Molecular Analysis of *Salmonella enteritidis* by Pulsed-Field Gel Electrophoresis and Ribotyping

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A total of 61 isolates of *Salmonella enteritidis* were analyzed by the techniques of pulsed-field gel electrophoresis (PFGE) and ribotyping. Twenty-three of the isolates were from Zurich, Switzerland, and 38 isolates were from the University Hospital, Kuala Lumpur, Malaysia. Five of the Malaysian isolates were hospital-related outbreak strains and were shown to be indistinguishable by PFGE analysis following digestion with three different restriction endonucleases, *XbaI* (5′-TCTAGA-3′), *SphI* (5′-ACTAGT-3′), and *AvrII* (5′-CGCTAG-3′). The PFGE pattern of an isolate from a suspected carrier staff nurse was found to be identical to those of the hospital outbreak isolates. These isolates were also indistinguishable by ribotyping with *SmaI* and *SphI*. The same single PFGE pattern was also detected in 29 of 32 sporadic isolates of *S. enteritidis*. Four closely related ribotypes were detected among these 29 isolates. Similarly, outbreak-related strains from Switzerland showed close genetic identity by PFGE and ribotyping. Strains obtained from poultry showed more variations in their PFGE patterns and ribotypes, although the patterns were still closely related. In addition, *SphI* ribotypes A and D among the Swiss strains correlated with phage types 4 and 8, respectively. No correlation of phage types with PFGE pattern was noted. Both PFGE and ribotyping indicate that the *S. enteritidis* strains circulating in Malaysia and Switzerland are very similar and may be clonally related. Comparison of the PFGE patterns with the ribotypes for 23 Swiss and 16 Malaysian isolates showed that there was a 69% concordance in the grouping of isolates. Overall analysis showed that one or two PFGE patterns and five ribotypes (using *SphI*) were detected among 38 Malaysian isolates and two to four PFGE patterns and four ribotypes (using *SphI*) were present among 23 Swiss isolates, thus suggesting that PFGE is slightly less sensitive than ribotyping with regard to the ability to discriminate between isolates. We conclude that the close genetic similarity observed between epidemiologically unrelated and outbreak-related isolates of *S. enteritidis* suggests that both PFGE and ribotyping are of limited value in the epidemiological analysis of these particular isolates, possibly because of the highly clonal nature of pathogenic strains of *S. enteritidis*.

Nontyphoidal salmonellosis, a disease caused by salmonellae other than *Salmonella typhi*, is an important food-borne infection with a worldwide distribution (4, 20) and an estimated annual incidence of 1.3 billion cases and 3 million deaths. The two most common causes of nontyphoidal salmonellosis are *Salmonella typhimurium* and *Salmonella enteritidis*. In the past 10 years there has been an increased incidence of gastrointestinal infections caused by *S. enteritidis* (20, 21), which has now become the predominant serotype in many countries. A number of recent studies have shown that the increased incidence of *S. enteritidis* may be related to the ingestion of raw, undercooked, or contaminated eggs or egg products (8, 10, 22, 26). Most cases of *S. enteritidis* food poisoning occur sporadically or as limited outbreaks, but recent reports of large, hospital- and nursing home-associated outbreaks emphasize the importance of salmonellosis as a major public health problem (13, 24).

Because of the recent prominence of *S. enteritidis*, a number of molecular typing methods have been used to improve the identification of this food-borne infection and also to differentiate strains below the level of serotyping (subtyping). Standard methods for identifying and typing *S. enteritidis* include serotyping, biotyping, and phage typing, which may not be discriminative enough, because more than 75% of the salmonellae isolated during an outbreak belonged to a single phage type (11). In more recent years, the use of DNA-related techniques such as plasmid analysis (14, 23), ribotyping (1, 8, 17, 26), and pulsed-field gel electrophoresis (PFGE) (19, 25) have proved to be useful in discriminating isolates of *Salmonella* species. For example, both PFGE and ribotyping have become useful tools for typing and differentiating strains for epidemiological studies with *S. typhi* (1, 25), *Salmonella brandenburg* (2), *S. enteritidis* (13, 14, 19), *Salmonella poona* (6), and *Salmonella brandenpur* (7). Here we report the use of PFGE and ribotyping to assess the relatedness of Malaysian and European isolates of *S. enteritidis* and provide an evaluation of the usefulness of these two techniques in the epidemiological analysis of this important bacterial pathogen.

**MATERIALS AND METHODS**

**Bacterial strains.** A total of 61 isolates of *S. enteritidis* were analyzed. Thirty-eight isolates were obtained from the Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia. Among the Malaysian isolates, five were isolates from the blood of patients involved in an outbreak in the University Hospital between 23 and 29 August 1993, and one isolate was obtained from the stool of a staff nurse on duty in the operation theater during the outbreak. None of the five patients had a history of diarrhea but peripheral or central lines were inserted into the patients in the operation theater. Thirty-two other isolates were sporadic strains isolated throughout 1993 from patients admitted to the hospital for various diseases including septicaemia, cholangitis, and acute gastroenteritis. All except nine of the sporadic strains were from blood. Among the 23 strains from Zurich, Switzerland, 7 were from patients whose