cline disc to the edge of confluent growth (table). In this study, we further characterised the tetM genes of these TRNG strains.

Isohn et al. previously reported the primer pair (A: 5'-GGGCGAGCACGACAACCTC-3' and B: 5'-TGCTTGCTGGTTACTGCT-3') for detection of tetM in *N. gonorrhoeae*. These sequences were derived from that of the *Ureaplasma* strain type I. More recently, the nucleotide sequences of the tetM genes of American and Dutch type plasmids have been determined and suggested that the tetM determinant found in the American type plasmid has a different origin from that in the Dutch type.1 Because the base sequence of the tetM gene from Dutch type plasmid which corresponds to the primer B is different from that of American type plasmid, primer B (5'-TGCTTGCTGGTTACTGCT-3') was used instead of the primer B to detect Dutch type tetM. The cells grown on a Kellogg's agar medium were lyzed in 100 μl of distilled water for 10 minutes at 94°C, and a 0.5 ml of lyase was added to a polymerase chain reaction (PCR) mixture. The PCR mixture contained 0.2 mM (each) deoxynucleoside triphosphate, 0.5 pmol of each oligonucleotide primer, Takara Taq DNA polymerase (Takara Shuzo, Kyoto, Japan), and buffers provided by the manufacturer in a total volume of 50 μl. The mixture was overlaid with 50 μl of mineral oil and heated in a DNA thermal cycler (PT-2000) at 94°C, 60 seconds at 58°C, and 60 seconds at 72°C. PCR amplification using the primer pair of A and B gave a product of the predicted size of 765 bp. Restriction digestion with SfaNI of 540, 140, and 90 bp amplified products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Detection Kit (Perkin-Elmer Corp CT, USA) and the ABI 310 Genetic Analyzer (Perkin-Elmer Corp), and that from 60061, 6010 and 5120 were identical to corresponding sequence of the tetM gene from American and Dutch type plasmids, respectively.

It is of interest that the isolation rate of TRNG was quite low and both American and Dutch type tetM genes were found in Tokyo and Kanagawa, Japan during the study. The strain, which was isolated in 1985 in Japan and was transported from other countries, but not spread in Tokyo and Kanagawa area. Isohn et al. found two types of HpaII (MspI) digestion pattern of the PCR products from tetM in TRNG strains. In this study we clearly distinguished the American type tetM gene from the Dutch type one using the PCR with the sets of the primer pairs. Further investigations will be needed to elucidate the prevalence of each type of tetM gene in *N. gonorrhoeae* infections.


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MATTERS ARISING

Epidemiology of genital Chlamydia trachomatis

Simms et al. in their review of the epidemiology of genital Chlamydia trachomatis in England and Wales states that "the high prevalence and case finding studies carried out over the past 20 years were critically assessed in terms of study design and testing methodologies." However, the authors, however, do not make explicit how the cited literature was obtained, sifted, and appraised. As a consequence they fail to identify all relevant published papers published during the period. I recently reviewed the literature relating to the prevalence of *C. trachomatis* infection in women attending British general practice (which was an earlier review of the literature) and concluded that the following criteria, for infection in the presence of the identified studies. This led to 15 relevant prevalence studies being identified and appraised. Simms et al., in contrast, identified only six of these studies. As far as general practice was concerned nine studies which met defined criteria were included in the review. It was concluded that the best current estimate of the prevalence of genital chlamydia in women attending general practice is 3% to 4%. In 50 family planning clinics this prevalence ranged from 2% to 12%.

This conclusion was based on the results of two large general practice prevalence studies which were not quoted by Simms et al. Six studies were identified from a review of British family planning literature with estimated prevalence rates of women attending family planning clinics ranging from 3% to 7%. The methodological quality of prevalence studies of genital chlamydia infection in women in general practice and at family planning clinics is, however, unsatisfactory. Common features of the studies reviewed were non-random sampling, small sample sizes, unclear inclusion/exclusion criteria, and use of different testing methods. This conclusion is in agreement with that of Simms et al. The discrepancy between my findings and those of Simms et al. supports the argument made by the Eirec and British family planning Working Group that all reviews of the medical literature should make explicit how the cited literature was obtained, sifted, and appraised. Failure to do so is likely to lead to important papers being missed.

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