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Characterization of the Outbreak-Derived *Salmonella enterica* Serovar Enteritidis Strains with Atypical Triple Sugar Iron and Simmons Citrate Reactions

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In May 1996, an outbreak of diarrhea occurred at a nursery school in Mie Prefecture, Japan, involving 54 patients whose major symptoms were diarrhea, abdominal pain and fever. In this case, *Salmonella enterica* serovar Enteritidis showing atypical biochemical properties was isolated from the patients. When fecal samples from 39 patients were tested, bacterial organisms forming S-type colonies (semi-transparent in the periphery and producing small amounts of hydrogen sulfide) on desoxycholate hydrogen sulfide lactose (DHL) agar were isolated from 29 patients. The slant of the triple sugar iron (TSI) agar, from which this strain was isolated, was yellow, suggesting fermentation of saccharose or lactose, or both. Citrate utilization was negative when examined using Simmons citrate medium. The other biochemical features of the isolates were as follows: glucose catabolism was positive; hydrogen sulfide production, positive; lysine, positive; indole production, negative; urea catabolism, negative; methyl red (MR) test, positive; and voges proskauer (VP) reaction, negative. Carbohydrate catabolism was negative when examined with Andrade peptone water containing 1% saccharose or lactose. When yeast extract was added to TSI agar at 1%, the slant of the TSI agar became red, indicating that both saccharose and lactose catabolism were negative. When 1% yeast extract was added to Simmons cirate medium, no change was observed (negative). The 16s-rDNA sequence (1) of the atypical strain shared 99% identity with those of the 16S rRNA gene of *S*. Typhimurium LT2 (GenBank accession no. AF233324). This bacterium showed agglutination to *Salmonella* antiserum O9 and H-G and H-m, (Denka Seiken, Co., Ltd., Tokyo, Japan) and was thus identified as *S*. Enteritidis (2). These results suggest that atypical strains were *S*. Enteritidis. It seems highly probable that this strain would be misdiagnosed as *Citrobacter* sp. or hydrogen sulfide-producing *Escherichia coli* if tested using TSI agar, lisin indole motility (LIM), sulfide indole motility (SIM) medium, Simmons citrate medium, urea medium or MR-VP medium (3). A small number of *Salmonella* sp. strains capable of fermenting of saccharose and lactose (4,5) have been reported. Unlike such strains, however, strains fermented in the current research essentially do not ferment saccharose and lactose. Strains with the same properties as the current bacterium have yet to be reported. Also, during this outbreak, the public health center that first tested samples from patients was not able to identify the pathogen, the source of infection or the infection route because the test results did not conform to any of the known properties of major known pathogens for diarrhea (e.g., *Salmonella* sp.). If bacterial strains with similar properties are isolated from multiple patients during outbreaks of infec-

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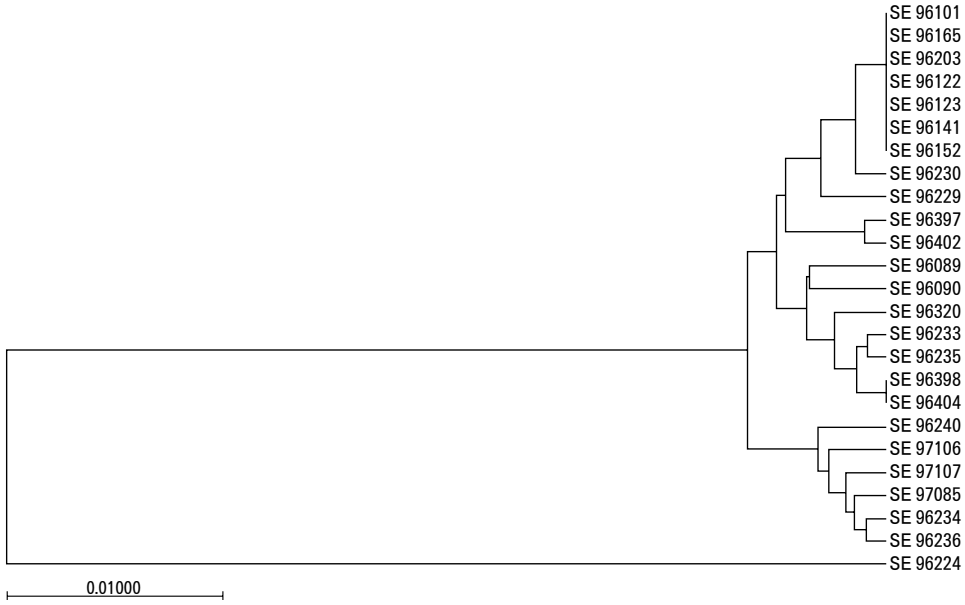

Fig. 1. Dendrogram constructed from the PFGE pattern of *Bln*I-digested *Salmonella* Enteritidis.
SE96101 isolated from present outbreak; SE96089, SE96141, SE96165, isolated from 3 food poisoning cases; other, isolated from sporadic case.

tion, it is essential to consider the possibility that the isolated strains are involved in the infection; exploration of the source and route of infection is therefore necessary. This examination can be done by utilizing biochemical properties as markers even when the strains have not been identified as a known species.

The phage type (PT) (6) of the *S*. Enteritidis isolated during the present outbreak was PT1 in 5 strains and untypable in 19 strains, while reacted but did not conform (RDNC) was seen in the remaining 5 strains. Extraction of plasmids was performed by a modified Kado-Liu method (7). All strains isolated from this outbreak had 2 plasmids (60 and 24 kb, respectively), and several strains isolated from other outbreaks have indicated the same plasmid profile. Pure, cultured bacteria were then processed by a *Bln*I restriction enzyme for analysis by pulsed-field gel electrophoresis (PFGE) with Bio-Rad CHEF-DRIII (Bio-Rad Laboratories, Hercules, Calif., USA) (8). The PFGE pattern of the DNA digested with *Bln*I was the same for all strains isolated during the present outbreak, indicating that they constituted a single cluster. This cluster included 6 strains isolated during other outbreaks. (Fig. 1) The current research used 3 methods to identify epidemiological markers. Regardless of the fact that they had specific properties, strains from the incident in question displayed no characteristic findings in terms of the plasmid profile and PFGE in comparison to strains from other incidents isolated in 1996, so it is possible that the present strain was a mutation of normal *S*. Enteritidis. In phage typing, the strains were divided into 3 groups; the presence of 3 types of characteristic strains like the current bacterium in 1 mass incident seems implausible, and the PFGE patterns were also identical, so the inability to obtain consistent results may have been due to a metabolic abnormality.

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