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Short Communication

Salmonella Typhimurium DT104 from Livestock in Japan

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SUMMARY: We examined the distribution of multidrug-resistant *Salmonella enterica* serotype Typhimurium definitive phage type 104 (DT104) among Japanese livestock from1973 to 1998. The 144 *S.* Typhimurium field isolates were tested for susceptibility to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, kanamycin, trimethoprim, nalidixic acid, and norfloxacin. Thirty-six of 68 strains which exhibited resistance to five or more antimicrobials (ACSSuT+) were identified as DT104. Results of plasmid profiling showed that all DT104 strains retain a 90-kb virulence plasmid, while 20 of 36 strains possessed a few additional small plasmids ranging from 2 to 4 kb. These results showed that DT104 strains have existed in Japanese livestock since 1990, and that this phage type may be an important pathogen for cattle in Japan.

The multidrug-resistant *Salmonella enterica* serotype Typhimurium definitive phage type 104 (DT104) has been an important zoonotic pathogen in Europe and North America since 1990. DT104 is known to have a wide host range and to be pathogenic for humans and cattle (1). Cattle are considered to be an important reservoir for *Salmonella* Typhimurium DT104 (1-3). But the worldwide distribution of DT104 in livestock, including that in Japan, is not well known. In Japan, human non-typhoid salmonellosis caused by *S.* Typhimurium DT104 is known as serious problem for public health (4,5). In this study, we examined the distribution of DT104 among Japanese livestock from 1973 to 1998. Before 1990, *S.* Typhimurium was thought to be primarily associated with calf diarrhea. But after 1990, salmonellosis caused by *S.* Typhimurium among cattle increased considerably. *Salmonella* serotypes isolated from cattle were mainly Typhimurium, with a few Dublin. We tested the antimicrobial susceptibility of 144 *S.* Typhimurium isolates, 68 of which were resistant to five or more antimicrobials (ACSSuT+). These 68 strains were further examined by phage typing.

The 144 *S.* Typhimurium strains were isolated during the period of 1973 through 1998, from epidemiologically independent clinical specimens submitted to livestock hygiene service centers, which are distributed throughout Japan. The 144 strains consisted of 125 strains of cattle origin, 9 of poultry, and 10 from the environment (premise 6, feeder 3, and dust 1). For all the strains, antibiotic susceptibility was evaluated by MICs. The MICs of ampicillin (A), chloramphenicol (C), streptomycin (S), sulfamethoxazole (Su), tetracycline (T), kanamycin (K), trimethoprim (Tp), nalidixic acid (N), and norfloxacin (Nx), determined using the two-fold serial agar dilution method, ranged from 100 to 0.025 μg/ml on Sensitivity Disk Agar (Nissui Pharmaceutical, Tokyo) according to the Approved Standard of the Japan Society of Chemotherapy (6). All 68 strains which exhibited ACSSuT+ resistance pattern were further tested by phage typing and plasmid profiling. Phage typing was performed according to the methods and schemes described previously (7).

The 144 *S.* Typhimurium strains were divided into 22 resistance patterns, with the patterns resistant to four or more antimicrobials shown in Table 1. Of 144 strains, 68 (47.2%) showed the ACSSuT+ resistance pattern. In particular, ACSSuT and ACSSuTK were the major resistance patterns in cattle.

As a result of phage typing, 36 of 68 (52.9%) ACSSuT+ strains were identified as phage type DT104 (Table 2). The first DT104 strain detected in this study was isolated from poultry in 1990, and showed a resistance pattern of ACSSuTTp. This atypical resistance pattern has also been isolated in England and Wales (3). But the epidemiological relationship in this study was not clarified. Except for another atypical strain, shown as ACSSuTK isolated from cattle in 1996, 34

Table 1. Antibiotic resistance patterns of *S.* Typhimurium isolated from Japanese livestock

Resistance pattern	Cattle	Poultry	Environment	Total
ACSSuTKTp	1			1
ACSSuTK N	4			4
ACSSuT TpN	1			1
ACSSuT Tp		1		1
ACSSuTK	18	1		19
ACSSuT	38	1	3	42
A SSuTK N	2			2
AC SuTK	3			3
A SSuTK	7	1	1	9
ACSSu	9		1	10
A SSuT	14		1	15
CSSuT	1			1
Others	27	5	4	36
Total	125	9	10	144

A: Ampicillin, C: Chloramphenicol, S: Streptomycin, Su: Sulfamethoxazole, T: Tetracycline, K: Kanamycin, Tp: Trimethoprim, N: Nalidixic acid

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Table 2. Number of *S.* Typhimurium DT104 isolated from Japanese livestock 1981-1998

Source	1981-1990		1991-1998		Total	
	No. of tested	No. of DT104	No. of tested	No. of DT104	No. of tested	No. of DT104
Cattle	13	0	49	31	62	31
Poultry	1	1	2	1	3	2
Environment			3	3	3	3
Total	14	1	54	35	68	36

of 36 DT104 strains showed a typical resistance pattern of ACSSuT.

As shown in Table 2, cases of cattle salmonellosis associated with *S.* Typhimurium clearly increased in the 1990s, with no *S.* Typhimurium strains showing ACSSuT+ resistance patterns isolated before 1980. From 1981 through 1990, no cattle strains of ACSSuT+ were identified as DT104. But from 1991 through 1998, 31/49 (63.3%) were identified as DT104. These results suggest that DT104 is an important cattle pathogen in Japan.

S. Typhimurium var. Copenhagen is known as a pigeon host-adapted type of *Salmonella* and is lacking an O5 antigen (8). In the United States, outbreaks of *S.* Typhimurium var. Copenhagen infections linked to dairy products were reported (9), and this strain has also been identified as DT104. Therefore, the relationship between *S.* Typhimurium var. Copenhagen and DT104 in Japan was examined using the presence or absence of O5 antigen. Among the 68 ACSSuT+ strains, O5 antigen was observed in 22% (8/36) of DT104 examples and in 19% (6/32) of other strains, suggesting that var. Copenhagen occurs with similar frequency in DT104 and non-DT104 strains of *S.* Typhimurium.

Plasmid profiling was performed on the 36 DT104 isolates using the method described by Kado and Liu (10). The plasmids were separated by electrophoresis using 0.8% agarose and photographed under UV illumination. Results of plasmid profiling showed that all strains retain a 90-kb plasmid, known as a virulence plasmid (11). Forty-four percent (16/36) of the strains examined had a 90-kb plasmid only, and 20 others possessed a few additional small plasmids ranging from 2 to 4 kb. These plasmid patterns differed slightly from those described in a previous report from the United Kingdom (12), but was in agreement with these described in a report from the United States (1).

In conclusion, these results showed that DT104 strains have existed in Japanese livestock since 1990, and that this phage type is an important pathogen for cattle. It is now considered to be widely distributed throughout Japan. Most of the 36 DT104 strains exhibited the antibiotic resistance pattern ACSSuT, but one strain showed an additional Tp resistance and another strain showed an additional K resistance. This atypical K resistance may be related to the use of K in Japanese livestock.

This wide distribution of DT104 among Japanese livestock may represent a potential public health risk. Fortunately, food-borne illnesses associated with *S.* Typhimurium decreased in the 1990s in Japan (4,13) and the DT104 strains isolated in this study were all highly susceptible to Nx.

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