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Outbreak of Food Poisoning Caused by Lunch Boxes Prepared by a Company Contaminated with Multidrug Resistant *Salmonella* Typhimurium DT104

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Salmonella enterica serovar Typhimurium definitive phage type (DT) 104 was first isolated in the United Kingdom in 1984. It was isolated widely from men and animals in Europe and the United States in the 1990s (1-3). Though it was first isolated in Japan in 1987 (4), prior to 2003 there were relatively few cases of food poisoning by the organism, and no large-scale food poisoning outbreak such as that caused by *S. Enteritidis* (5). However, in September 2003, a large-scale outbreak of food poisoning by *S. Typhimurium* broke out in Osaka through the distribution of cooked food.

On September 4, a local public health bureau in Kyoto Prefecture received a report that many employees of an institution were absent due to abdominal pain, diarrhea, and fever. It was found that they had consumed lunch boxes distributed by a cooked food-provider (Company A) in Osaka. On September 9, a medical facility in Osaka Prefecture reported to the local health bureau on food poisoning cases in a kindergarten. The patients exhibited abdominal pain, vomiting, and diarrhea. They also had consumed lunch boxes supplied by the same Company A, which daily distributed 18,681 lunch boxes to 3,081 institutions and 1,100 lunch boxes to 28 kindergartens. The September outbreak affected 144 institutional workers and 214 kindergarteners. The chief symptoms were diarrhea (355 patients, or 99% of the total), abdominal pain (292 patients, 83%) and fever (256 patients, 72%). The distribution of the cases indicated that lunch boxes prepared by Company A on September 1 and 4, respectively, caused the outbreaks in the institutions and kindergartens.

S. Typhimurium was isolated from 77 of the 91 patients from whom stool samples were taken. However, 46 cooked food specimens for institutions prepared in August 27-September 2 and 40 specimens for kindergartens prepared in September 1-5 that were retained according to the Japanese food regulatory rule were negative for the organism. Ten swab specimens from the facilities of Company A were also negative. The responsible food material could not be identified by analysis of the consumption pattern. Investigation of the food processing facilities indicated that the cause of the outbreak may have been insufficient sterilization of machines, such as dishwashers or vacuum coolers. The difficulty of detecting the causative bacteria from the food materials in the present case may have been due to the low-level or uneven distribu-

tion of the contamination, as suggested by the low incidence of affected individuals, i.e., only 358 patients among nearly 20,000 consumers.

A total of 49 isolates, 8 from the affected institutions (5 isolated by the Osaka Prefectural Institute of Public Health and 3 by the Kyoto Prefectural Institute of Hygienic and Environmental Sciences) and 41 isolates from kindergartens (all isolated by the Osaka City Institute of Public Health and Environmental Sciences), were examined for antimicrobial susceptibility and pulsed-field gel electrophoresis (PFGE) pattern and compared with 4 isolates from 3 outbreaks in Osaka Prefecture in 2003 and 2 isolates from sporadic cases also in Osaka. The drug resistance pattern obtained by using BD Sensi-Disc antimicrobial discs (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) following the NCCLS guideline of the United States (6) revealed that all the 49 isolates from the present outbreak were resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT), and one isolate from a sporadic case showed the same drug resistance pattern (Table 1). Eight isolates from the institutions, 5 isolates from kindergartens, 4 isolates from other outbreaks and 2 isolates from sporadic cases were submitted to the PFGE analysis of the total DNA (7). All the isolates from the present outbreak and one isolate from a sporadic case showed the same pattern, i.e., Ba type in *BlnI* digestion and Xa pattern in *XbaI* digestion (both restriction enzymes were products of Roche Diagnostics, Mannheim, Germany) (Fig. 1). Other isolates showed dissimilar patterns. Five isolates each from the institutions and kindergartens and one isolate from a sporadic case were used for bacteriophage typing (8). All 10 isolates from the present outbreaks were typed as DT104, but the sporadic isolate was typed as DT104B.

Most *S. Typhimurium* DT104 isolates implicated in food poisoning in Europe and the United States were resistant to ACSSuT, and the resistance genes were clustered within 13 kb on the chromosome (9). The isolates from the present outbreak also showed the same drug resistance pattern, although the isolates from other outbreaks in 2003 had a different drug resistance pattern.

One isolate from a sporadic case in 2003 shared the same drug resistance and PFGE patterns with the isolates from the present outbreak. However, this isolate was DT104B, rather than DT104 as in the present case. However, DT104B is considered closely related to DT104; in fact, DT104B isolates have been reported to have the same drug resistance pattern (10) and PFGE pattern (11) as DT104 strains.

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Table 1. Characteristics of *S. Typhimurium* isolates in 2003 analyzed in the present report

Case No.	Outbreak month	Implicated food	Antimicrobial susceptibility		PFGE			Bacteriophage typing	
			No. of isolates examined	Resistance type ¹⁾	No. of isolates examined	Type by <i>BlnI</i>	Type by <i>XbaI</i>	No. of isolates examined	Phage type
Food poisoning A	September	lunch box (Institutions)	8	ACSSuT	8	Ba	Xa	5	DT104
		lunch box (Kindergartens)	41	ACSSuT	5	Ba	Xa	5	DT104
Food poisoning B	September	not identified	1	sensitive	1	Bb	Xb		not done
Food poisoning C	August	not identified	1	STmSuT	1	Bc	Xc		not done
Food poisoning D	August	not identified	2	sensitive	2	Bd	Xd		not done
Sporadic case A	July	not identified	1	ACSSuT	1	Ba	Xa	1	DT104B
Sporadic case B	October	not identified	1	KT	1	Be	Xe		not done

¹⁾: A, ampicillin; C, chloramphenicol; S, streptomycin; Tm, trimethoprim; Su, sulfisoxazole; K, kanamycin; T, tetracycline.

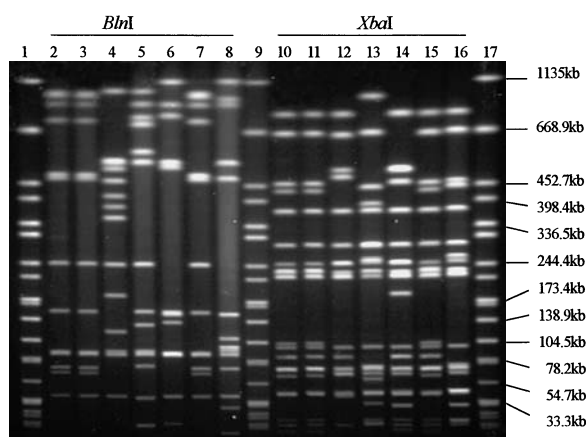


Fig. 1. PFGE patterns of *S. Typhimurium* isolates. Lanes 1, 9 and 17: DNA size standard *Salmonella enterica* serovar Braenderup H9812; lanes 2 and 10: an isolate from the institutions implicated in the present outbreak; lanes 3 and 11: an isolate from the kindergartens implicated in the present outbreak; 4 and 12, 5 and 13, and 6 and 14: isolates from other outbreaks, B-D; 7 and 15, and 8 and 16: isolates from sporadic cases.

Our results suggest that close monitoring of DT104 *S. Typhimurium* may be necessary.

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REFERENCES

- World Health Organization (1996): Zoonoses control-*Salmonella Typhimurium* infections humans. Wkly. Epidemiol. Rec., 71,138-139.
- Glynn, M. K., Bopp, C., Dewitt, W., Dabney, P., Mohter, M. and Angulo, F. (1998): Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N. Engl. J. Med.,

- 338, 1333-1337.
- Horby, P. W., O'Brien, S. J., Adak, G. K., Graham, C., Hawker, J. I., Hunter, P., Lane, C., Lawson, A. J., Mitchell, R. T., Reacher, M. H., Threlfall, E. J., Ward, L. R. and PHLS Outbreak Investigation Team (2003): A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. Epidemiol. Infect., 130, 169-178.
- Matsushita, S., Konishi, N., Arimatsu, M., Kai, A., Yamada, S., Morozumi, S., Izumiya, H., Terajima, J. and Watanabe, H. (1999): Drug-resistance and definitive type 104 of *Salmonella* serovar Typhimurium isolated from sporadic cases in Tokyo, 1980-1998. J. Jpn. Assoc. Infect. Dis., 73, 1087-1094 (in Japanese).
- National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare (2003): Salmonellosis in Japan as of June 2003. Infect. Agents Surveillance Rep., 24, 179'-180'.
- National Committee for Clinical Laboratory Standards (2000): Performance standards for antimicrobial disk susceptibility tests; approved standard. 7th ed. document M2-A7. National Committee for Clinical Laboratory Standards. Wayne, Pa.
- Ribot, E. M., Wierzbza, R. K., Angulo, F. J. and Barrett, T. J. (2002): *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. Emerg. Infect. Dis., 8, 387-391.
- Anderson, E. S., Ward, L. R., de Saxe, M. J. and J. D. de Sa, J. D. (1977): Bacteriophage typing designation of *Salmonella typhimurium*. J. Hyg., 78, 297-300.
- Briggs, C. E. and Fratamico, P. M. (1999): Molecular Characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother., 43, 846-849.
- Izumiya, H., Terajima, J., Matsushita, S., Tamura, K. and Watanabe, H. (2001): Characterization of multidrug-resistant *Salmonella enterica* serovar Typhimurium isolated in Japan. J. Clin. Microbiol., 39, 2700-2703.
- Gorman, R. and Adley, C. C. (2004): Characterization of *Salmonella enterica* serotype Typhimurium isolates from human, food, and animal sources in the Republic of Ireland. J. Clin. Microbiol., 42, 2314-2316.