Molecular Analysis of Environmental and Human Isolates of *Salmonella typhi*

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Molecular characterization of a total of 54 isolates of *Salmonella typhi* from Santiago, Chile, was performed by pulsed-field gel electrophoresis (PFGE) after digestion of chromosomal DNA with three restriction endonucleases: *XbaI* (5'-TCTAGA-3'), *AvrII* (5'-CCTAGG-3'), and *SpeI* (5'-ACTAGT-3'). Thirteen of the 54 isolates were obtained from environmental sources (sewage and river water), and the rest were isolates from clinical cases of typhoid fever. Considerable genetic diversity was detected among the human isolates obtained in 1994, as evidenced by the presence of 14 to 19 different PFGE patterns among 20 human isolates, with *F* (coefficient of similarity) values ranging from 0.69 to 1.0 (*XbaI*), 0.61 to 1.0 (*AvrII*), and 0.70 to 1.0 (*SpeI*). A total of eight phage types were detected among these 20 isolates, with 50% possessing the E1 or 46 phage type. There was no correlation between PFGE pattern and phage types. Similar diversity was seen among 21 isolates obtained in 1983, with 17 to 19 PFGE patterns detected and *F* values of 0.56 to 1.0 (*XbaI*), 0.55 to 1.0 (*AvrII*), and 0.67 to 1.0 (*SpeI*). Comparison of these two groups of human isolates obtained 11 years apart indicated that certain molecular types of *S. typhi* are shared and are able to persist for considerable periods; a similar degree of genetic diversity was also detected among the environmental isolates of *S. typhi*, which 10 to 12 different PFGE patterns were detected among the 13 isolates analyzed, with *F* values ranging from 0.56 to 1.0 (*XbaI*), 0.52 to 1.0 (*AvrII*), and 0.69 to 1.0 (*SpeI*). Certain molecular types present among the environmental isolates of *S. typhi* were also found among the human isolates from the same time period, providing evidence for the epidemiological link between environmental reservoirs and human infection.

In many developing countries, typhoid fever remains an important public health problem, with 16.6 million cases and 600,000 deaths annually (14). In areas where typhoid fever is endemic, water from rivers or lakes which is used for public consumption and is sometimes contaminated by raw sewage is the main source of infection (9). It has been clearly demonstrated that the incidence of typhoid fever decreases dramatically with the provision of clean water through chlorination and filtration (9). Thus, in many developing countries where the use of raw river water remains widespread, this pathway of transmission remains an important factor in disease epidemiology. However, despite the clear importance of environmental sources of *Salmonella typhi*, little is known about the biochemical and molecular characteristics of such strains of *S. typhi* and the mechanisms of survival employed by this pathogen. Very few molecular studies have been carried out on these strains of *S. typhi* mainly because of the well-known difficulty in isolating this organism from environmental sources (3, 5, 15). Multiple resistance to antibiotics among *S. typhi* strains has become an important problem in recent times (14), and it is conceivable that transfer of antibiotic resistance can occur in aquatic environments. It is clear that a better definition of the molecular epidemiology of *S. typhi*, including analysis of strains obtained from environmental sources, would benefit greatly from the application of recently developed molecular typing techniques. We report here the results of molecular analysis by pulsed-field gel electrophoresis (PFGE) on environmental and human isolates of *S. typhi* from Santiago, Chile. PFGE analysis was undertaken to determine (i) whether the isolates were identical or different, (ii) whether environmental isolates were similar or identical to human isolates, and (iii) the extent of genetic diversity among the isolates.

Environmental and human isolates of *S. typhi* were used in this study. A total of 13 environmental isolates were obtained during the summer of 1983 (January to March) from sewage or water from the Mapocho River, Santiago, Chile, using Moore swabs as described previously (5). A total of 41 human isolates from blood were also obtained from sporadic cases of typhoid fever in Santiago, Chile; 21 isolates were obtained in 1983, and 20 were obtained during 1994. The organisms were isolated, maintained, and identified by standard methods (6). The isolates studied belonged to multiple phage types, and typing was performed according to standard procedures by the Salmonella Reference Centre at the Institute for Medical Research, Kuala Lumpur. As reported previously, most of the isolates belonged to phage types E1 and 46 (22). Repeated subculturing of isolates was avoided, and stocks of the primary isolates were maintained at -70°C. All *S. typhi* isolates tested were sensitive to ampicillin, amikacin, chloramphenicol, kanamycin, carbenicillin, cephalothin, cefamandole, gentamicin, neomycin, tetracycline, trimethoprim, streptomycin, spectinomycin, sulfonamides, nitrofurans, and nalidixic acid, as determined by standard disk diffusion procedures to measure resistance (12). None of the isolates studied contained any plasmids, as determined by a standard alkaline lysis procedure and by PFGE of undigested DNA (see below).

DNA for PFGE analysis was prepared by a modification of the method of Smith et al. (19) as described previously (20).

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