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Plasmid Profile Analysis in Epidemiological Studies of Animal *Salmonella typhimurium* Infection in Japan

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Plasmid profiles were investigated in 65 isolates of *Salmonella typhimurium* derived from animal outbreaks during the period of 1978 through 1983 in Japan. Incidence of plasmids, drug-resistance, and conjugative R plasmids were extraordinarily high in these isolates. This high incidence reflects the prophylactic and therapeutic use of antibiotics. Most isolates from diseased animals, cohabiting animals, and each farm showed the same or similar plasmid patterns. However, there was a difference in plasmid patterns within strains isolated from each of several animals. It may be that one or two plasmids were introduced or deleted in these strains, leading to the difference discerned in strains isolated from the same animal. It was also shown that during one epidemic, two strains of *S. typhimurium* were involved that could be distinguished by plasmid profile analysis. Our conclusion is that when *S. typhimurium* strains isolated from animals reared in limited areas exhibit identical or similar plasmid patterns, they are derived from the same source and that when strains isolated in a limited area exhibit quite a different plasmid pattern, these strains are derived from independent sources.

Since the prevalence of plasmids in *Salmonella* species was reported by Taylor et al. in 1982 (19), there have been many reports that plasmid profile analysis was useful as an epidemiological tool in outbreaks of salmonellosis (13–15, 20). Plasmid profile analysis of *Salmonella typhimurium* was thought to be at least as reliable as phage typing in identifying related or unrelated isolates from outbreaks (1, 4). Compared with phage typing, which requires specialized laboratories, plasmid profile analyses are more rapid and more practical. However, almost all strains of *Salmonella* examined by plasmid analysis were from human clinical isolates, and only a few reports of its application to animals have been made (12, 16, 23). Therefore, we investigated the plasmids in *S. typhimurium* strains isolated in Japan from 1978 through 1983 and compared the profiles of various strains isolated from each animal and at each rearing house and farm to check the uniformity of plasmids.

MATERIALS AND METHODS

***Salmonella* strains.** *Salmonella* strains were isolated from diseased animals at local Livestock Animal Hygiene Centers by local public employees. These *Salmonella* isolates were sent to the Salmonella Center at the National Institute of Animal Health, Tsukuba, Japan. From the isolates which were typed as *S. typhimurium*, we selected 65 strains. The source of each bacterium was established and the data were epidemiologically significant. Prefectures (regions) in which the salmonellae were isolated were Hokkaido, Miyagi, Nagano, Toyama, Wakayama, Tokushima, Kumamoto, and Gifu.

The strains were divided into the following three groups according to their sources and epidemiological significance: (i) multiple isolates from the same diseased animal; (ii) multiple isolates from different animals in the same rearing house; and (iii) multiple isolates from different farms in the same prefecture which were associated with epidemics.

An epidemic occurred at Toyama Prefecture during 1981 and 1982. *S. typhimurium* was isolated from calves introduced at 11 farms in Himi City, Fukumitsu, Fukuno, and Fukuoka, at one Agriculture Cooperative in Fukumitsu, and from a local market, dairy farms, and ordinary farms (Fig. 1). At that time, some calves were introduced from neighboring Ishikawa and Gifu Prefectures, and one strain (L-534) of *S. typhimurium* was also isolated in Gifu Prefecture.

Strain storage. At the National Institute of Animal Health, isolates of *S. typhimurium* were incubated in Trypticase soy broth (BBL Microbiology Systems) for 18 h at 37°C and were stored at −80°C. At our laboratory, the isolates were stored in soft Nutrient Agar (Difco Laboratories) at 4°C.

Japanese cattle-rearing practices. On most calf-rearing farms, male calves were introduced from local markets or other sources at less than 7 days of age and were reared to the age of about 1 or 1.5 years before they were sold. In the rearing houses, calves were given substitute milk until they were 1 month old, and then they were given artificial milk until they were 3 months old. After that, they were given regular feed.

Antibiotics were administered as follows. Substitute milk contained (per ton) 420,000 to 840,000 U of zinc-bacitracin and 25 to 40 g of colistin or 40 to 50 g of tetracycline or both. Artificial milk contained (per ton) 168,000 to 336,000 U of zinc-bacitracin, 50 g of fradiomycin, and 5 to 20 g of colistin or 40 to 50 g of tetracycline or both. Artificial milk always contained tetracycline.

In addition to these antibiotics, most calves less than 5 weeks old were given 100 to 2,500 mg of colistin or 40 to 120 mg of ampicillin per kg of body weight per day for the prevention of diarrhea due to *Escherichia coli* and *Salmonella* species in drinking water or feed additives. Most calves less than 3 months old were also fed 200 to 400 g of tetracycline per ton of feed for prevention of pneumonia.

Test for drug resistance and conjugative R plasmids. Tests

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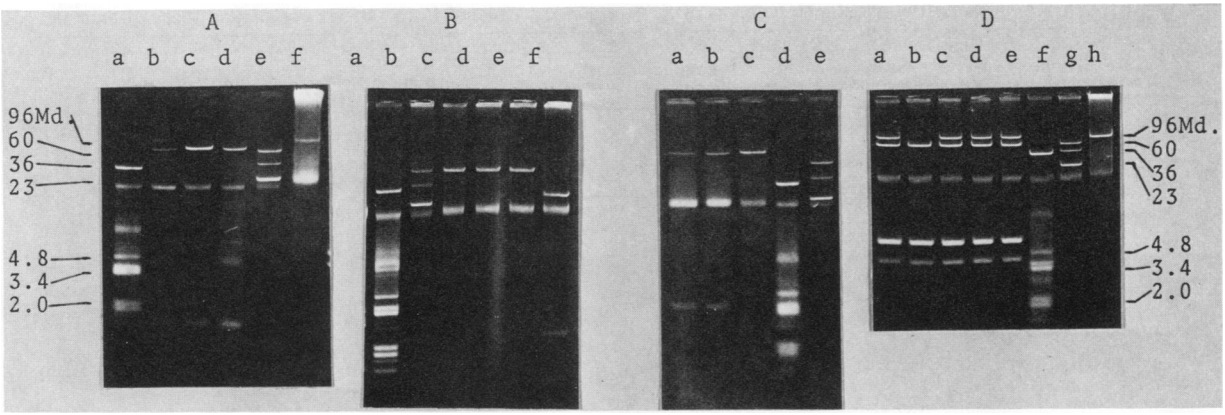

FIG. 1. Agarose gel electrophoresis of plasmid DNAs from *S. typhimurium* isolated from each of four animals. (A) Animal 1 (calf): lane a, *E. coli* V517; lane b, L-112; lane c, L-113; lane d, L-115; lane e, *S. typhimurium* LT2 (cryptic plasmid, RP4, Rs-a); lane f, *E. coli* C600 (R40a). (B) Animal 2 (cow): lane a, *E. coli* V517; lane b, *S. typhimurium* LT2; lane c, L-518; lane d, L-519; lane e, L-520; lane f, L-521. (C) Animal 6 (foal): lane a, L-564, lane b, L-565; lane c, L-566; lane d, *E. coli* V517; lane e, *S. typhimurium* LT2. (D) Animal 10 (calf): lane a, L-719, lane b, L-720; lane c, L-721; lane d, L-722; lane e, L-723; lane f, *E. coli* V517; lane g, *S. typhimurium* LT2; lane h, *E. coli* C600 (R40a).

for drug resistance and conjugative R plasmids were performed as described elsewhere (11). Antimicrobial agents were used in the following amounts (micrograms per milliliter): ampicillin (Ap), 25; chloramphenicol (Cm), 25; kanamycin (Km), 25; streptomycin (Sm), 25; sulfadimethoxine (Su), 400; and tetracycline, 25. *E. coli* ML1410 was used as the recipient bacterium in plasmid transfer studies.

Plasmid profile analysis. Plasmids were detected by a modified rapid method described by Kado and Liu (6). Bacterial cells were grown overnight in 10 ml of L broth at 37°C, harvested by centrifugation, and suspended in 1 ml of E buffer (40 mM Tris acetate, 2 mM EDTA [pH 7.9]). The cells were then lysed by the addition of 2 ml of freshly prepared lysing solution (3 g of sodium dodecyl sulfate, 0.6 g of Tris, and 6.4 ml of 2 N NaOH in 100 ml of distilled water), incubated for 1 h at 55°C, and extracted with 6 ml of phenol-chloroform (1:1 [vol/vol]). After centrifugation, the supernatant was subjected to agarose gel electrophoresis for the detection and sizing of plasmid DNA. The molecular weight standards were R27 (112 megadaltons [MDa]), R40a (96 MDa), cryptic plasmid of *S. typhimurium* LT2 (60 MDa), RP4 (36 MDa), Rs-a (23 MDa), and small plasmids of *E. coli* V517.

RESULTS

Incidence of drug resistance and plasmids. The incidence of drug resistance was extraordinarily high in *S. typhimurium* isolates from animals in Japan (Table 1). All strains were resistant, and conjugative R plasmids were found in 25 (38.5%) of 65 resistant strains. Moreover, 100% of strains had one or more plasmids.

Comparison of isolates from diseased animals. Plasmid and resistance patterns were compared among isolates from various organs or feces of the same animal. In 7 of 11 animals (animals 3, 4, 5, 7, 8, 9, and 11), *S. typhimurium* isolates showed identical plasmid and resistance patterns, although isolates from animal 4 displayed two different resistance patterns (Table 2). There was a difference in plasmid patterns in strains from each of the other four animals (animals 1, 2, 6, and 10), although 11 strains from each of those animals shared some of the same plasmids. For example, four isolates (strains L-112, L-113, L-114, and L-115) from animal 1 shared the 62- and 1.3-MDa plasmids. In addition to these plasmids, strain L-112 harbored 90-, 7.0-, and 4.2-MDa plasmids, and strain L-115 harbored 7.0- and 4.2-MDa plasmids (Fig. 1A). Four isolates (strains L-518, L-519, L-520, and L-521) from animal 2 shared the 60-MDa plasmid; one of the isolates also harbored plasmids of 30 and 2.4 MDa (Fig. 1B). One isolate (strain L-566) of three from animal 6 lacked the 3.6-MDa plasmid, although the remaining two isolates harbored this plasmid (Fig. 1C). In five isolates from animal 10, there were differences due to the presence or absence of conjugative R plasmids (Fig. 1D).

Comparison of isolates from cohabiting animals at each rearing house. *S. typhimurium* isolates from five rearing houses showed identical or very similar plasmid patterns in strains from each of those houses; a slight difference due to the presence or absence of conjugative R plasmids (in isolates from houses 2 and 3) was noted (Table 3). It was of interest that the isolates from rearing houses 3, 4, and 5 showed quite different plasmid patterns. Calves from these places were introduced to Tokushima Prefecture at almost the same time (November 11 and 18). This finding suggests that plasmid content reflected the place of origin.

Comparison of isolates associated with an epidemic. Plasmid patterns were investigated in 24 isolates derived during the prolonged epidemic in the northwestern part of Toyama Prefecture in the northern part of central Japan. These strains were isolated at 12 farms between May 1981 and November 1982. Farm 1 is located at Fukumitsu in the western part of Toyama Prefecture, farms 2 through 10 are in

TABLE 1. Incidence of drug resistance and plasmids in *S. typhimurium* strains (n = 65)

No. of drugs evoking resistance	No. of strains		
	Resistant[a]	Harboring conjugative R plasmid[b]	Harboring plasmid[c]
6	10	8	10
5	11	9	11
4	25	3	25
3	3	0	3
2	11	4	11
1	5	0	5

[a] Total, 65 (100%) of 65.
[b] Total, 24 (36.9%) of 65.
[c] Total, 65 (100%) of 65.

TABLE 2. Plasmid and drug resistance patterns of *S. typhimurium* isolated from various organs and feces of animals

Animal no.	Prefecture	Date of collection	Kind of animal	Source	No. of strains found	Drug resistance[a]	Transferred drug resistance	Mass of plasmids (MDa)	Strain no.
1	Kumamoto	10/17/78	Calf	Heart	1	Sm Tc	Sm Tc	90, 62,[b] 7.0, 4.2, 1.3	L-112[c]
			Calf	M-L[d]	1	Sm Tc	Sm Tc	62[b] 1.3	L-113[c]
				Liver	1	Sm Tc	Sm Tc	62[b] 1.3	L-114[c]
				Kidney	1	Sm Tc	Sm Tc	62,[b] 7.0, 4.2, 1.3	L-115[c]
2	Wakayama	10/14/81	Cow	Feces	3	Sm		60	L-518, L-519, L-520
				Feces	1	Sm		60, 30, 2.4	L-521
3	Wakayama	10/14/81	Cow	Feces	4	Sm Su		5.2, 2.4	L-522, L-523, L-524, L-525
4	Wakayama	10/14/81	Cow	Feces	3	Sm Su		5.2, 2.4	L526, L-528, L-529
				Feces	1	Ap Km Sm Su		5.2, 2.4	L-527
5	Miyagi	11/25/81	Calf	M-L	1	Ap Km Sm Su Tc		82, 60, 7.4	L-535
				Intestine	2	Ap Km Sm Su Tc		82, 60, 7.4	L-536, L-537
6	Kumamoto	05/21/82	Foal	Liver	1	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	85,[b] 3.6	L-564
				Lung	1	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	85,[b] 3.6	L-565
				Feces	1	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	85[b]	L-566
7	Hokkaido	08/31/81	Foal	Lung	1	Ap Cm Km Sm Su Tc		60, 5.4, 4.0	L-516[c]
				Feces	1	Ap Cm Km Sm Su Tc		60, 5.4, 4.0	L-517
8	Toyama	08/30/82	Calf	Liver	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	L-617[c]
				Spleen	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	L-618[c]
9	Toyama	08/30/82	Calf	Liver	1	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	80,[b] 6.6, 3.8	L-621[c]
				Heart	1	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	80,[b] 6.6, 3.8	L-622[c]
10	Nagano	04/05/83	Calf	Liver	1	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.4	L-719
				Brain	1	Ap		60, 6.5, 2.4	L-720
				Spleen	1	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.4	L-721
				Intestine	2	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.4	L-722, L-723
11	Nagano	04/05/83	Calf	Heart	1	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.5	L-724
				Liver	1	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.4	L-725
				Spleen	1	Ap Cm Sm Su Tc	Cm Sm Su Tc	110,[b] 60, 6.5, 2.4	L-726

[a] Tests for ampicillin (Ap), chloramphenicol (Cm), kanamycin (Km), streptomycin (Sm), sulfadimethoxine (Su), and tetracycline (Tc).
[b] Size of conjugative R plasmids.
[c] *S. typhimurium* subsp. *copenhagen*.
[d] M-L, Mesenteric lymph node.

Himi City, and farms 11 (Fukuno) and 12 (Fukuoka) are between Fukumitsu and Himi City (Fig. 2).

In May 1981, two *S. typhimurium* outbreaks occurred at farm 1, and the two strains (L-538 and L-539) involved were characterized by six plasmids ranging from 120 to 3.9 MDa (plasmid pattern A) and by resistance to ampicillin, streptomycin, sulfadimethoxine, and tetracycline (Table 4). Subsequently, in July 1981, one strain (L-541), showing quite

TABLE 3. Plasmid and drug resistance patterns of *S. typhimurium* strains[a] isolated from cohabiting calves at rearing houses

House no.	Prefecture	Date of collection	Source	Drug resistance[b]	Transferred drug resistance	Mass of plasmids (MDa)	Strain no.
1	Toyama	11/20/81	M-L[c]	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	L-538[d]
			Feces	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	L-539[d]
2	Toyama	12/19/81	Spleen	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	L-544[d]
			Feces	Ap Km Sm Su Tc	Ap Km Sm Tc	120, 95,[e] 60, 6.0, 5.1, 4.9, 3.9	L-545[d]
3	Tokushima	12/17/82	Feces			60, 2.0, 1.4	L-674
			Feces	Ap Cm Km Sm Su Tc	Ap Sm Su Tc	85,[e] 60, 2.0, 1.4	L-675
4	Tokushima	12/17/82	Feces	Ap Sm Su Tc	Ap Sm Su Tc	75,[e] 2.8	L-676
			Feces	Ap Sm Su Tc	Ap Sm Su Tc	75,[e] 2.8	L-677
5	Tokushima	12/17/82	Feces	Sm Su Tc		110, 60, 6.5, 4.2	L-678
			Feces	Sm Su Tc		110, 60, 6.5, 4.2	L-679
			Feces	Sm Su Tc		110, 60, 6.5, 4.2	L-680

[a] Only one strain was isolated from each calf.
[b] See Table 2, footnote *a*.
[c] M-L, Mesenteric lymph node.
[d] *S. typhimurium* subsp. *copenhagen*.
[e] Size of conjugative R plasmids.

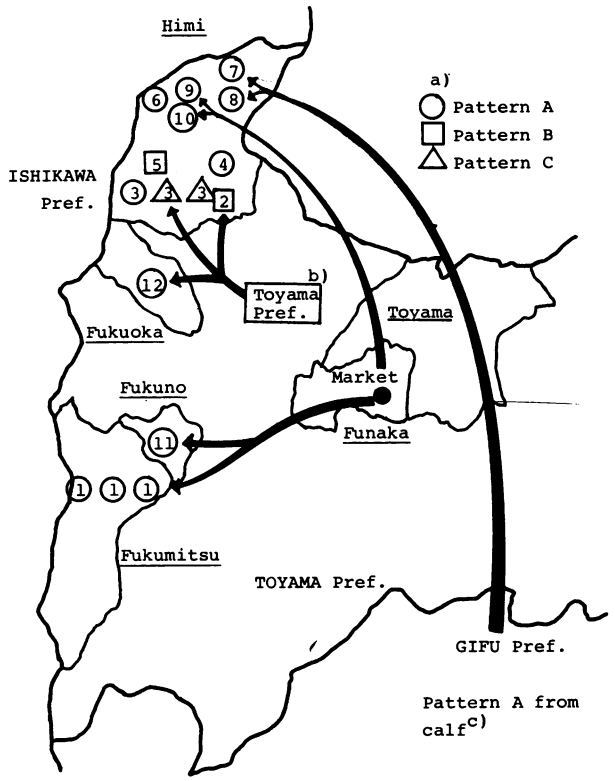

FIG. 2. Transport of calves and plasmid patterns of strains of *S. typhimurium* isolated from calves. Arrows indicate the transport of diseased calves. (a) Farm number as given in Table 3 is indicated. (b) Calves were introduced from an unknown place in this prefecture. (c) In Gifu Prefecture, *S. typhimurium* exhibiting plasmid pattern A was isolated from a calf.

different plasmid (plasmid pattern B) and resistance (to chloramphenicol, streptomycin, sulfadimethoxine, and tetracycline) patterns, was isolated at farm 2 in Himi City, and one strain with the same plasmid pattern was isolated at farm 5 in Himi City in December 1981. At farm 3, strains showing plasmid and resistance patterns the same as or similar to those of strains L-538 and L-539 were isolated in December 1981. About 1 year later, strains showing different resistance (to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfadimethoxine, and tetracycline) and plasmid (plasmid pattern C) patterns were isolated. At farms 4 and 6 through 12, *Salmonella* isolates showed plasmid pattern A and resistance to ampicillin, streptomycin, sulfadimethoxine, and tetracycline.

Investigation of the transport of diseased calves in Toyama Prefecture showed that some diseased calves could be traced (Fig. 2). Although a positive correlation between plasmid patterns of the isolates and the routing of calves could not be demonstrated clearly, it became evident that *S. typhimurium* strains characterized by plasmid pattern A were prevailing in this prefecture and that some calves were introduced from neighboring prefectures. It was also demonstrated that one strain having plasmid pattern A was isolated on 30 October 1981 in Gifu Prefecture, from which some calves were introduced to farms 7 and 8 in Himi City.

Incidence of *S. typhimurium* subsp. *copenhagen*. All strains isolated in Toyama Prefecture, one strain in Gifu Prefecture, four strains in Kumamoto, and two strains in Hokkaido were *S. typhimurium* subsp. *copenhagen*.

DISCUSSION

This study shows that the incidence of plasmids, drug resistance, and conjugative R plasmids was extraordinarily high in *S. typhimurium* strains isolated in Japan. Similar data on *Salmonella* isolates including *S. typhimurium* have been published elsewhere (8, 22). We isolated *S. typhimurium* strains from apparently healthy male calves and found a high incidence of multiple drug resistance and plasmids of various sizes (10). The high incidence of plasmids and multiple drug resistance suggest that the prophylactic and therapeutic uses of antimicrobial agents created a serious economic loss in calf rearing in Japan.

To our knowledge, there have been no comparisons of plasmid patterns with strains of *S. typhimurium* isolated from the same diseased animal. In most instances, plasmid and resistance patterns of the isolates from each of the animals were identical. However, there were some differences in plasmid patterns with isolates from certain animals (animals 1, 2, 6, and 10). The isolation of one or more different plasmids suggests that they may have been introduced or deleted spontaneously. This is a first report to demonstrate a difference of plasmid carriage in isolates from the same animal. Moreover, there is some evidence that the difference of plasmids in isolates from the same animal can be attributed to the presence or absence of conjugative R plasmids (animal 10), a finding that was true of the isolates from cohabiting animals reared in houses 2 and 3. It is possible that such R plasmids were introduced by conjugation or deleted independently of the presence of other coexisting plasmids. Genetic instability of certain R plasmids has been reported elsewhere (3). We also demonstrated that IncH1 R plasmid in *S. typhimurium* isolates was less stable against sodium dodecyl sulfate, novobiocin, and ethidium bromide than resident plasmids (10). We also found a difference in plasmids in *S. enteritidis* isolates due to R plasmids in the strains isolated from the same animal (unpublished data). These findings suggest a more dynamic nature for certain plasmids, including R plasmids in *S. typhimurium*.

Most *S. typhimurium* isolates during epidemics exhibited the same plasmid pattern (plasmid pattern A). However, some isolates displayed different types of plasmid patterns. These results indicated that those outbreaks that lasted a long time were caused by more than one strain and that the strains could be distinguished by their plasmid profiles. It is possible that a long-term epidemic due to two or three strains, introduced independently, might occur even within the limited areas because some healthy animals in Japan were *Salmonella* carriers (5, 7, 10, 17). It has been reported elsewhere that in *Salmonella* infections on a pig farm, the *S. typhimurium* variety changed, indicating the occurrence of a new infectious source on the same farm (5). During this epidemic, one strain with plasmid pattern A and resistance to ampicillin, streptomycin, sulfadimethoxine, and tetracycline was isolated in Gifu Prefecture on 30 October 1981. About 8 months later, strains with the same plasmid pattern were isolated at farms 7 and 8 in Himi City from calves introduced from Gifu Prefecture.

Plasmid analysis is accepted as a means of identifying relatedness or unrelatedness of strains of the genera *Salmonella* (1, 2, 4, 13–15), *Shigella* (18), *Enterobacter* (9), and *Campylobacter* (21). Grouping by plasmid pattern is possible because of the variation among plasmids of size and of stability in given strains of *S. typhimurium* (1, 4). We have already reported that several resident plasmids in *S. typhimurium* isolates from healthy calves were stable (10).

TABLE 4. Plasmid and drug resistance patterns of *S. typhimurium* strains isolated from calves at farms in Toyama Prefecture

Location	Farm no.	Date of isolation	Source	No. of strains	Drug resistance[a]	Transferred drug resistance	Mass of plasmids (megadaltons)	Plasmid pattern	Strain[b] no.
Fukumitsu	1	05/14/81	M-L[c]	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-538
		05/14/81	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-539
		12/08/81	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-547
Himi	2	07/23/81	Feces	1	Cm Sm Su Tc	Tc	96,[d] 2.0, 1.5	B	L-541
	3	11/29/81	Spleen	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-544
			Feces	1	Ap Km Sm Su Tc	Ap Km Sm Tc	120, 95,[d] 60, 6.0, 5.1, 4.9, 3.9	A′	L-545
		08/22/82	Feces	2	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	80,[d] 6.6, 3.8	C	L-621, L-622
		10/01/82	Liver	2	Ap Cm Km Sm Su Tc	Ap Cm Km Sm Su Tc	80,[d] 6.6, 3.8	C	L-643, L-644
	4	12/09/81	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-548
	5	12/10/81	Feces	1	Cm Sm Su Tc	Tc	96,[d] 2.0, 1.5	B	L-549
	6	06/14/82	Feces	2	AP Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-578, L-579
	7	06/15/82	Feces	2	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-585, L-586
	8	06/15/82	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-587
	9	06/18/82	Feces	2	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-588, L-589
	10	06/18/82	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-590
Fukuno	11	12/07/81	Feces	1	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-546
Fukuoka	12	08/16/81	Feces	2	Ap Sm Su Tc		120, 60, 6.0, 5.1, 4.9, 3.9	A	L-617, L-618, L-620

[a] See Table 2, footnote *a*.
[b] All strains were *S. typhimurium* subsp. *cophenhagen*.
[c] M-L, Mesenteric lymph node.
[d] Size of conjugative R plasmids.

However, this study demonstrated that certain plasmids of *S. typhimurium* isolates may be spontaneously introduced or deleted.

Thus we may conclude that *S. typhimurium* strains isolated from animals reared in limited areas and exhibiting identical or similar plasmid patterns originated from one source and that isolates from such limited areas exhibiting quite different plasmid patterns were derived from different sources.

In the present study, we detected at least one plasmid in all 65 isolates. In the United States, Rilay et al. (15) could not detect plasmids in all *S. typhimurium* isolates checked. Holmberg et al. (4) also failed to detect plasmids in 50 (21%) of 228 strains examined. Although the comparison between *S. typhimurium* in the United States and Japan is difficult, since the U.S. isolates were derived from humans and the Japanese isolates were derived from animals, it is of interest that all isolates of *S. typhimurium* from animals in Japan have plasmids. This high incidence of plasmids may reflect the prophylactic and therapeutic uses of antimicrobial agents and recommends the plasmid analysis of *S. typhimurium* isolates as useful for epidemiological study.

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