovar was determined for each sample based on the electrophoresis patterns.

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Table 1. Distribution of *Chlamydia trachomatis* serovars identified among female patients in Thailand and Japan in the mid-90s

		No. of specimens with each serovar								
		Ba	D	E	F	G	H	I	J	K
Prostitutes in Thailand	(n = 56)	1	8	11	16[1)]	4	3[1)]	1	3	10
Non-prostitutes in Thailand	(n = 15)	0	1	2	9	0	2	0	0	1
Non-prostitutes in Japan	(n = 47)	2	15[1)]	8[1)]	8	7	3	1	0	4

[1)]: includes one case of mixed-serovar infection.

RESULTS

The results of the typing of *C. trachomatis* serovars are shown in Table 1. Nine serovars, Ba, D, E, F, G, H, I, J and K, were identified. Two specimens demonstrated an electrophoretic pattern that indicated mixed serovar infection; one was infection with serovars F and H in a prostitute of Thailand, and the other was infection with serovars D and E in a non-prostitute from Japan. Serovar F was the most frequently identified in Thai patients (35.2%; 25/71), followed by serovars E (18.3%; 13/71), K (15.5%; 11/71) and D (12.7%; 9/71). Differences were not revealed by comparison of identified serovars between prostitutes and non-prostitutes from Thailand. Serovar F was most prevalent, followed by serovar E in both groups. On the other hand, serovar D was the most frequently identified serovar (31.9%; 15/47) in non-prostitutes in Japan, followed by serovars F (17.0%; 8/47), E (17.0%; 8/47) and G (14.9%; 7/47).

DISCUSSION

C. trachomatis is currently classified into 18 serovars, i.e., A, B, Ba, C, D, Da, E, F, G, H, I, Ia, J, K, L1, L_2, L_2a and L_3 (1). Until recently, serotyping of *C. trachomatis* had been done by micro-immunofluorescent assay using panels of monoclonal antibodies. In the last several years, however, molecular techniques have increasingly been used to identify serovars. The antigenic determinants of serovar specificity reside in the outer membrane proteins (MOMP). MOMP genes are divided into five conserved domains and four variable domains (VD I through VD IV). The region from VD I to III is amplified by the PCR-RFLP method used in this study.

The serovar distribution of *C. trachomatis* in Thailand was first reported in 2001 by Bandea et al. (9) using urine obtained from 45 asymptomatic pregnant women in 1996. Their results showed that serovar F was the most frequently identified (25%), followed by D (22.6%), H (11.7%) and K (11.7%). Their results were similar to ours, in that the most frequently identified serovars were the same. However, they also identified Ia (7%), B (7%) and Ja (4.5%), which were not found in our study. Neither Ia nor Ja is typable by our PCR-RFLP method. Differences in identifying these minor serovars could be attributed to the methodology used in each study. Standardization of these methods is necessary. Wongworapat et al. (10) also reported *C. trachomatis* serovar distribution using endocervical swabs taken from female sex workers who visited the Venereal Disease and AIDS Control Center in Chiang Mai, northern Thailand. The specimens were taken between 1997 and 1999. The most frequent serovar was D (34.5%), followed by F (21.4%) and K (13.1%). The serovars that they identified were almost the same as those we identified, except that serovar D was the most frequently found according to their data.

The serovar distribution of *C. trachomatis* among asymptomatic Japanese pregnant women in the northern island, Hokkaido, was reported by Ikehata et al. in 2000 (12). Frequently identified serovars were D (24.3%), F (17.9%), E (11.0%), G (6.9%) and H (6.9%), results which were similar to our data on non-prostitutes in Japan. On the other hand, Yoshizawa et al. (13) indicated that the most frequently identified serovar had changed from serovar D to F in one district close to the New Tokyo International Airport in Japan during the 1980s and 1990s. Because our specimens in the Japanese population were collected within 2 years, we were not able to find any change in *C. trachomatis* serovars during the collection period. The clinical significance of longitudinal surveillance of *C. trachomatis* serovars has not been determined and comparison of these data from various countries during different time periods is warranted.

The relationship between certain *C. trachomatis* serovars and clinical manifestations has been controversial. There are several reports on the possible relationship between certain serovars and virulence. Batteiger et al. (4) reported that serovars F and G were infrequently isolated among men with fewer than three polymorphonuclear leukocytes on endourethral smears. However, they found no relationship between serovar distribution and cervicitis. Workowski et al. (5) reported that patients with lower genital infection with serovar F exhibited fewer signs of cervical infection. They also indicated an association between race and genital infection with either *C. trachomatis* serovar D or Ia (6). On the other hand, Persson and Osser (7) reported a lack of a relationship between *C. trachomatis* serovars and genital symptoms and diseases. In a recent study using a large number of samples in combination with a review of the literature, Geisler et al. (8) reported that *C. trachomatis* serovars did not strongly influence clinical manifestations. In the present study, although clinical data were not available, the serovar distribution of prostitutes was similar to that of non-prostitutes in Thailand.

In conclusion, the distribution of *C. trachomatis* serovars in Thailand and Japan during the same period was successfully clarified in this study, and the results suggested that prospective surveillance of *C. trachomatis* serovars would be beneficial.

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