Analysis of Salmonella typhi Isolates from Southeast Asia by Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis (PFGE) revealed that multiple genetic variants of Salmonella typhi are simultaneously present in Southeast Asia and are associated with sporadic cases of typhoid fever and occasional outbreaks. Comparative analysis of PFGE patterns also suggested that considerable genetic diversity exists among S. typhi strains and that some PFGE patterns are shared between isolates obtained from Malaysia, Indonesia, and Thailand, implying movement of these strains within these regions of Southeast Asia, where they are endemic.

Typhoid fever continues to pose an important public health challenge in many developing countries, with an annual incidence of 16 to 17 million cases and approximately 600,000 deaths. This threat is especially pronounced in the Southeast Asian region, with its rapid pace of economic development. As a consequence of economic growth, extensive, reciprocal movements of migrant workers are occurring between the neighboring countries of Malaysia, Thailand, and Indonesia, which has one of the highest incidences of typhoid fever in the world at more than 1,000 cases per 100,000 inhabitants. This points to the very real possibility of movement of Salmonella typhi strains among these countries. The problem of the movement of strains is made more urgent by the increasing incidence of antibiotic-resistant strains and the observation of more severe clinical disease among typhoid fever cases in Indonesia (6), which may be associated with more virulent strains. The potential for movement of S. typhi strains underpins the need for effective epidemiological surveillance as a basis for the development of rational control strategies. With regard to the molecular epidemiology of bacterial pathogens, we have witnessed an increasing interest in the development of molecular approaches which are reproducible and highly discriminatory for differentiating individual strains of bacterial pathogens. These approaches include multilocus enzyme electrophoresis, restriction endonuclease analysis, ribotyping, pulsed-field gel electrophoresis (PFGE), PCR-based profiling, and nucleotide sequence analysis (7). PFGE, in particular, has been widely used recently in molecular epidemiological investigations of infections caused by a large number of bacterial pathogens, including S. typhi (15). In a previous study, we used PFGE to analyze S. typhi isolates from sporadic cases and from outbreaks in Malaysia (15). It has been proposed recently that PFGE is able to differentiate between clonally related strains (one or two band differences) and strains which represent independent clones (differences in three or more bands) (8). We report here the use of PFGE in a comparative molecular characterization of S. typhi isolates from Malaysia, Thailand, and Indonesia. We show the considerable diversity and sharing of molecular types of S. typhi in Southeast Asia, implying extensive movement of strains among these three countries, where S. typhi is endemic.

S. typhi isolates were obtained from the University Hospital (120 isolates) and the Institute for Medical Research (60 isolates), Kuala Lumpur, Malaysia; Siriraj Hospital, Bangkok, Thailand (10 isolates); and the Cipto Mangunkusumo Hospital, Jakarta, and the Dr. Sutomo Hospital, Surabaya, Indonesia (50 isolates) (Table 1). All isolates were obtained from sporadic cases of typhoid fever within the same time period (1987 to 1994). The organisms were isolated, maintained, and identified by standard biochemical and serotyping methods (3). All isolates were tested for antibiotic susceptibility by standard disk diffusion procedures for measuring resistance (3) and were susceptible to ampicillin, chloramphenicol, kanamycin, streptomycin, co-trimoxazole, and tetracycline. Preparation of DNA for restriction endonuclease digestion and subsequent analysis by PFGE were done as described previously (15). S. typhi chromosomal DNA was digested with restriction endonucleases XbaI (5'-TCTAGA-3'), DpnI (5'-ACTAGT-3'), and AvaII (5'-CCTAGG-3'), and DNA fragments were separated by a contour-clamped homogeneous electric field gel electrophoresis method on a CHEF DR-II or CHEF DR-III system (Bio-Rad Laboratories, Richmond, Calif.). PFGE patterns were visually assessed, assigned arbitrary pattern types, and compared by calculating a similarity coefficient (F, proportion of shared fragments between two isolates) (4). The F value was calculated by using the following formula: \( F = 2n_{xy}(n_x + n_y) \), where \( n_x \) is the total number of DNA fragments from isolate \( x \), \( n_y \) is the total number of DNA fragments from isolate \( y \), and \( n_{xy} \) is the total number of DNA fragments that were identical in the two isolates. Isolates were considered to be genetically similar if there was complete concordance of the DNA fragment profiles and were considered different, for pattern comparison only, if there was a difference of one or more DNA bands. With this method, an \( F \) value of 1.0 indicates identical patterns and an \( F \) value of 0 suggests complete dissimilarity. PFGE patterns were also analyzed by using a computer program for analysis of electrophoretic patterns.