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Short Communication

An Increase in Multi-Drug-Resistant Isolates of *Salmonella* Typhimurium from Healthy Carriers in Aichi, Japan

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SUMMARY: To investigate the prevalence of drug-resistant isolates of *Salmonella* Typhimurium in Aichi, Japan, we performed antimicrobial susceptibility tests for 148 isolates from healthy carriers, and from sporadic and outbreak cases of salmonellosis from 1980 to 1999. We found an increase in drug-resistant isolates from 56% (37/66) in the 1980s to 74% (61/82) in the 1990s due to increasing examples of four-, five-, and six-drug resistances. Of 98 resistant isolates in 1980 - 1999, 12 were identified as ampicillin (A)-, chloramphenicol (C)-, streptomycin (S)-, sulfonamide (Su)-, and tetracycline (T)-resistant *S.* Typhimurium (4 in the 1980s, 8 in the 1990s), whose pattern was identical to that of multi-drug-resistant *S.* Typhimurium definitive phage type 104 (DT104) which has been recently detected in various developed countries. Six-drug-resistance ACSSuTP (piperacillin), in which P was added to the core pattern of the ACSSuT, was also found in four isolates in the 1980s and seven in the 1990s. Another six-drug-resistant pattern, ACSSuTN (nalidixic acid), appeared in five isolates in the 1990s. These multi-drug-resistant isolates were predominately found in healthy carriers (21/28), suggesting that in Aichi the multi- (five- or six-) drug-resistant isolates of *S.* Typhimurium have existed in healthy carriers as well as in diarrhea patients in 1980 to 1999.

In various developed countries, a marked increase in the number of multi-drug-resistant *Salmonella* Typhimurium isolates belonging to definitive phage type 104 (DT104) from sporadic and outbreak cases of gastroenteritis has been reported starting around 1990. The organism has a core pattern of resistance to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su), and tetracycline (T) (1,2). Japanese researchers demonstrated the involvement of multi-drug-resistant *S.* Typhimurium DT104 in sporadic cases of illness in the past 20 years. Four DT104-related outbreaks were recognized between 1997 and 1998 in Tokyo (3,4). The multi-drug-resistant isolate of *S.* Typhimurium DT104 is thus an enteric pathogen which causes serious public health problems. Despite wide investigation of the bacteria, the reasons for the prevalence of the pathogen with multi-drug-resistance among healthy carriers in Japan are not fully understood. In the current study, we tested the drug-resistance of 148 *S.* Typhimurium isolates from healthy carriers as well as sporadic and outbreak cases of salmonellosis in Aichi from 1980 to 1999, although we could not perform phage typing for *S.* Typhimurium.

Among the 148 isolates examined, 90 were from healthy carriers whose occupation was food handler, 36 from patients with sporadic illness, and 22 from 11 outbreak cases (one to three isolates per one outbreak). All isolates were obtained in our laboratory, laboratories of regional health centers, and clinical laboratories of general hospitals in Aichi. Antimicrobial susceptibility tests were performed using the agar dilution method (5). Antibiotics used in this study and their minimum inhibitory concentrations interpreted as resistance were as follows: A (16 μg/ml), C (8 μg/ml), S (16 μg/ml), sulfamethoxazole (SX) (256 μg/ml), T (8 μg/ml), gentamycin (G) (4 μg/ml), nalidixic acid (N) (16 μg/ml), piperacillin (P) (64 μg/ml), trimethoprim (Tp) (8 μg/ml) (2,6). All antibiotics were purchased from Sigma Chemical, St. Louis, Mo., USA.

The Table summarizes the number of antibiotics to which the isolates were resistant and the patterns of drug resistance. Among 66 isolates in 1980 - 1989, 37 (56%) were resistant to one to six antibiotics tested, but the majority (73%) were resistant to less than three antibiotics. In contrast, 61 (74%) of 82 isolates in 1990 - 1999 were found to be resistant. Moreover, most were resistant to four or more antibiotics and thus the ratio of the isolates, which showed one to three drug-resistances, was significantly less than that in 1980 - 1989 (36% versus 73%). Of 98 resistant isolates from 1980 to 1999,

Table. Rate of drug-resistant strains and their resistance patterns of *S.* Typhimurium

Period of isolation	No. of isolates tested	No. of drug-resistant isolates	Three or less	Four	Five		Six	
					ACSSXT	Others	ACSSXTP	ACSSXTN
1980-1989	66	37(56%)	27(73%)	1	4	1	4	0
1990-1999	82	61(74%)	22(36%)	14	8	5	7	5

Resistance symbols: A, ampicillin; C, chloramphenicol; S, streptomycin; SX, sulfamethoxazole; T, tetracycline; P, piperacillin; N, nalidixic acid

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12 were identified as ACSSuT-resistant *S.* Typhimurium (4 in the 1980s, 8 in the 1990s), whose pattern was identical to that of the multi-drug-resistant *S.* Typhimurium DT104, which has been recently detected in various advanced nations. These were from 10 healthy carriers, followed by two sporadic cases. Six-drug-resistance (ACSSuTP), in which P was added to the core pattern of the ACSSuT, was found in four isolates in the 1980s and seven in the 1990s. The eleven with the six-drug-resistance were isolated mainly from healthy carriers (nine isolates). Another six-drug-resistant pattern (ACSSuTN) appeared in five isolates in the 1990s, but it had not emerged in the 1980s. The two isolates were from healthy carriers and three from an outbreak in 1999.

These results clearly indicated that the ratio of drug-resistant isolates of *S.* Typhimurium in Aichi has been increasing from 1980 to 1999 due to increases in four-, five- and six-drug-resistance. The patterns of multi-drug-resistance also differed in the 1990s from those in the 1980s. In addition, the ACSSuT-resistant *S.* Typhimurium had already existed in Aichi in the 1980s. These results agreed closely with several reports of the ACSSuT -resistant *S.* Typhimurium DT104 from Japan as well as other advanced countries (1-4). The ACSSuT-, ACSSuTP-, and ACSSuTN-resistant *S.* Typhimurium isolates were predominately obtained from healthy carriers, suggesting that the five- and six-drug-resistant *S.* Typhimurium isolates have disseminated to healthy asymptomatic carriers as well as to sporadic and outbreak cases of diarrhea patients. Therefore, we need to immediately perform phage typing of those multi-drug-resistant isolates of *S.* Typhimurium in collaboration with National Institute of Infectious Diseases, Tokyo to further reveal the prevalence of multi-drug-resistant *S.* Typhimurium DT104 in Aichi.

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REFERENCES

1. Threlfall, E. J., Frost, J. A., Ward, L. R. and Rowe, B. (1996): Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet, 347, 1053-1054.
2. Health Protection Branch-Laboratory Centre for Disease Control (1998): Human isolates of *Salmonella typhimurium* DT104 in Ontario. Can. Commun. Dis. Rep., Vol.24, No.3, 1 February.
3. Matsushita, S., Konishi, N., Arimatsu, M., Kai, A., Yamada, S. and Morozumi, S. (1999): Drug-resistance and definitive type 104 of *Salmonella* Serovar Typhimurium isolated from sporadic cases in Tokyo, 1980-1998. Jpn. J. Assoc. Infect. Dis., 73, 1087-1094 (in Japanese).
4. Izumiya, H., Tamura, K., Terajima, J. and Watanabe, H. (1999): *Salmonella enterica* serovar. Typhimurium phage type DT104 and other multi-drug resistant strains in Japan. Jpn. J. Infect. Dis., 52, 133.
5. National Committee for Clinical Laboratory Standards (1993): Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa., USA.
6. Llanes, C., Kirchgesner, V. and Plesiat, P. (1999): Propagation of TEM- and PSE-type β-lactamases among amoxicillin-resistant *Salmonella* spp. isolated in France. Antimicrob. Agents Chemother., 43, 2430-2436.