An Epidemiological Study of *Salmonella enteritidis* by Pulsed-Field Gel Electrophoresis (PFGE): Several PFGE Patterns Observed in Isolates from a Food Poisoning Outbreak

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Abstract: An epidemiological analysis of *Salmonella enteritidis* from a food poisoning was done using pulsed-field gel electrophoresis (PFGE) of BlnI- or XbaI-digested fragments of chromosomal DNA of isolates. *S. enteritidis* isolates obtained from 19 patients had identical PFGE patterns. Therefore, a strain giving the same pattern was considered to be the causative agent of this outbreak. In addition, four isolates that had different BlnI-digested PFGE patterns were obtained from three patients, suggesting that the observed variations in PFGE patterns might occur as the result of some point mutations of chromosomal DNA during growth or from the existence of several *S. enteritidis* strains from various sources. Subsequent PFGE analysis of continuously subcultured strains supported the former possibility. These observations indicate that PFGE analysis on multiple numbers of colonies from each patient are necessary for the epidemiologic investigation of *S. enteritidis*.

Key words: *Salmonella enteritidis*, Pulsed-field gel electrophoresis (PFGE), Epidemiology

Over the past decade, the incidence of food poisoning caused by *Salmonella enteritidis* has markedly increased in Japan (1). Strains belonging to phage type (PT) 34, PT 4, and PT 1 are particularly prevalent in most outbreaks (1). It is therefore necessary to characterize further those clinical isolates of *S. enteritidis* even within a single PT. Several recent studies demonstrated that pulsed-field gel electrophoresis (PFGE) is a useful and reliable method for subtyping many clinical isolates of *S. enteritidis* within a single PT (4, 5), in which all isolates obtained from a single outbreak showed identical PFGE patterns (4). In this study, we further evaluated use of PFGE on *S. enteritidis* isolates from an outbreak of a food poisoning which occurred in a nursing home for aged people in April 1995. Twenty-five of the 53 people developed a gastrointestinal illness in this case. Initially, eggs consumed by the people were suspected to have caused the outbreak, although viable cells of *S. enteritidis* were not isolated from any of them.

A total of 28 isolates, each obtained from the feces of 19 patients in the outbreak, were examined in this study. *Salmonella* single colonies on a DHL agar plate were picked and identified biochemically and serologically by standard bacteriological methods (3). All isolates produced acid from glucose, mannitol, sorbitol, rhamnose, melibiose, arabinose, xylose, trehalose, glycerol, dulcitol, maltose, fructose, galactose and mannose, but not from inositol, sucrose, amygdalin, adonitol, cellobiose, raffinose, inulin, salicin or lactose. Disk diffusion tests were performed with antibiotic-containing disks obtained from Becton Dickinson Microbiology Systems, Cockeysville, Md., U.S.A. These were all resistant to streptomycin; moderately susceptible to tetracycline; and susceptible to piperacillin, ceftazolin, ofloxacine, fosfomycin, gentamicin, ampicillin, kanamycin and sulfamethoxazole-trimethoprim. The isolates were also subjected to phage typing, and all determined to belong to PT 1. Plasmid DNA was isolated using the procedure of Kado and Liu (2). Each isolate had plasmid of approximately 60 kb except for one, which had additional plasmid larger than 60 kb.

Intact chromosomal DNA was prepared from ran-

Abbreviations: PFGE, pulsed-field gel electrophoresis; PT, phage type.
domly selected single colonies grown on the plates. All chromosomal DNA were digested with two different restriction enzymes, XbaI and BlnI, and analyzed by PFGE as described previously (4). The gel was stained with ethidium bromide and photographed under UV illumination. The DNA size standards used were a bacteriophage lambda ladder consisting of concatemers with an increment of 48.5 kbp (New England BioLabs, Beverly, Mass., U.S.A.).

All isolates had appreciable restricted digestion patterns ranging from approximately 40–700 kb. A PFGE of XbaI-digested fragments of all S. enteritidis chromosomal DNA generated identical patterns (data not shown). BlnI-digested fragment showed five distinguishable patterns (Fig. 1; arbitrarily designated as Pattern A. In addition, four isolates giving different patterns, B–E, were obtained from three patients (12, 13, and 14, arrow heads). Values on the right (in kilobase pairs) refer to the positions of marker bands.

Powell et al (5) reported similar observations in the PFGE patterns of chromosomal DNA digested with XbaI among S. enteritidis PT 4 isolated in England and Wales between 1967 and 1992. They also considered that inversions and transpositions may include alterations in the position of the restriction enzyme recognition site, resulting in changes in the PFGE patterns. Tenover et al (6) suggested that an isolate would be considered to be closely related to the outbreak strain if its PFGE pattern differed from the outbreak pattern by changes consistent with a point mutation. It was therefore hypothesized that the observed variations in PFGE patterns reflected chromo-

![Fig. 1. PFGE patterns of BlnI-digested chromosomal DNA of Salmonella enteritidis isolates from patients involved in a food poisoning.](image1)

![Fig. 2. Changes in PFGE patterns of BlnI-digested chromosomal DNA of isolates obtained from patients 7 and 11 after subculturing 12 times.](image2)
somal rearrangement, possibly due to some point mutations of the chromosomal DNA of the bacterium during in vivo or in vitro growth.

In order to evaluate this hypothesis, nine of the isolates initially having Pattern A were continuously subcultured at 37°C on heart-infusion agar plates 12 times at intervals of 1~2 days. After this subculturing process, PFGE patterns of DNA from randomly selected colonies digested by XbaI or BlnI were re-examined. It was found that the BlnI-digestion patterns of two isolates were not only different from the original pattern but also different from any of the patterns previously observed (Fig. 2). However, there were no changes in XbaI-digestion patterns (data not shown), indicating that choice of the restriction enzyme might be critical for analysis by PFGE.

From the evidence, analysis of one colony per plate by PFGE is capable of leading to the misinterpretation of an outbreak which, in this case, had been actually caused by a single strain of S. enteritidis rather than heterogeneous strains. It is therefore necessary to examine multiple numbers of colonies on plates by PFGE for more comprehensive epidemiological investigation.

References