Epidemiologic Analysis of Sporadic *Salmonella typhi* Isolates and Those from Outbreaks by Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis (PFGE) was used to compare and analyze 158 isolates of *Salmonella typhi* from five well-defined outbreaks of typhoid fever in Malaysia and also isolates involved in sporadic cases of typhoid fever occurring during the same period. Digestion of chromosomal DNAs from these *S. typhi* isolates with the restriction endonucleases XbaI (5'-TCTAGA-3'), SpeI (5'-ACTAGT-3'), and AvrII (5'-CCTAGG-3') and then PFGE produced restriction endonuclease analysis (REA) patterns consisting of 11 to 24 DNA fragments ranging in size from 20 to 630 kbp. Analysis of the REA patterns generated by PFGE after digestion with XbaI and SpeI indicated that the *S. typhi* isolates obtained from sporadic cases of infection were much more heterogeneous (at least 13 different REA patterns were detected; Dice coefficient, between 0.73 and 1.0) than those obtained during outbreaks of typhoid fever. The clonal nature and the close genetic identities of isolates from outbreaks in Alor Setar, Penang, Kota Kinabalu, Johor Bahru, and Kota Bahru were suggested by the fact that only a limited number of REA patterns, which mostly differed by only a single band, were detected (one to four patterns; Dice coefficient, between 0.82 and 1.0), although a different pattern was associated with each of these outbreaks. Comparison of REA patterns with ribotyping for 18 *S. typhi* isolates involved in sporadic cases of infection showed a good correlation, in that 72% of the isolates were in the same group. There was no clear correlation of phage types with a specific REA pattern. We conclude that PFGE of *S. typhi* chromosomal DNA digested with infrequently cutting restriction endonucleases is a useful method for comparing and differentiating *S. typhi* isolates for epidemiological purposes.

In many developing countries in the tropical parts of the world, typhoid fever remains an important cause of morbidity and mortality, with an estimated annual global incidence of 21 million cases and more than 700,000 deaths. In Malaysia, the disease is endemic, and its incidence appears to be increasing, with periodic outbreaks occurring recently in various parts of the country (5). The emergence of antibiotic-resistant strains of *Salmonella typhi* (13) and the increased incidence of typhoid fever in human immunodeficiency virus type 1-infected individuals are further causes for concern. In relation to effective surveillance and the development of rational control strategies for this important human disease, the availability of detailed and accurate data related to the molecular epidemiology of *S. typhi* is crucial. However, epidemiological investigations used to determine the source and spread of *S. typhi* have been hampered by the absence of reliable and sufficiently discrimi-

nate methods of differentiating individual strains beyond the species level. Methods that have been used include antibiotic resistance patterns, biochemical reactions, phage typing, and plasmid analysis. More recently, there has been an increasing interest in the application of molecular techniques to type bacterial pathogens (35). With *S. typhi*, techniques such as multilocus enzyme electrophoresis (27, 32), lipopolysaccharide (16) and envelope protein profiles (11), chromosomal restriction endonuclease digestion patterns (11), and ribotyping (1, 24) have been used. Many of these techniques are not sufficiently sensitive for distinguishing individual strains and investigators often find them tedious to perform. The recent development of pulsed-field gel electrophoresis (PFGE) (31, 33, 34) has provided another approach for obtaining molecular fingerprints which may be useful in epidemiological studies (22). PFGE has been used successfully to perform comparative chromosomal DNA analysis of several bacterial pathogens for epidemiological investigations (8, 10, 12, 14, 15, 20, 23, 26, 29, 30) and is believed to possess a discriminating capacity greater than those of ribotyping and other probe-based restriction fragment length polymorphism methods (35). The restriction endonuclease analysis (REA) patterns generated by PFGE generally consist of a fairly small number of well-separated bands which present fewer difficulties of interpretation and ambiguities compared with the patterns generated by conventional agarose gel electrophoresis. PFGE is also useful for assessing the extent of molecular diversity within a species, physical and genetic mapping of bacterial chromosomes, and estimating genome sizes (28). We report here that PFGE following XbaI and SpeI restriction digestion of *S. typhi* chromosomal DNA is a useful method for differentiating individual isolates of *S. typhi* and in the molecular analysis of isolates involved in outbreaks of typhoid fever. To our knowledge, this represents the first study in which a wide variety of epidemiologically distinct *S. typhi* isolates have been studied by PFGE.

**MATERIALS AND METHODS**

**Bacterial strains.** Isolates of *S. typhi* from either the blood or stools of humans were used in the present study. The organisms were isolated, maintained, and identified by standard methods (7). Multiple isolates were obtained during well-documented outbreaks in various parts of Malaysia between

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