Characterization of Salmonella Serovars by PCR–Single-Strand Conformation Polymorphism Analysis

Satheesh Nair,1,4*, Thong Kwai Lin,2 Tikki Pang,3 and Martin Altviegg4

Institute of Postgraduate Studies & Research1 and Institute of Biological Sciences,2 University of Malaya, Kuala Lumpur, Malaysia; World Health Organization, Geneva, Switzerland3; and Department of Medical Microbiology, University of Zurich, Zurich, Switzerland4

Received 25 October 2001/Returned for modification 16 February 2002/Accepted 29 March 2002

Salmonellae are the etiologic agents of different diseases collectively referred to as salmonellosis. Human salmonellosis can be divided into four syndromes: enteric fever (typhoid-like disease), gastroenteritis (food poisoning), bacteremia with or without gastroenteritis, and the asymptomatic carrier state.

On a global scale, the incidence of typhoid fever is decreasing, while that of nontyphoidal salmonellosis is increasing, although both remain major health problems. The World Health Organization has estimated that annually there are close to 17 million cases of typhoid fever, with nearly 600,000 deaths, and 1.3 billion cases of acute gastroenteritis or diarrhea due to nontyphoidal salmonellosis, with 3 million deaths (8, 23, 26).

To curb both typhoidal and nontyphoidal salmonellosae, laboratory-based surveillance of human and animal infections is a necessary first step of any prevention strategy. Phenotypic methods play an important role in the identification to the genus level. Serotyping is used for primary typing of strains, while phage typing and antibiogram are used for subdivision of serotypes (33). However, serotyping of Salmonella is laborious due to the large number of recognized serotypes, i.e., over 2,400 (1, 27).

In addition, a number of molecular typing methods have also been used to try to improve the identification of salmonellae and also to differentiate strains below the level of serotypes. These DNA-related techniques include ribotyping (3), pulsed-field gel electrophoresis (PFGE) (18, 20, 32), IS200 fingerprinting (4), PCR-ribotyping (12), ribosomal DNA intergenic spacer amplification and heteroduplex analysis (9), amplified fragment length polymorphism (1, 21), automated 5' nucleic PCR assay (7), and random amplified polymorphic DNA analysis (30).

In recent times, various molecular techniques that detect base sequence changes in bacteria have been used as tools in epidemiological typing. One of the most widely used techniques for the identification of point mutations, due to its simplicity, sensitivity, and rapidity, is PCR–single-strand conformation polymorphism (PCR-SSCP) (6, 22). SSCP was first designed to detect mutations in oncogenes and allelic variations in the human genome (22). Since then, this technique has played a role in bacterial typing (35) and in Salmonella studies (11, 34). Briefly, amplified double-stranded DNA is denatured to single-stranded DNA and subjected to nondenaturing polyacrylamide gel electrophoresis. The mobility of the single-stranded DNA in the gel is dependent not only on its length but also on its secondary structure, as determined by nucleotide sequence (6).

Here, we investigate the possibility of using PCR-SSCP to differentiate Salmonella strains both at the serovar level (interserovar) and at the intraserovar level, using nucleotide variation in the groEL gene, which encodes a heat shock protein (GroEL) that is a member of the stress response protein (HSP60) family (36).

MATERIALS AND METHODS

Bacterial strains. Forty-one epidemiologically unrelated strains from 10 different Salmonella serovars were studied (Table 1). These strains were kindly provided by Andre Burnens from the Swiss National Reference Laboratory for Foodborne Diseases, University of Berne, Berne, Switzerland. Five of the serovars (Salmonella enterica serovar Typhimurium, S. enterica serovar Newport, S. enterica serovar Hadar, S. enterica serovar Infantis, and S. enterica serovar Virchow) were the most common serovars isolated in Switzerland at the time of the study. The other five serovars (S. enterica serovar Enteritidis, S. enterica serovar Typhi, S. enterica serovar Arizonae, S. enterica serovar Paratyphi A, and S. enterica serovar Paratyphi B) consisted of strains that belonged to the reference collection. All of the strains had been identified, biochemically tested, and serotyped at the institution from which they were obtained.

The bacteria were maintained on Luria-Bertani (LB) agar plates. Repeated subculturings of isolates was avoided, and for long-term maintenance, all the isolates were kept in LB broth with 20% glycerol at -70°C (2).