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Change of drug resistance patterns and genetic properties of R plasmids in *Salmonella typhimurium* of bovine origin isolated from 1970 to 1979 in northern Japan

By S. MAKINO, N. ISHIGURO, G. SATO

Department of Veterinary Public Health, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, *Japan*

AND N. SENO

Tokachi Livestock Hygiene Service Centre, Obihiro 080, *Japan*

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SUMMARY

A total of 321 *Salmonella typhimurium* strains of bovine origin obtained in northern Japan during the period 1970–1979 were tested for drug resistance and detection of conjugative R plasmids. Three hundred and eighteen (99·1 %) of these strains were resistant to one or more drugs. The isolation frequency of multiply drug-resistant strains tended to increase year by year. Two hundred and thirty-seven (74·5 %) of these resistant strains carried conjugative R plasmids. A total of 308 R plasmids including 174 (56.5 %) thermosensitive (ts) R plasmids were derived from the 237 drug-resistant strains, indicating that 71 (30·0 %) strains have two different conjugative R plasmids in a single host cell. Of the 308 R plasmids examined for fertility inhibition (*fi*), 167 ts and 131 non-ts R plasmids were fi^-. Of the 60 ts R plasmids examined for incompatibility, 50 were classified into H1 group and 10 into H2 group. Of the 52 non-ts R plasmids examined, 35 were classified into the Iα group and the remaining plasmids were untypable in our tests. Mercury resistance marker was found in about 20 % of H1 R plasmids coding for multiresistance, and all of H2 R plasmids coded for resistance to tellurite. The clonal distribution of an *S. typhimurium* strain which carried an H1 R plasmid coding for resistance to six drugs and mercury was recognized in 1978 and 1979.

INTRODUCTION

The extensive use of various drugs for preventive, therapeutic and nutritive purposes in domestic animals has contributed to the development of drug-resistant populations of enterobacteria and in such conditions there is a potential risk to man. Most of these drug-resistant enterobacteria carried conjugative R plasmids, and the resistance might be transferred among bacterial genera or from strains colonizing animals to man (Anderson, 1968; Davies & Stewart, 1978; Hirsh & Wigner, 1977; Levy *et al.* 1976).

The higher incidence of drug-resistant salmonella has been reported more in cattle than in other animals (Sojka *et al.* 1977; Timony, 1978), and the most

frequent serovar in bovine salmonella strains is *Salmonella typhimurium* (Kashiwazaki *et al.* 1974; Sojka *et al.* 1977; Stephan *et al.* 1977; Terakado *et al.* 1980). Threlfall *et al.* (1978b) reported that the rise in drug resistance in *S. typhimurium* phage type 204 and rapid spread of the resistant strain occurred in cattle and humans in England during recent years. Sato & Terakado (1977) reported the change of properties of R plasmids in *S. typhimurium* isolated from calves for 5 years in confined environment. However, few studies have considered a change of the incidence of drug-resistant salmonella strains over a period of years or the properties of R plasmids isolated for a long term in a certain area.

This paper deals with the annual change of drug resistance patterns and of the properties of conjugative R plasmids in salmonella isolated from bovine sources, mainly diseased calves, from 1970 to 1979 in Northern Japan.

MATERIALS AND METHODS

Salmonella strains examined

A total of 321 *S. typhimurium* strains including 192 (59·8%) strains of *S. typhimurium* subserovar *copenhagen* was included in this study. These strains were isolated principally from calves in 25 farms and 1 rendering plant in northern Japan, mainly Hokkaido, during the years 1970–1979. The number of salmonella strains tested per year is shown in Table 2. In particular, the longitudinal study was carried out in two farms (Uenae and Memuro) over 9 years 1970–1979 and 5 years 1975–1979, respectively. These strains were kept on Dorset's egg slopes (Nissui).

Bacterial strains, plasmids and phages used for genetic experiments

Escherichia coli K-12 derivatives used in this study and the reference R plasmids used for incompatibility tests are shown in Table 1. pOH1477 derivative conferring resistance to ampicillin and chloramphenicol of pOH1476 derived from *S. typhimurium* OH1476 strain was obtained by acridine orange treatment by the method of Datta *et al.* (1979), and was identified as incompatibility group H1 in this study. The phages f2, λ, T4 and T7 were used.

Media

Nutrient broths used in this study were Penassay broth (PAB., Difco) and L Broth (Lennox, 1955). Heart infusion agar (Eiken), Mueller-Hinton agar (Eiken) and Deoxycholate-hydrogen sulphide-lactose (DHL) agar (Eiken) were also used as basal media as previously described (Ishiguro *et al.* 1980*b*). The selection media used for citrate utilization (Cit) were Simmons citrate agar (Eiken) plates, supplemented with methionine (50 μg/ml) and nalidixic acid (50 μg/ml) when necessary (Ishiguro *et al.* 1979).

Antibiotic sensitivity tests and detection of conjugative R plasmids

Sensitivity testing of *S. typhimurium* was done as previously described (Ishiguro *et al.* 1980*b*), using 10 antibiotics at the following final concentrations (μg/ml): ampicillin (Ap), 25; chloramphenicol (Cm), 25; kanamycin (Km), 25; streptomycin (Sm), 12·5; sulfadimethoxine (Su), 800; tetracycline (Tc), 25; furatrizine (Ft), 6·3; nalidixic acid (Na), 25; rifampin (Rif), 50; and colistin (Cl), 12·5 U/ml.

Table 1. *Bacterial strains and plasmids employed*

Bacterial strains or plasmids	Relevant genetic markers*	Reference
E. coli K-12		
ML1410	F^-, *met*, nal^r	
SG1	Rifampin-resistant mutant of Hfr W1895	
SG3	Rifampin-resistant mutant of *E. coli* 921 *met*, *thr*, *thi*, *leu*, *lac*, r_k^-, m_k^-	
Plasmids		
R386 (FI)†	Tc	Jacob *et al.* (1977)
F'lac (FI)	lac^+	Jacob *et al.* (1977)
R100 (FII)	CmSmSuTcHg	Jacob *et al.* (1977)
R124 (FIV)	Tc	Jacob *et al.* (1977)
R27 (H1)	Tc	Jacob *et al.* (1977)
R478 (H2)	CmKmTcTer	Jacob *et al.* (1977)
R144 (Iα)	Km(Tc)	Jacob *et al.* (1977)
R391 (J)	Km	Jacob *et al.* (1977)
R387 (K)	CmSm	Jacob *et al.* (1977)
R446b (M)	SmTc	Jacob *et al.* (1977)
R14 (B)	ApSmSuTc	Jacob *et al.* (1977)
RP4 (P)	ApKmTc	Jacob *et al.* (1977)
Rs-a (W)	CmKmSmSu	Jacob *et al.* (1977)
pOH4047-2 (FII)	ApCmKm	Ishiguro *et al.* (1980*a*)
pOH806-2 (Iα)	Cm	Ishiguro *et al.* (1980*b*)
pOH3123 (H1)	KmTcCit$^+$	Ishiguro *et al.* (1979)
pOH1477 (H1)	ApCm	This paper

* *met*, methionine; *thr*, threonine; *thi*, thiamine; *leu*, leucine; *nal*, nalidixic acid; r_k^- m_k^-, restriction and modification deficient.

† incompatibility group.

Transfer experiments were done by modifying the methods of Sato & Terakado (1977). Matings were carried out at 28 and 37 °C between each resistant salmonella strain and the *E. coli* recipient strain ML 1410. Each salmonella strain (donor) was incubated in 2 ml of PAB at 28 °C overnight. *E. coli* ML1410 was cultured in a similar way. Then, 0·2 ml of donor culture and an equal amount of recipient culture were mixed in 1·5 ml of PAB. The mixture was incubated at 28 and 37 °C for 18 h. A loopful of the mixture was inoculated on a selective agar plate containing Na (50 μg/ml) and a suitable drug. The selection was carried out by using those drugs to which each donor strain was resistant, in order to detect as many transconjugants with different resistance patterns as possible. If the transconjugants were more frequently detected by the mating made at 28 °C than that at 37 °C, they were considered to carry thermosensitive (ts) conjugative R plasmids. To determine the resistance patterns of transconjugants obtained, 5 colonies of transconjugants from each selective medium were inoculated on non-selective DHL agar plates and then replicated on drug agar plates. After that, the colonies showing different resistance patterns were purified, and were used for further genetic experiments. When any transconjugants could not be obtained by the method described above, the quantitative transfer experiments were performed as previously described (Ish-

iguro *et al.* 1980*b*). Donor and recipient were cultured for 4 h at 28 °C with frequent shaking. Then, 0·1 ml of donor and 1·0 ml of recipient were mixed in 4·5 ml of fresh PAB, and incubated for 2 h at 28 °C or 37 °C with gentle shaking. Then, 0·1 ml of appropriate dilutions in saline was plated on selective agar plates.

Tests for mercury resistance, tellurite resistance and citrate utilization

The transconjugants of *E. coli* ML1410 obtained in transfer experiments were tested for resistance to mercury (Hg) by the method of Nakahara *et al.* (1977) and to tellurite (Ter) by the method of Summers & Jacoby (1977). Citrate-utilizing ability (Cit) on Simmons citrate agar was determined according to method of Ishiguro *et al.* (1979).

Fertility inhibition (fi) *and phage-inhibition tests*

The *fi* character of R plasmids was examined by the method of Ishiguro *et al.* (1980*b*), using the male-specific phage f2. Phage-inhibition test was done according to the method of Taylor & Grant (1976), using phages λ, T4 and T7.

Incompatibility tests

The incompatibility of conjugative R plasmids derived from *S. typhimurium* was examined by the method of Ishiguro *et al.* (1980*b*). To examine the incompatibility group of ts R plasmids carrying resistance to six drugs (Ap, Cm, Km, Sm, Su and Tc), the Cit character of pOH3123 (incompatibility group H1) was used as selective marker for incompatibility tests (Ishiguro *et al.* 1980*c*). The H group was subsequently divided into two subgroups, H1 and H2, by the interaction with F factor. Group H2 plasmids are compatible with F factor, whereas H1 plasmids are incompatible with F factor (Smith *et al.* 1973). For subgrouping so, F'lac was used as a reference plasmid.

RESULTS

Antibiotic resistance in S. typhimurium *strains of bovine origin*

Table 2 shows the percentage of *S. typhimurium* resistant to each of the drugs tested. Tc and Sm resistances were the most frequent in this study. Resistance to Ap, Cm or Km appeared in 1973. The increase of salmonella strains harbouring Ap and Km resistances was especially notable; most salmonella strains obtained in 1979 were resistant to Ap and Km. In contrast with the increase of Ap- or Km-resistant strains, there was considerable variation in the isolation frequency of Cm-resistant strains. Salmonella strains conferring Ft and Na resistances were rare in this study.

Table 3 lists the drug resistance patterns of the 321 *S. typhimurium* strains. All except three strains were resistant to one or more of the drug(s) tested. Twenty-eight resistance patterns were found in this study. By 1971, the most frequent resistance pattern was SmSuTc [18 (43·9 %) of 41 strains in 1970 and 1971]. During 1973–1975, CmSmSuTc pattern was the predominant [60 (42·6 %) of 142 strains during these periods]. Since, in each of several years during this survey, salmonella strains tested were obtained from only one or two farms including a farm (Uenae) in which a longitudinal study was carried out, the possibility that our data were partially distorted by testing a number of prevalent strains with the same properties isolated

Table 2. *Percentage of resistance markers in* S. typhimurium *examined in this study*

	Percentage resistance									
Drug	1970	1971	1972	1973	1974	1975	1977	1978	1979	Total
Tc	87·0	88·2	100	98·7	100	89·2	100	90·5	100	93·4
Sm	95·7	88·2	100	88·6	100	100	65·5	97·6	100	92·8
Su	78·3	41·2	0	59·5	100	56·8	17·2	35·7	92·2	62·3
Cm	0	0	0	44·3	95·8	40·5	69·0	42·9	31·3	40·6
Km	0	0	0	12·7	0	24·3	65·5	81·0	92·2	41·5
Ap	0	0	0	2·6	0	0	0	85·7	95·3	30·5
Cl	4·3	0	0	1·3	0	16·2	0	16·7	3·1	5·3
Ft	0	11·8	0	0	0	0	0	0	0	0·6
Na	0	0	0	0	0	0	0	0	4·7	0·9
Rif	0	0	0	0	0	0	0	0	0	0
No. of resistant strains (%)	23 (100)	17 (94·4)	3 (100)	79 (97·5)	24 (100)	37 (100)	29 (100)	42 (100)	64 (100)	318 (99·1)
No. of strains tested	23	18	3	81	24	37	29	42	64	321

Table 3. *Change in antibiotic resistance patterns of* S. typhimurium *by year of isolation*

Resistance patterns	No. of strains per year									
	1970	1971	1972	1973	1974	1975	1977	1978	1979	Total
ApCmKmNaSmSuTc	.	.	.	.	.	.	.	.	3 (2)*	3 (2)
ApClCmKmSmSuTc	.	.	.	.	.	.	.	.	1	1
ApCmKmSmSuTc	.	.	.	.	.	.	.	15 (2)	15 (3)	30 (5)
ApClCmSmTc	.	.	.	1	.	.	.	.	.	1
ApClKmSmTc	.	.	.	.	.	.	.	4 (1)	.	4 (1)
ApKmSmSuTc	.	.	.	.	.	.	.	.	40 (7)	40 (7)
ClCmSmSuTc	.	.	.	.	.	2 (1)	.	.	.	2 (1)
ClKmSmSuTc	.	.	.	.	.	1	.	.	.	1
CmKmSmSuTc	.	.	.	7 (1)	.	.	1	.	.	8 (2)
ApClSmTc	.	.	.	.	.	.	.	2 (1)	1	3 (2)
ApCmSmTc	.	.	.	1	.	.	.	.	1	2 (2)
ApKmSmTc	.	.	.	.	.	.	.	12 (1)	.	12 (1)
ClKmSmTc	.	.	.	.	.	2 (1)	.	1	.	3 (2)
ClSmSuTc	1	.	.	.	.	.	.	.	.	1
CmKmSmTc	.	.	.	.	.	.	10 (1)	2 (1)	.	12 (1)
CmSmSuTc	.	.	.	26 (3)	23 (1)	11 (1)	.	.	.	60 (4)
FtSmSuTc	.	2 (1)	.	.	.	.	.	.	.	2 (1)
KmSmSuTc	.	.	.	2 (2)	.	5 (2)	.	.	.	7 (4)
CmKmTc	.	.	.	.	.	.	8 (1)	.	.	8 (1)
KmSmTc	.	.	.	1	.	1	.	.	.	2 (2)
SmSuTc	14 (1)	4 (2)	.	7 (3)	1	.	4 (2)	.	.	30 (7)
ApCm	.	.	.	.	.	.	.	1	.	1
ClSm	.	.	.	.	.	1	.	.	.	1
CmTc	.	.	.	.	.	.	1	.	.	1
SmSu	3 (1)	1	.	5 (1)	.	2 (1)	.	.	.	11 (2)
SmTc	4 (1)	7 (1)	3 (1)	17 (5)	.	11 (2)	4 (1)	2 (1)	3 (1)	51 (9)
Sm	.	1	.	3 (1)	.	1	.	3 (1)	.	8 (2)
Tc	1	2 (1)	.	9 (2)	.	.	1	.	.	13 (4)
(S)	.	1	.	2 (2)	.	.	.	.	.	3 (2)
Total	23 (1)	18 (2)	3 (1)	81 (7)	24 (2)	37 (3)	29 (2)	42 (5)	64 (12)	321 (26)

* No. of isolation source.

(S), Susceptibility to all antibiotics used in this study.

from the farm can not be completely denied. However, the strains which were obtained from the farm in 1970–1971, were considered to have been derived from calves introduced from different sources on the basis of phage types and biotypes (Sato & Terakado, 1977). On the other hand, 30 salmonella strains obtained in 1978 and 1979 showed resistance to six drugs (ApCmKmSmSuTc), and, in 1979, 40 strains showed resistance to five drugs (ApKmSmSuTc). In 1979, 3 strains carrying resistance to seven drugs (ApCmKmSmSuTcNa) were observed. In the later stage of this survey, the number of farms yielding multiresistant strains increased. The frequency of multiresistant salmonella strains tended to increase year by year.

Detection of conjugative R plasmids in S. typhimurium *strains*

Two hundred and thirty-seven (74·5 %) of the 318 resistant strains were able to transfer all or part of their resistance to *E. coli* ML1410 (Tables 4 and 5). Although, as described above, our data might be partially distorted by investigating a number of prevalent strains with the same properties obtained from the particular farm, the low frequencies of R^+ salmonella strains seem quite obvious during the first 2 years. The evident decrease of the frequency in 1979 was due to the emergence of large population of salmonella strains resistant to five drugs (ApKmSmSuTc) not carrying conjugative R plasmids. It should be noted that R plasmids coding for resistance to six drugs appeared in 2 of 4 farms in 1978 and in 5 of 12 farms in 1979.

A total of 308 R plasmids was obtained from the 237 resistant salmonella strains. Table 4 indicates that (40.0 %) of the 237 R^+ strains had two R plasmids coexisting in a single host cell. The following 8 patterns of markers of coexisting R plasmids were found in order of decreasing isolation frequency; (CmSmSuTc . Tc), (CmKmTc . Tc), (SmSuTc . Tc), (CmKmSmSuTc . Tc), (KmSmTc . SmSu), (Tc . SmSu), (SmSu . Tc), and (ApCm . Tc) (Underline indicates ts R plasmids). As indicated in Table 5, a total of 13 resistance patterns of markers of R plasmids were detected. One hundred and seventy-four (56.5 %) of 308 R plasmids detected showed ts transfer. One hundred and fifty-three (87·9 %) of these ts R plasmids coded for resistance to more than 3 kinds of drugs, indicating that ts R plasmids had more complicated resistance patterns than non-ts R plasmids. R plasmids carrying resistance patterns with Ap-, Cm- or Km-resistance marker were more frequently associated with ts transfer than those without the markers.

Mercury (Hg) resistance, tellurite (Ter) resistance and citrate utilization (Cit)

Of 124 R plasmids examined, 32 (25.8 %) coded for resistance to Hg and 10 (8·1 %) coded for resistance to Ter, plasmids conferring these characters were always ts R plasmids. The 32 R plasmids coding for Hg-resistance were isolated from 6 farms and 1 rendering plant. Ten R plasmids coding for Ter-resistance were detected from 2 farms in 1978 and 1979, most of them were derived from Uenae farm in 1978 (Table 6). In that farm, though 103 salmonella strains were examined during 1970–1978, no R plasmid coding for resistance to Ter had been obtained before 1978. Although the 27 R plasmids coding for resistance to six drugs were tested for Cit ability, none of them showed Cit ability (data not shown).

Table 4. *Transferred antibiotic resistance patterns in* S. typhimurium

Period of isolation	No. of resistant strains	No. of R^+ strains (%)	No. of sources	Drug resistance patterns of R plasmids
1970	23	6 (26·1)	1	(Tc . SmSu)*-1† (Tc . SmSuTc)-3; Tc-2
1971	17	10 (58·8)	2	(Tc*** . SmSu)-1; (Tc . SmSuTc)-1; Tc-8
1972	3	3 (100)	1	SmTc-3
1973	79	69 (87·3)	6	(CmKmSmSuTc . Tc)-7; (CmSmSuTc . Tc)-24; CmSmSuTc-1; KmSmTc-2; (ApCm . Tc)-1; (Tc . SmSu)-1; (Tc . SmSuTc)-2; ApCm-1; Tc-5; Tc-25
1974	24	24 (100)	2	CmSmSuTc-23; (Tc . SmSu)-1
1975	37	34 (91·9)	4	CmSmSuTc-12; (KmSmTc . SmSu)-6; KmSmTc-3; SmSu-2; Tc-1; Tc-10
1977	29	29 (100)	2	(CmKmSmSuTc . Tc)-1; (CmKmTc . Tc)-18; (Tc . SmSuTc)-3; CmTc-1; Tc-6
1978	42	38 (90·5)	5	ApCmKmSmSuTc-15; ApKmSmTc-15; KmSmTc-1; ApSmTc-3; (CmKmTc . Tc)-1; CmKmTc-1; SmTc-2
1979	64	24 (37·5)	12	ApCmKmSmSuTc-19; ApSmTc-1; ApCm-1; Tc-3
Total	318	237 (74.5)	26	

* Indicating R plasmids coexisting in a single host cell.
† No. of R^+ strains.
*** Underline shows thermosensitive R plasmid.

Table 5. *Demonstration of R plasmids carrying Hg^r and Ter^r in* S. typhimurium *and their resistance patterns*

Resistance patterns of R plasmids	R plasmids detected			No. of R plasmids tested (ts)	No. of Hg^r R plasmids (ts)	No. of Ter^r R plasmids (ts)
	No.	%	ts			
ApCmKmSmSuTc	34	11·0	34	27 (27)	26 (26)	—
CmKmSmSuTc	8	2·6	8	3 (3)	1 (1)	—
ApKmSmTc	15	4·9	15	7 (7)	—	7 (7)
CmSmSuTc	60	19·5	60	13 (13)	—	—
ApSmTc	4	1·3	4	2 (2)	—	2 (2)
CmKmTc	20	6·5	20	4 (4)	4 (4)	—
KmSmTc	12	3·9	12	7 (7)	—	—
SmSuTc	9	2·9	0	5	—	—
CmTc	1	0·3	1	1 (1)	1 (1)	—
SmTc	5	1·6	2	2 (1)	—	1 (1)
SmSu	12	3·9	0	9	—	—
ApCm	3	1·0	1	3 (1)	—	—
Tc	125	40·6	17	41 (6)	—	—
Total	308		174	124 (72)	32 (32)	10 (10)
%	—		56·5	—	25·8 (44·4)	8·1 (13·9)

Hg^r, mercury resistance.
Ter^r, tellurite resistance.

Genetic properties of conjugative R plasmids examined

The *fi* character of 308 R plasmids including 174 ts and 134 non-ts R plasmids was investigated. Seven (4·0%) of the ts R plasmids and 3 (2·2%) of the non-ts R plasmids were *fi*$^+$. The remaining 298 ts and non-ts R plasmids were *fi*$^-$.

A total of 112 *fi*$^-$ and *fi*$^+$ R plasmids was tested for incompatibility. The 60 ts R plasmids tested were classified into H1 (50 plasmids) and H2 (10 plasmids). Thirty-five *fi*$^-$ non-ts R plasmids were classified into incompatibility group Iα (31·3% of the 112 plasmids tested). Fourteen *fi*$^-$ non-ts R plasmids and 3 *fi*$^+$ non-ts R plasmids were found to be untypable (UT) using the limited number of reference plasmids shown in Table 1 (15·2% of the 112 plasmids tested). *E. coli* K-12 strains carrying H2 R plasmids inhibited development of phages λ and T7, and reduced phage T7 plaque size to 1–2 mm (Table 6). Moreover, these H2 R plasmids were found to code for Ter-resistance. The genetic properties of R plasmids in two representative farms are shown chronologically in Table 6. Thermosensitive H1 R plasmids had been obtained from 1971 in Uenae farm, but H2 R plasmids appeared for the first time in 1978.

The R plasmids coding for resistance to six drugs (ApCmKmSmSuTc) were incompatible with pOH3123 and classified into incompatibility group H1 and most (96.3%) of them coded for resistance to Hg (Table 5). Five ts R plasmids coding for resistance to two to five drugs and Hg were obtained from Memuro farm in 1977 and 1978, and typed as incompatibility group H1 (Table 6).

In this study, conjugative Iα plasmids and H1 plasmids were the predominant incompatibility groups among R plasmids tested. However, the isolation frequency of the Iα plasmids tended to decrease year by year, whereas that of the H group plasmids tended to increase.

Table 6. *Genetic properties of R plasmids derived from* S. typhimurium *isolated on 2 farms*

Farm and period of isolation	Phenotype of R plasmids	No. of R plasmids tested (ts)	fi	Relative EOP* of bacteriophage (R^+/R^-)			Incompatibility group
				λ	T4	T7	
Uenae 1970	SmSuTc	2	—	NT	NT	NT	UT
	SmSu	1	—	NT	NT	NT	UT
	Tc	5	—	1	1	0·2-1†	Iα
1971	SmSuTc	1	—	NT	NT	NT	UT
	SmSu	1	—	NT	NT	NT	UT
	Tc	1 (1)	—	1	1	1	H1
	Tc	1 (1)	+	1	1	1	H1
	Tc	1	—	1	1	1	Iα
1973	CmSmSuTc	1 (1)	—	1	1	1	H1
	Tc	1 (1)	—	1	NT	NT	H1
	Tc	2	—	0·5-1	1	1	Iα
1974	CmSmSuTc	3 (3)	—	1	1	1	H1
1978	ApkmSmTcTer	4 (4)	—	10^{-3}-1	1	0·5-1†	H2
	ApKmSmTcTer	3 (3)	+	0·5-1	1	0·2-1†	H2
	SmTcTer	1 (1)	—	0·1	1	0·1†	H2
Memuro 1975	CmSmSuTc	3 (3)	—	1	1	1	H1
	KmSmTc	4 (4)	—	1	1	1	H1
	SmSu	4	—	NT	NT	NT	UT
1977	CmKmSmSuTcHg	1 (1)	—	NT	NT	NT	H1
	CmKmTc	3 (3)	—	1	1	1	H1
	CmTcHg	1 (1)	—	1	1	1	H1
	Tc	5	—	1	1	1	Iα
1978	CmKmTcHg	1 (1)	—	1	1	1	H1
1979	ApCmKmSmSuTcHg	2 (2)	—	1	1	1	H1

* EOP: efficiency of plating.
† Phage T7 plaques have a pin-point morphology.
\+ Phage T7 plaques are reduced 1–2 mm on the inhibiting R^+ strains.
NT, not tested. UT, untypable.

Clonal distribution of S. typhimurium *carrying H1 R plasmids (ApCmKmSmSuTcHg) among calves*

As shown in Table 4, R plasmids coding for resistance to six drugs appeared for the first time in 1978 and were distributed widely in 1979. Moreover, 26 of the 27 H1 plasmids coded for Hg-resistance (Table 5). H1 R plasmids coding for Hg-resistance (CmKmSmSuTcHg, CmKmTcHg, CmTcHg) were found during 1977–1978 in a farm (Memuro) (Table 6). Although it is not clear whether these plasmids became progenitors of the above described R plasmids coding for resistance to six drugs, there is no doubt that a clone of *S. typhimurium* carrying an H1 R plasmid coding for resistance to six drugs (ApCmKmSmSuTc) and mercury was distributed in calves in Hokkaido district. The 40 strains resistant to ApKmSmSuTc emerged on 7 of 12 farms in 1979. In this study, detection of conjugative R plasmids from these strains was unsuccessful, but further genetical study (for example, the confirmation of plasmid-mediation) is needed for the strains with the uniform resistance pattern.

DISCUSSION

In the present study, the annual change of drug resistance patterns and of transferred resistance patterns in *S. typhimurium* of bovine origin was presented. Our results indicate that the frequency of multiply drug-resistant *S. typhimurium* strains tends to increase year by year, as shown in other countries (Threlfall *et al.* 1978*b*; Timony, 1978). *S. typhimurium* strains resistant to seven drugs including Na were obtained in 1979 (Table 3). In Japan, increasing isolation of Na resistant salmonella or *E. coli* of bovine origin has been reported since 1973 (Ishiguro *et al.* 1980*c*; Takahashi, 1976; Terakado *et al.* 1980). This may be interpreted as the result of selection pressure due to the widespread use of nalidixic acid or similar drugs. In addition, Tc and Sm resistances were most frequent, as reported in elsewhere (Sojka *et al.* 1977; Stephan *et al.* 1977).

In this study, the R plasmids derived from *S. typhimurium* of bovine origin were classified into incompatibility group H, Iα or UT. This result is almost the same as that of R plasmids of swine salmonella (Ishiguro *et al.* 1980*b*; Terakado *et al.* 1980). We indicated that 56·5% of R plasmids tested showed ts transfer and all of these ts R plasmids were classified as incompatibility group H. H1 plasmids coding for Tc-resistance emerged in Japan in 1971, 2 years earlier than the description by Terakado & Sato (1978).

Terakado *et al.* (1980) reported that R plasmids conferring multiresistance including Cm belonged to H1 and Iα. However, we found that most of the R plasmids carrying Cm-resistance belonged to incompatibility group H1. It is presumed that there is an interrelationship between the R plasmids of group H1 and the widespread distribution of Cm-resistance among salmonella.

Taylor & Grant (1976) reported that *E. coli* K-12 carrying H2 plasmids inhibited development of phages λ and T7. *E. coli* K-12 carrying H2 plasmids was found to inhibit development of these phages in this study. All of the H2 plasmids coded for resistance to Ter. The inhibition of development of bacteriophages λ and T7, and Ter-resistance marker in ts R plasmids might be subsidiary indicators of incompatibility group H2 in *S. typhimurium* of bovine origin.

We obtained R plasmids of H2 group in 1978 in Uenae farm, in which H1 plasmids had persisted. The emergence of H2 plasmids might be associated with the introduction of calves from a new source. Also, it is probable that the clonal distribution of *S. typhimurium* carrying H1 R plasmids coding for resistance to six drugs among calves in 1978 and 1979 was caused and facilitated by the transport of infected calves from the same source. Moreover, the similarity of the genetic properties of R plasmids between human source and animal source has been reported. Transfer of distinguishable *E. coli* strains with particular R plasmids from calves (Hirsh & Wiger, 1977) and chickens (Levy *et al.* 1976) to humans was demonstrated experimentally. Threlfall *et al.* (1978*a*) isolated *S. typhimurium* phage types 204 and 193 carrying particular R plasmids from calves and humans during epidemics of bovine salmonellosis. Davies & Stewart (1978) reported a close similarity between R plasmids in enteric bacteria from domestic pets and man. A close genetic relationship of R plasmids between salmonella and *E. coli* from pigs was found by Ishiguro *et al.* (1980*a*). These reports suggest the possibility of transfer of enterobacteria carrying R plasmids between animals and humans,

and of transfer of R plasmids among enterobacterial genera. Genetic characters of R plasmids seem to be an indispensable epidemiological marker.

Clonal distribution of *S. typhimurium* or *S. wien* of human origin carrying FI*me* plasmids coding for resistance to six drugs (ApCmKmSmSuTc) has been reported (Anderson *et al.* 1977; McConnell *et al.* 1979). Threlfall *et al* (1978*a*) and Rowe *et al.* (1979) reported that the recent prevalence of multiresistant strains of *S. typhimurium* phage types 204 and 193 among calves and humans in England and in European countries was caused by the transport of infected calves. In addition, Rowe *et al.* (1979) showed that *S. typhimurium* resistant to ApCmKmSmSuTc with or without trimethoprim(Tp) emerged by the progressive acquisition of R plasmids such as H2 and I_1 group and Tp plasmids. As described above, we frequently isolated H1 plasmids coding for resistance to six drugs in 1978 and 1979. In northern Japan, H1 R plasmids coding for single Tc-resistance have occurred since 1971 and H1 plasmids coding for resistance to 2–5 kinds of drugs and mercury emerged in 1977. Which of these H1 plasmids was the progenitor of the R plasmids coding for resistance to six drugs (ApCmKmSmSuTcHg) remains unsolved. However, there is no doubt that the widespread use of various drugs created a strong selection pressure among the drug-resistant salmonella and facilitated the emergence and spread of the H1 plasmids coding for resistance to six drugs.

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REFERENCES

ANDERSON, E. S. (1968). Drug resistance in *Salmonella typhimurium* and its implications. *British Medical Journal* **3**, 333–339.

ANDERSON, E. S., THRELFALL, E. J., CARR, J. M., MCCONNEL, M. M. & SMITH, H. R. (1977). Clonal distribution of resistance plasmid-carrying *Salmonella typhimurium*, mainly in the Middle East. *Journal of Hygiene* **79**, 425–448.

DATTA, N., HUGHES, V. M., NUGENT, M. E. & RICHARDS, H. (1979). Plasmids and transposons and their stability and mutability in bacteria isolated during an outbreak of hospital infection. *Plasmid* **2**, 182–196.

DAVIES, M. & STEWART, P. R. (1978). Transferable drug resistance in man and animals: Genetic relationship between R-plasmids in enteric bacteria from man and domestic pets. *Australian Veterinary Journal* 54, 507–512.

HIRSH, D. C. & WIGNER, N. (1977). Effect of tetracycline upon transfer of R plasmids from calves to human being. American Journal of Veterinary Research **38**, 1137–1139.

ISHIGURO, N., GOTO, J. & SATO, G. (1980*A*). Genetical relationship between R plasmids derived from *Salmonella* and *Escherichia coli* obtained from a pig farm, and its epidemiological significance. *Journal of Hygiene* **84**, 365–379.

ISHIGURO, N., MAKINO, S., SATO, G. & HASHIMOTO, K. (1980b). Antiobiotic resistance and genetic properties of R plasmids in *Salmonella* isolates of swine origin in Japan. American Journal of Veterinary Research **41**, 46–50.

ISHIGURO, N., OKA, C., HANZAWA, Y. & SATO, G. (1979). Plasmids in *Escherichia coli* controlling citrate-utilizing ability. *Applied and Environmental Microbiology* **38**, 956–964.

ISHIGURO, N., SATO, G., ICHIJO, S. & KONISHI, T. (1980*c*). Incompatibility of R plasmids derived from *Salmonella* and *Escherichia coli* isolated simultaneously from a bovine fecal sample. *American Journal of Veterinary Research* **41**, 1982–1986.

JACOB, A. E., SHAPIRO, J. A., YAMAMOTO, L., SMITH, D. I., COHEN, S. N. & BERG, D. (1977). Plasmids studies in *Escherichia coli* and other enteric bacteria. In *DNA Insertion Elements, Plasmids, and Episomes* (ed. A. I. Bukhari, J. A. Shapiro and S. L. Adhya). *Cold Spring Harbor Laboratory, USA.*, p. 607.

KASHIWAZAKI, M., NAMIOKA, S., HECHELMANN, H. & LEISTNER, L. (1974). Incidence of R factors among *Salmonella* strains isolated from animals in Japan. *National Institute of Animal Health Quarterly* **14**, 29–30.

LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190–206.

LEVY, S. B., FITZGERALD, G. B. & MACONE, A. B. (1976). Spread of antibiotic resistance plasmids from chicken to chicken and from chicken to man. *Nature, London* **260**, 40–24.

MCCONNELL, M. M., SMITH, H. R., LRONARDOPOULOS, J. & ANDERSON, E. S. (1979). The value of plasmid studies in the epidemiology of infections due to drug-resistant *Salmonella wien*. *Journal of Infectious Diseases* **139**, 178–190.

NAKAHARA, H., ISHIKAWA, Y., SARAI, Y., KONDO, I. & KOZUKUE, H. (1977). Mercury resistance and R plasmids in clinical isolates of *Klebsiella pneumoniae*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, Abteilung I: Orig. A **238**, 51–58.

ROWE, B., THRELFALL, E. J., WARD, L. R. & ASHELY, A. S. (1979). International spread of multiresistant strains of *Salmonella typhimurium* phage types 204 and 193 from Britain to Europe. *Veterinary Record* **105**, 468–469.

SATO, G. & TERAKADO, N. (1977). R factor types found in *Salmonella typhimurium* and *Escherichia coli* isolated from calves in a confined environment. *American Journal of Veterinary Research* **38**, 743–747.

SMITH, H. R., GRINDLEY, N. D. F., HUMPHREY, G. O. & ANDERSON, E. S. (1973). Interactions of group H resistance factors with the F factor. *Journal of Bacteriology* **115**, 623–628.

SOJKA, W., WRAY, D. & HUDSON, E. B. (1977). A survey of drug resistance in salmonellae isolated from animals in England and Wales during 1973 and 1974. *British Veterinary Journal* **133**, 292–311.

STEPHAN, R., BULLING, E. & STEINBECK, A. (1977). Die entwicklung der Antibiotikaresistanz von der Bundesrepublik Deutschland einschließlich Berlin (West). *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, Abteilung I: Orig. A **237**, 264–273.

SUMMERS, A. O. & JACOBY, G. A. (1977). Plasmid-determined resistance to tellurium compounds. *Journal of Bacteriology* **129**, 276–281.

TAKAHASHI, I. (1976). Drug resistance of *Salmonella* isolated from domestic animal and fowl in Japan (in Japanese). *Modern Media*, Tokyo **22**, 248–259.

TAYLOR, D. E. & GRANT, R. B. (1976). Inhibition of bacteriophage Lambda, T1 and T7 development by R plasmids of the H incompatibility group. *Antimicrobial Agents and Chemotherapy* **10**, 762–764.

TERAKADO, N., OHYA, T., UEDA, H. & ISAYAMA, Y. (1980). A survey of drug resistance and R plasmids in *Salmonella* isolated from domestic animals in Japan. *Japanese Journal of Veterinary Science* **42**, 543–550.

TERAKADO, N. & SATO, G. (1978). Detection of an R plasmid of incompatibility group H in *Salmonella typhimurium* of bovine origin. *National Institute of Animal Health Quarterly* **18**, 180–181.

THRELFALL, E. J., WARD, L. R. & ROWE, B. (1978*a*). Spread of multiresistant strains of *Salmonella typhimurium* phage types 204 and 193 in Britain. *British Medical Journal* **2**, 997.

THRELFALL, E. J., WARD, L. R. & ROWE, B. (1978*b*). Epidemic spread of a chloramphenicol-resistant strain of *Salmonella typhimurium* phage type 204 in bovine animals in Britain. *Veterinary Record* **103**, 438–440.

TIMONY, J. F. (1978). The epidemiology and genetics of antibiotic resistance of *Salmonella typhimurium* isolated from diseased animals in New York. *Journal of Infectious Diseases* **137**, 67–73.