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Genetical relationship between R plasmids derived from *Salmonella* and *Escherichia coli* obtained from a pig farm, and its epidemiological significance

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SUMMARY

A total of 475 *Salmonella* strains belonging to 5 serovars, isolated from a pig farm which had been heavily contaminated with *Salmonella* for the past 2 years were tested for antibiotic susceptibility and detection of R plasmids. Thirty-three *Escherichia coli* isolates from the same farm were also examined in a similar way.

Out of 475 strains 348 (73·2 %) were resistant to one or more antibiotics such as tetracycline (Tc), streptomycin (Sm), sulfadimethoxine (Su), chloramphenicol (Cm) and kanamycin (Km), and 247 (85·2 %) out of 290 strains belonging to 3 serovars examined harboured conjugative R plasmids. There was no change in the pattern of drug resistance during this survey nor any variation in the pattern of resistance of R plasmids, whatever the serovar. The antibiogram pattern Tc Sm Su, mainly *S. typhimurium*, was common among *Salmonella* strains. Among the transferred resistance patterns, the thermosensitive R plasmids conferring the Tc marker detected in this study were Fi^-, and belonged to incompatibility group H1, whereas the R plasmids conferring Sm Su resistances which coexisted in the same host were Fi^+, and compatible with the reference R plasmids tested. The Iα plasmid conferring Cm resistance alone was isolated from *S. anatum* and the FII plasmid conferring Sm Km resistances was also isolated from *S. typhimurium*. In contrast, the 33 *E. coli* strains examined were resistant to three or five antibiotics and most of the resistance markers were located on conjugative R plasmids. Iα plasmids conferring Cm resistance alone or FII plasmids conferring Cm or Km markers were common in the *E. coli* strains. H1 and H2 plasmids conferring multiple resistance markers were also found in them. The genetic properties of R plasmids derived from *Salmonella* or *E. coli* strains are compared, and the potential spread of R plasmids between strains of *Salmonella* and *E. coli* is discussed.

INTRODUCTION

Salmonellosis is recognized to be a global problem for man, livestock and pets. In particular, the association between salmonellosis in man and the infection in food animals has been also investigated by many workers (Chau, Shortridge & Huang, 1977; Goto, 1976; Watson, 1975). Poultry and pork have been considered

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to be the most important sources of salmonellosis for man (Wilcock & Olander, 1978).

During the past years, the widespread use of various antimicrobial agents in the feed has been the commonly recommended procedure for treatment and control of porcine salmonellosis. On the other hand, the use of antibiotics as feed additives in domestic animals has increased the resistance of *Salmonella* and coliforms to these drugs (Timoney, 1978; Smith & Tucker, 1975). In fact, the *Salmonella* strains obtained from domestic animals in Japan were resistant to antibiotics including chloramphenicol, and they harboured conjugative R plasmids (Sato *et al.* 1977; Sato & Terakado, 1977). Recently the R plasmids detected from different sources have been characterized and classified by various methods such as the fertility inhibition (Fi) character, phage inhibition and incompatibility tests for epidemiological interpretations (Anderson & Threlfall, 1974).

In the previous paper (Ishiguro *et al.* 1979), we investigated the distribution and mode of spread of *Salmonella* on a conventional pig farm by differentiation of serovars of *Salmonella* strains, biotyping of *S. typhimurium*, and their drug susceptibility. It was demonstrated that the differentiation of serovars of *Salmonella* strains and the method of biotyping of *S. typhimurium* were useful in investigating the sources and perpetuation of *Salmonella* infection on the farm, whereas the antibiogram of *Salmonella* strains was not useful as a marker in the epizootiological study. Since antibiotic resistance patterns are of limited value in the identification of R plasmids, it is necessary to assay the genetic properties of R plasmids derived from *Salmonella* strains from an epidemiological viewpoint. There has been no report involving detection of R plasmids from *Salmonella* strains obtained from a pig farm for long term or genetical relationship between R plasmids derived from *Salmonella* and *Escherichia coli* obtained from the same farm.

This paper deals with a longitudinal study of detection of R plasmids from *Salmonella* strains and genetic properties of R plasmids derived from *Salmonella* or *E. coli* on a pig farm.

MATERIALS AND METHODS

The farming status of the K farm investigated

Farm K was an intensive pig breeding–fattening unit and comprised several subunits, such as farrowing house, weanling house, fattening house HI and HII, sow house, boar house, isolation house, sow house yard, fattening-house-II yard, equipment stores and office. Since the first outbreak of clinical salmonellosis occurred in December 1972, the survey of *Salmonella* isolation from various specimens on K farm was carried out for two years. The details of epidemiological studies of *Salmonella* infection on K farm were reported in the previous paper (Ishiguro *et al.* 1979).

Salmonella *strains examined*

A total of 475 *Salmonella* strains of 5 different serovars obtained from K farm during 1972–4 were tested in this study. These strains were isolated from composite fecal samples, rectal fecal samples of market pigs, environmental swab samples, sewage samples, manure samples and feeding stuffs during the survey. The details of the isolation procedure were reported in the previous paper (Ishiguro *et al.* 1979).

E. coli *strains examined*

The 33 strains of *E. coli* were isolated from composite fecal samples collected from the floor of 33 pens of a farrowing house in June 1973. They were obtained by direct cultivation, using MacConkey agar (Eiken) plates, and identified as *E. coli* by 34 biochemical tests (Ishiguro, Oka & Sato, 1978).

Bacterial strains, plasmids and phages used for genetical experiments

E. coli K-12 derivatives used in this study for genetical experiments of R plasmids are shown in Table 1. The reference R plasmids used are also given in Table 1. The RST10-1 (incompatibility group H1) derived from *E. coli* OH3052 (being designated as KE10 previously) was obtained from nitrosoguanidine treatment (Terakado & Sato, 1978*a*). The male specific phages used in this study were f1 and f2, and phages λ, T4 and T7 were also used for the phage inhibition tests of R plasmids.

Media

Heart infusion agar (Eiken) was used for antibiotic susceptibility tests, except in the test with sulphadimethoxine (Su), in which Mueller–Hinton agar (Eiken) was used. The nutrient broth used for conjugative experiments was penassay broth (Difco). Deoxycholate-hydrogen sulphide-lactose agar (DHL; Eiken) was used as basal medium of selective plates for ampicillin (Ap), chloramphenicol (Cm), kanamycin (Km) and streptomycin (Sm); heart infusion agar for Tc; and Mueller–Hinton agar for Su. To the heart infusion or Mueller–Hinton agar were added 4 ml of a 0·2% BTB solution and 1·5 g of lactose per 100 ml. L broth (LB; Lennox, 1955), LB agar and soft agar were used for growth and titration of phages. In this study, $CaCl_2$ was added to LB or LB agar at a final concentration of 0·0025 M.

Antibiotic susceptibility tests and detection of conjugative R plasmids

Antibiotic susceptibility testing of *Salmonella* or *E. coli* strains was routinely carried out by the agar dilution method, using 12 antibiotics at the following final concentrations (μg/ml): Ap, 25; Cm, 25; Km, 25; Sm, 12·5; Tc, 25; cephaloridine, 25; gentamicin, 12·5; colistin, 12·5; furazolidon 6·3; nalidixic acid (Nal), 25; rifampin, 25; and Su, 800. A strain was recorded as resistant if its growth was not inhibited by these concentrations of drugs.

R plasmids were detected by the procedures described by Ishiguro *et al.* (1978). *E. coli* ML1410 was used as a recipient. Each of the strains was cultivated in

Table 1. *Bacteria and plasmids employed*

Bacterial strain or plasmid	Relevant genetic* markers	Reference
E. coli K-12		
ML 1410	F⁻, *met*, *nal*	Ishiguro, Oka & Sato, 1978
SG1	Rifampin-resistant mutant of Hfr *E. coli* W1895	Terakado & Sato, 1978*a*
SG3	Rifampin-resistant mutant of *E. coli* 921 (*met*, *thr*, *thi*, *leu*, *lac*, r_k^- m_k^-)	
Plasmid		
RA1 (A)†	TC SM	Taylor & Grant, 1977
R40a (C)	Su Km Ap	Datta, N., 1977
R386 (FI)	Tc	Datta, N., 1977
R100 (FII)	Tc Sm Su Cm	Datta, N., 1977
R124 (FIV)	Tc	Taylor & Grant, 1977
R144 (Iα)	Km	Datta, N., 1977
R391 (J)	Km	Datta, N., 1977
R387 (K)	Sm Cm	Datta, N., 1977
RN3 (N)	Tc Sm Su	Datta, N., 1977
RP4 (P)	Tc Km Ap	Datta, N., 1977
RS-a (W)	Sm Su Cm Km	Datta, M., 1977
R27 (H1)	Tc	Datta, N., 1977
RST10-1 (H1)	Sm Su Cm Km	Terakado & Sato, 1978*a*
R478 (H2)	Tc Cm Km	Taylor & Grant, 1977
R446-b (M)	Tc Sm	Datta, N., 1977
R14 (O)	Tc Sm Su Ap	Datta, N., 1977
Rts1 (T)	Km	Datta, N., 1977
R6k (X)	Ap	Taylor & Grant, 1977
R471a (L)	Ap	Datta, N., 1977

* Drug resistance symbols: Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulfadimethoxine; Nal, nalidixic acid.

† Incompatibility group.

penassay broth at 25 °C for 18 h. *E. coli* ML1410 was cultured in a similar way. Two millilitres of broth were inoculated with 0·2 ml of each donor broth culture and an equal amount of recipient culture. The mixture was incubated at 25 or 37 °C for 18 h. A loopful of each mixed culture was subcultured onto a selective agar plate containing Nal (50 μg/ml) and one of the above drugs to which the strain tested was resistant. The selective media were incubated at 37 °C for 24 h. To examine transconjugant recipients and their resistance patterns, five colonies of transconjugants on each selective media were purified on the same selective medium and tested for resistance to the antibiotics applied.

To further study the transfer of R plasmids to *E. coli* K-12, the quantitative transfer experiments were also performed by the standard method. Donor and recipient were cultured for 4 h at 25 °C with shaking. Then 0·1 ml of donor and 1 ml of recipient were mixed in 4·5 ml of fresh penassay broth, and incubated for 2 h at 25 °C or at 37 °C with gentle shaking. Then 0·1 ml of appropriate dilutions in saline were plated on selective agar plates. The 20 transconjugants thus obtained

were purified on the same selective plate, and tested for drug resistance by the method described above.

Fertility inhibition (Fi) tests and phage inhibition tests

The Fi character of R plasmids derived from *Salmonella* or *E. coli* strains were examined by the surface spot method, using the male specific phages f1 and f2. The R plasmids to be tested were transmitted to *E. coli* SG1 from transconjugant *E. coli* ML1410 carrying the R plasmid by conjugative experiments. An overnight L broth culture of SG1 (R^+) was streaked on the LB agar plates, and f1 or f2 phage lysate was spotted on the lawn. If a lytic zone developed, the Fi character of R plasmid was regarded as the Fi^-, otherwise as the Fi^+ type.

Phage inhibition tests were done as described by Taylor & Grant (1977). The R plasmids to be tested were transmitted to *E. coli* SG3 from ML1410 carrying the R plasmid. The R plasmids were then tested for ability to reduce both the number of plaques, and the plaque size of phages λ, T4 and T7, using *E. coli* SG3 as an indicator strain.

Incompatibility tests

The compatibility of R plasmids derived from *Salmonella* or *E. coli* strains was examined by the method of Datta (1977). A list of the reference R plasmids used in this study is given in Table 1. *E. coli* ML1410 was used as donor strain and *E. coli* SG3 was used as a recipient. Transfer frequencies were determined from 2 h mating at 25 or 37 °C, measured as the number of transconjugants per donor. In each mating, the 20 transconjugant clones obtained on selective plates were picked and purified by successive single-colony isolations on the same selective plate, and then the purified clones were tested for the presence of incoming and resident plasmids. If both resistance markers were present and stable in the transconjugants and were separately transferable to another strain of *E. coli* K-12, both plasmids were recorded as compatible; that is the plasmid under test was classified into an untypable group.

RESULTS

Antibiotic susceptibility and detection of conjugative R plasmids in Salmonella *strains*

The results of antibiotic susceptibility tests of 475 *Salmonella* strains examined are shown in Table 2. It is seen that 306 (92·2%) out of 332 strains of *S. typhimurium*, 33 (37·5%) out of 88 strains of *S. anatum* and 9 (19·5%) out of 46 strains of *S. senftenberg* were resistant to drugs such as Tc, Sm, Su, Cm and Km, whereas the 6 strains of *S. livingstone* and 2 of *S. infantis* were susceptible to the drugs tested. All *Salmonella* strains examined were susceptible to Ap, cephaloridine, colistin, furazolidon, gentamicin, Nal and rifampin. Of the 348 resistant *Salmonella* strains only 2 of *S. anatum* were resistant to Cm. The most predominant resistance patterns in the resistant strains were Tc Sm Su and Tc alone. Transferred drug resistance from the resistant *Salmonella* strains to *E. coli* ML1410 is also shown in Table 2. Of 290 resistant *Salmonella* strains examined for R plasmid, 247 (85·2%)

Table 2. *Drug resistance patterns and transferred drug resistance in* Salmonella *strains examined on K farm*

Serovar	No. of strains examined	Drug resistance: Resistance pattern	Drug resistance: No. of strains	No. of strains examined for R plasmid	Transferred drug resistance: Resistance pattern	Transferred drug resistance: No. of R^+ strains (ts)*
S. typhimurium	332	Tc Sm Su Km	1	254	Tc Sm Km	1 (1)
		Tc Sm Su	185		Tc Sm Su	152 (152)
		Tc Sm	17		Tc Sm	2 (2)
		Sm Su	24		Sm Su	21
		Tc	76		Tc	60 (60)
		Sm	3			
S. anatum	88	Tc Sm Su	1	32	Tc Sm Su	1 (1)
		Tc Sm	3		Tc Sm	1 (1)
		Tc Cm	2		Tc Cm	2 (2)
		Tc	5		Tc	3 (3)
		Sm	22			
S. senftenberg	46	Tc Sm Su	1	4	Tc Sm Su	1 (1)
		Tc Sm	1		Tc	3 (3)
		Tc	4			
		Sm	3			
S. livingstone	6					
S. infantis	2					
Total	475		348 (73·2%)	290		247 (85·2%)

* (ts), R plasmids showing thermosensitive transfer.

Table 3. *Drug resistance pattern and transferred drug resistance in* 33 E. coli *strains examined on K farm*

Drug resistance		Transferred drug resistance	
Resistance pattern	No. of strains	Resistance pattern	No. of R^+ strains (ts)*
Tc Sm Su Cm Km Ap	2	TC Su Cm Km	1 (1)
		Tc Cm Km Ap	1
Tc Sm Su Cm Km	7	Tc Sm Su Cm Km	6 (6)
		Tc Sm Cm Km	1
Tc Su Cm Km Ap	1	Tc Cm Km Ap	1
Tc Sm Su Cm Ft	4	Tc Sm Su Cm	3 (3)
		Sm Su	1
Tc Sm Su Cm	4	Tc Sm Su Cm	3 (2)
Sm Su Cm Km Ap	1	Sm Su Cm Ap	1
Tc Sm Su Km Ft	2	Sm Su Km	2
Tc Sm Su Km	1	—	
Tc Sm Su Ft	1	Sm Su	1
Tc Sm Km Ft	3	Tc Sm Km	1
Cm Km Ap Ft	1	Cm Ap	1
Tc Su Cm	1	Tc Su Cm	1 (1)
Su Cm Ft	1	Cm	1
Tc Sm Su	1	—	
Tc Su	1	—	
Km	1	—	
(S)	1		

* (ts), R plasmids showing thermosensitive transfer.
—, Resistance not transferred.
(S), Susceptibility to antibiotics used in this study.

harboured conjugative R plasmids. Among the 3 serovars, 236 (92·9%) out of 254 resistant strains of *S. typhimurium* had conjugative R plasmids. All conjugative R plasmids conferring Tc resistance detected in this study showed thermosensitive transfer, and their genetic characters will be discussed later in this paper.

Antibiotic susceptibility and detection of conjugative R plasmids in 33 E. coli *strains*

Antibiotic susceptibility and transferred drug resistance in 33 *E. coli* strains examined are shown in Table 3. All *E. coli* strains but one were resistant to one or more drugs such as Tc, Sm, Su, Cm, Km, Ap and Ft, and they exhibited multiple resistance. None of the *E. coli* strains were resistant to cephaloridine, colistin, gentamicin, Nal and rifampin. The *E. coli* strains tended to have a greater multiple resistance than *Salmonella* strains, as shown in Tables 2 and 3. Of the multiple resistant *E. coli* strains, most had conjugative R plasmids and most resistance markers of resistant *E. coli* strains were also transmitted to *E. coli* ML1410 (Table 3). Moreover, 13 (52 %) of 25 R^+ strains carried thermosensitive R plasmids.

Change of resistance patterns or transferred resistance patterns in Salmonella *isolated during* 2 *years*

Table 4 shows a summary of drug resistance patterns and transferred resistance

Table 4. *Summary of drug resistance patterns of* Salmonella *strains from fattening house I and their transferred resistance pattern*

	Month of sampling (1973–1974)																		
Resistance pattern	1	2	3	4	5	6	7	8	9	10	12	1	2	3	5	6	8	12	Total
Tc Sm Su Km	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Tc Sm Su	3	3	—	3	1	1	3	8	8	13	5	4	2	2	2	6	1	—	65
Tc Sm	1	2 1*	2	4	—	—	—	—	1	—	—	—	—	—	—	—	—	—	11
Tc Cm	—	—	—	—	—	—	—	—	—	1†	—	—	—	—	—	—	—	—	1
Sm Su	—	1	—	2	2	—	—	—	—	1	2	—	—	1	1	—	—	1	11
Tc	3	3	—	1	1	—	—	—	1	3	4	—	2	—	1	2	—	—	21
Sm	—	—	—	—	—	—	—	—	2†	1†	—	—	—	—	—	—	—	—	3
(S)	—	—	—	2	1	1	—	—	1†	—	—	1*	—	—	—	1	—	—	7
No. of strains tested	7	10	2	12	5	2	3	8	13	19	12	5	4	3	4	9	1	1	120
Transferred resistance pattern																			
Tc Sm Km	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Tc Sm Su	2	3	—	2	1	—	2	6	3	9	5	2	2	2	2	6	1	—	48
Sm Su	—	1	—	1	2	—	—	—	1	—	—	—	—	1	1	—	—	1	8
Tc Cm	—	—	—	—	—	—	—	—	—	1†	—	—	—	—	—	—	—	—	1
Tc	3	3	1	5	2	1	—	—	—	1	2	1	2	—	1	1	—	—	23
Sm	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	2
(R⁻)	—	—	—	—	—	—	—	—	2†	1†	—	—	—	—	—	—	—	—	3
No. of strains tested	5	7	2	8	5	1	2	6	6	12	8	4	4	3	4	7	1	1	86

* No. of *S. senftenberg* strains. † No. of *S. anatum* strains.

patterns in *Salmonella* from fattening house I during 2 years. Fattening house I was the most persistently and heavily contaminated with *Salmonella* among the subunits on K farm. No change of resistance patterns of *Salmonella* strains was found during this survey, the most common was Tc Sm Su in the 120 *Salmonella* strains tested. One strain of *S. typhimurium* showing Tc Sm Su Km was encountered in those obtained in December 1973.

The transferred drug resistance patterns indicated in Table 4 were, in general, similar to those encountered in Table 2. One distinct and interesting difference was that one strain of *S. anatum* carrying conjugative Cm resistance plasmid was isolated in October 1973. Except for the isolation of *S. anatum*, there was no pattern of temporal variation in transferred resistance pattern of *Salmonella* strains, regardless of date of isolation.

Frequency of transfer of drug resistance from Salmonella *strains to* E. coli *K*-12

The results of quantitative transfer of drug resistance from *Salmonella* strains to *E. coli* K-12 are shown in Table 5. These 3 representative *Salmonella* strains were resistant to Tc, Sm and Su, in which the Tc resistance marker was more efficiently transferred to *E. coli* ML1410 at 25 °C than at 37 °C, and all transconjugants selected on Tc selective media were resistant to Tc alone. In contrast to transfer of Tc marker, Sm and Su resistance markers were transmitted to *E. coli* ML1410 with a low frequency at 25 or 37 °C, and the transconjugants selected for either Sm or Su selective media harboured resistance to both Sm and Su. These results indicate that the *Salmonella* strains carrying Tc, Sm and Su resistance patterns detected in this study harbour both a thermosensitive R plasmid conferring Tc resistance and the other R plasmid conferring Sm and Su resistances. Each plasmid derived from *Salmonella* strains was serially numbered in designation of pOH, and their genetic properties were studied in more detail.

Genetic properties of R plasmids derived from Salmonella *strains*

Since the transferred resistance patterns of *Salmonella* strains were unique in *Salmonella* strains detected in this survey, the R plasmids derived from 8 representative strains were used for genetical studies, and the results are shown in Table 6. All the thermosensitive R plasmids conferring Tc resistance showed the Fi^- character, and were incompatible with the known H1 plasmid (RST10–1), regardless of serovar. Since these thermosensitive R plasmids did not give a reduced titre and plaque size when compared with the SG3 (R^-) strain when phage λ, T4 and T7 were plated, they were classified into incompatibility group H1. Except that the pOH625-2 carrying Sm resistance alone were Fi^-, the R plasmids conferring Sm and Su resistances were Fi^+ and compatible with each reference R plasmid used in this study, indicating that they belong to an untypable group.

The pOH806-2 carrying Cm resistance derived from *S. anatum* was Fi^+ and incompatible with R144 (Iα), indicating that it belongs to incompatibility group Iα. Moreover, the pOH883-2 carrying Sm and Km resistances were Fi^+ and classified into incompatibility group FII. None of the R plasmids detected from *Salmonella* strains inhibit development of phage λ, T4 and T7 tested.

Table 5. *Frequency of resistance of transfer from* Salmonella *strains to* E. coli *ML1410 at 25 °C and 37 °C*

Strain designation	Source	Drug resistance pattern	Selective drug	At 25 °C		At 37 °C	
				Transfer frequency	Character of trans-conjugants	Transfer frequency	Character of trans-conjugants
S. typhimurium OH622	Fattening house I	Tc Sm Su	Tc	$8{\cdot}9 \times 10^{-3}$	Tc	$1{\cdot}3 \times 10^{-7}$	Tc
			Sm	$< 10^{-7}$	—	$7{\cdot}7 \times 10^{-7}$	Sm Su
			Su	$< 10^{-7}$	—	$2{\cdot}9 \times 10^{-6}$	Sm Su
S. typhimurium OH656	Fattening house II	Tc Sm Su	Tc	$6{\cdot}2 \times 10^{-3}$	Tc	$1{\cdot}7 \times 10^{-7}$	Tc
			Sm	$< 10^{-7}$	—	$2{\cdot}3 \times 10^{-6}$	Sm Su
			Su	$< 10^{-7}$	—	$3{\cdot}3 \times 10^{-6}$	Sm Su
S. typhimurium OH1023	Manure	Tc Sm Su	Tc	$6{\cdot}3 \times 10^{-3}$	Tc	$5{\cdot}0 \times 10^{-7}$	Tc
			Sm	$< 10^{-7}$	—	$9{\cdot}6 \times 10^{-7}$	Sm Su
			Su	$5{\cdot}6 \times 10^{-7}$	Sm Su	$2{\cdot}0 \times 10^{-5}$	Sm Su

Table 6. *Genetic properties of thermosensitive R plasmids (Tc marker) and the other R plasmids derived from* Salmonella *strains*

Strain designation	Source	Date of isolation	Plasmid designation	Drug resistance of R plasmid	Fi	Relative efficiency of plating of phage (R^+/R^-)			Incompatibility group
						λ	T4	T7	
S. t OH622	Fattening	25 Apr. 1973	pOH622–1	Tc	–	1	1	1	H1
(Tc Sm Su)	house I		–2	Sm Su	+	1	1	1	UT*
S. t OH625	Fattening	25 Apr. 1973	pOH625–1	Tc	–	1	1	1	H1
(Tc Sm)	house I		–2	Sm	–	1	NT†	NT	UT
S. t OH694	Fattening	31 July 1973	pOH694–1	Tc	–	1	1	1	H1
(Tc Sm Su)	house II		–2	Sm Su	+	1	NT	NT	UT
S. t OH703	Fattening	12 Aug. 1973	pOH703–1	Tc	–	1	1	1	H1
(Tc Sm Su)	house II yard		–2	Sm Su	+	1	NT	NT	UT
S. a OH806	Fattening	14 Oct. 1973	pOH806–1	Tc	–	1	1	1	H1
(Tc Cm)	house I		–2	Cm	+	1	1	1	Iα
S. t OH883	Fattening	5 Dec. 1973	pOH883–1	Tc	–	1	1	1	H1
(Tc Sm Su Km)	house I		–2	Sm Km	+	1	1	1	FII
S. s OH954	Manure	30 Jan. 1974	pOH954–1	Tc	–	1	1	1	H1
(Tc Sm Su)			–2	Sm Su	+	1	1	1	UT
S. t OH1019	Fattening	13 June, 1974	pOH1019–1	Tc	–	1	1	1	H1
(Tc Sm Su)	house I		–2	Sm Su	+	1	NT	NT	UT

S. t, *S. typhimurium*; S. a, *S. anatum*; S. s, *S. senftenberg*.
* UT, Untypable. † NT, Not tested.

Table 7. *Genetic properties of R plasmids derived from* E. coli *strains*

Strain designation	Drug resistance pattern	Plasmid designation	Resistance pattern of R plasmid	Fi	Relative efficiency of plating of phage (R^+/R^-) λ	T4	T7	Incompatibility group
OH3044	Tc Sm Su Cm	pOH3044–1	Tc Sm Su Cm	−	0·07	1	0·4*	H2
		–2	Cm	+	1	1	1	Iα
OH3046	Cm Su Ft	pOH3046	Cm	+	1	1	1	Iα
OH3047	Tc Su Cm Km Ap	pOH3047–1	Tc	−	NT†	NT	NT	NT
		–2	Cm Km Ap	+	1	1	1	FII
OH3049	Tc Sm Cm Km Ap	pOH3049–1	Tc Sm	+	1	1	1	Iα
		–2	Cm Ap	+	1	NT	NT	FII
OH3052	Tc Sm Su Cm Km	RST10–1	Sm Su Cm Km	−	1	1	1	H1
OH3053	Tc Sm Su Cm Km	pOH3053–1	Tc Sm Su Cm Km	−	1	NT	NT	NT
		–2	Cm	+	1	1	1	Iα
OH3063	Tc Sm Su Cm Km	pOH3063–1	Tc Sm Su Cm Km	+	1	NT	NT	NT
		–2	Cm	+	1	1	1	Iα
OH3064	Tc Sm Su Ft	pOH3064	Sm Su	+	1	NT	NT	UT‡
OH3073	Tc Sm Km Ft	pOH3073	Tc Sm Km	+	1	1	1	FII

* Phage T7 plaques were reduced 1–2 mm on the inhibiting R^+ strain.
† NT, Not tested. ‡ UT, Untypable.

Genetic properties of R plasmids derived from E. coli *strains*

The R plasmids detected from *E. coli* strains were serially numbered in designation of pOH as well as R plasmids derived from *Salmonella* strains, and they were tested for genetic properties. Genetic properties of the 14 R plasmids derived from 9 *E. coli* strains are shown in Table 7. The resistance patterns of R plasmids isolated from *E. coli* was variable, compared with those observed in *Salmonella*. In all, the R plasmids conferring Cm resistance alone were detected in 4 (44%) out of 9 *E. coli* strains tested. It should be noted that they were Fi^+ and classified into incompatibility group Iα. The 3 plasmids (pOH3047-2, pOH3049-2 and pOH3073) showing fertility inhibition and belonging to incompatibility group FII were found among *E. coli* strains tested in this study. It is of interest that H1 (RST10-1) and H2 (pOH3044-1) plasmids were isolated from the samples of the confined pig farm. It was demonstrated that incompatibility between the pOH-3044-1 and R478 was stronger than that between the pOH3044-1 and RST10-1 (data not shown). Also, the pOH3044-1 gave a reduced titre and plaque size, when phages λ and T7 were plated (Table 7). None of the R plasmids except pOH3044-1 reduced the plating efficiency of the phages tested.

DISCUSSION

In the present study, *S. typhimurium* was the most predominant resistant serovar among 5 serovars, and most of the resistance markers of resistant *S. typhimurium* strains were controlled by conjugative R plasmids. Of transferred resistance markers, thermosensitive H1 plasmids conferring Tc resistance were constantly detected from this farm during the survey. Since tetracycline was commonly used as a feed additive for promotion of growth before the legislative controls of the use of antimicrobial drugs in animals feeds became effective in January 1977 in Japan, it seems probable that Tc resistance of enteric organisms may have been due to the use of tetracyclines. It is well known that thermosensitive R plasmids are common in animal strains of *S. typhimurium* (Timoney, 1978; Terakado & Sato, 1978*b*; Ishiguro *et al.* unpublished data). Though the genetic or ecological significance of the thermosensitive R plasmids is not well understood, it seems that a thermosensitive transfer of R plasmid facilitates transmission of R plasmids outside the body by such means as sewage or river water (Anderson, 1975). Our observations also indicate that thermosensitive R plasmids are widely distributed in *Salmonella* strains from pigs in Japan.

Smith *et al.* (1973) reported that the H1 plasmids of thermosensitive H plasmids were incompatible with F plasmids in the autonomous state, whereas H2 plasmids are compatible with F. It was also demonstrated that inhibition of development of λ, T4 and T7 by R plasmids is characteristic and unique to the H2 subgroup of R plasmid (Taylor & Grant, 1977). The thermosensitive Tc plasmids isolated from *Salmonella* strains in this study were incompatible with RST10-1 (H1 plasmid) derived from *E. coli* OH3052, and they have also not been shown to inhibit the development of double-stranded deoxyribounucleic acid phages of λ and T7.

Therefore, these Tc plasmids are responsible for incompatibility group H1. Though the Sm Su plasmids showing Fi^+ character detected in *Salmonella* strains could not be classified into the known incompatibility groups, they have been isolated from a variety of bacterial species from many parts of the world in both transmissible and nontransmissible forms and are common in enteric organisms (Barth & Grinter, 1974).

More interestingly, the extensive distribution of Cm plasmids of incompatibility group Iα was observed in *E. coli* strains, and the Cm plasmid belonging to incompatibility group Iα was isolated from *S. anatum*. The Iα plasmid conferring Cm resistance observed in *S. anatum* may have been transmitted from *E. coli* strains carrying Iα plasmids. There is further evidence involving the transfer of drug resistance between strains of *Salmonella* and *E. coli*. It should be noted that the FII plasmid isolated from *S. typhimurium* was very common in *E. coli* strains obtained from this farm. These findings suggest that the transmission of R plasmids between naturally occurring strains of *Salmonella* and *E. coli* occurred.

Another interesting observation made during this study was that the H1 (RST10-1) and H2 (pOH 3044-1) plasmids were isolated from *E. coli* strains obtained on this confined pig farm. Smith, Parsell & Green (1978) reported that the relative distribution of H1 and H2 plasmids may differ in different countries. However, although mercury resistance was not associated with R plasmids derived from *E. coli* strains examined (unpublished data), it is of interest that citrate-utilizing ability had been found in *E. coli* OH3052 and the citrate-utilizing determinant was always transmitted together with the resistance determinant to *E. coli* K-12 (Sato *et al.* 1978). Subsequently, the 12 (36 %) out of 33 *E. coli* strains used in this study were found to be citrate positive (Ishiguro *et al.* 1978). In contrast, the R plasmids derived from *Salmonella* strains examined failed to express citrate utilization in *E. coli* K-12.

Sato & Terakado (1977) reported that the identification of R plasmids of *S. typhimurium* on the basis of incompatibility tests was useful only as a subsidiary epidemiological marker in a feedlot and there were differences of genetic properties of R plasmids in both *Salmonella* and *E. coli* isolated from calves in the feedlot. Resistance patterns or transferred drug resistance patterns of R plasmids of *Salmonella* strains were not useful as epidemiological markers to assay the occurrences of different exotic infection sources on this pig farm. However, the present study suggests that the identification of R plasmids on the basis of their genetic properties would have value for investigation of the transmission of R plasmids between strains of *Salmonella* and *E. coli* from an epidemiological viewpoint.

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REFERENCES

ANDERSON, E. S. (1975). The problem and implications of chloramphenicol resistance in the typhoid bacillus. *Journal of Hygiene* **74**, 289.

ANDERSON, E. S. & THRELFALL, E. J. (1974). The characterization of plasmids in the enterobacteria. *Journal of Hygiene* **72**, 471.

BARTH, P. T. & GRINTER, N. J. (1974). Comparison of the deoxyribonucleic acid molecular weights and homologies of plasmids conferring linked resistance to streptomycin and sulfonamides. *Journal of Bacteriology* **120**, 618.

CHAU, P. Y., SHORTRIDGE, K. F. & HUANG, C. T. (1977). *Salmonella* in pig carcasses for human consumption in Hong Kong: a study on the mode of contamination. *Journal of Hygiene* **78**, 253.

DATTA, N. (1977). Classification of R plasmids. In *R factor Drug resistance plasmid* (ed. S. Mitsuhashi). *University of Tokyo Press*, 255.

GOTO, I. (1976). Ecological study on the Genus *Salmonella*. *The Bulletin of Azabu Veterinary College* **31**, 327.

ISHIGURO, N., OKA, C. & SATO, G. (1978). Isolation of citrate-positive variants of *Escherichia coli* from domestic pigeons, pigs, cattle, and horses. *Applied and Environmental Microbiology* **36**, 217.

ISHIGURO, N., SATO, G., TAKEUCHI, K. & NAKAYAMA, A. (1979). A longitudinal epizootiological study of *Salmonella* infection on a piggery: a study on the mode of contamination by biotyping of *Salmonella typhimurium* and by the antibiogram. *The Japanese Journal of Veterinary Science* **41**, 261.

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

SATO, G., ASAGI, M., OKA, C., ISHIGURO, N. & TERAKADO, N. (1978). Transmissible citrate-utilizing ability in *Escherichia coli* isolated from pigeons, pigs and cattle. *Microbiology and Immunology* **22**, 357.

SATO, G., ISHIGURO, N., ASAGI, M., OKA, C., KAWANISHI, T. & INOUE, T. (1977). Biochemical characteristics and *in-vitro* drug sensitivity of *Salmonella typhimurium*, Copenhagen variety, isolated from domestic and feral pigeons, crows, a kite, chickens and animals in Japan. *The Japanese Journal of Veterinary Science* **39**, 521.

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.

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

SMITH, H. W., PARSELL, Z. & GREEN, P. (1978). Thermosensitive antibiotic resistance plasmids in Enterobacteria. *Journal of General Microbiology* **109**, 37.

SMITH, H. W. & TUCKER, J. F. (1975). The effect of feeding diets containing permitted antibiotics on the faecal excretion of *Salmonella typhimurium* by experimentally infected chickens. *Journal of Hygiene* **75**, 293.

TAYLOR, D. E. & GRANT, R. B. (1977). Incompatibility and bacteriophage inhibition properties of N-1, a plasmid belonging to the H2 incompatibility group. *Molecular and General Genetics* **153**, 5.

TERAKADO, N. & SATO, G. (1978*a*). Demonstration of the so-called Mexican type R plasmids in *Escherichia coli* isolated from domestic animals and pigeons. *Microbiology and Immunology* **22**, 227.

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

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

WATSON, W. A. (1975). Salmonellosis and meat hygiene: red meat. *The Veterinary Record* **96**, 374.

WILCOCK, B. & OLANDER, H. (1978). Influence of oral antibiotic feeding on the duration and severity of clinical disease, growth performance, and pattern of shedding in swine inoculated with *Salmonella typhimurium*. *Journal of American Veterinary medical Association* **172**, 472.