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Drug Resistance and Distribution of R Factors in *Salmonella* Strains

TOKUMITSU TANAKA,* KENGO IKEMURA, MITSUKO TSUNODA, ITARU SASAGAWA, AND SUSUMU MITSUHASHI

Department of Microbiology, School of Medicine, Gunma University, Maebashi, and Public Health Laboratory, Niigata Prefecture, Niigata, Japan*

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Drug resistance and the distribution of R factors in *Salmonella* strains were surveyed using 1,980 strains isolated in Japan from 1955 to 1973. Resistances were mostly restricted to sulfanilamide (SA), tetracycline (TC), and streptomycin (SM), and combinations thereof. The demonstrated frequency of strains resistant to chloramphenicol (CM) was very low as compared with that in *Escherichia coli* and *Shigella* strains. In relation to resistance to TC, CM, SM and SA, the frequency of isolation of single resistance was the highest, followed by triple, double, and quadruple resistance in that order. Low frequency of isolation of quadruple resistance was due to the low frequency of CM resistance in *Salmonella* strains and differed from the *E. coli* or *Shigella* group. R factors with single TC resistance was most common, followed by those with TC,SM,SA; SM,SA; TC,CM,SM,SA; and single (SM and SA) resistance, in that order. Kanamycin and ampicillin resistance was unusual and mostly transmissible.

The incidence of infections caused by shigellae had been very high in Japan up until the past 10 years, and many studies, including those of drug resistance, were focused on *Shigella* strains (4, 7). In parallel with the decrease in bacillary dysentery, however, much attention has been focused on *Salmonella* infections, which have not decreased to any great extent in spite of improved sanitary conditions and the concomitant use of antibiotics for clinical treatment. We have collected many strains of gram-positive and -negative bacteria and have studied their drug resistance and the distributions of drug-resistant plasmids (4-7).

This paper deals with drug resistance in *Salmonella* strains isolated in Japan from 1955 to 1973.

MATERIALS AND METHODS

Bacterial strains. *Salmonella* strains were all isolated in Japan from 1955 to 1973. Strains were isolated from sporadic infections, and also one strain from each epidemic was sent to this laboratory. These strains were purified and serotyped, and their drug resistances were determined prior to storage in cooked meat medium and by freeze drying. Two substrains of *Escherichia coli* K-12, ML1410 (*met*$^-$ *nal*^{r} F$^-$)(*nal*^{r}, resistant to nalidixic acid[NA]) and W3630 (*mal*$^-$), were used as the recipients of R-factor transfer.

Media. Brain heart infusion broth (Difco) was used for liquid culture of bacterial strains and for conjugation experiments. For the determination of drug resistance, heart infusion agar (Eiken Chemical Co., Tokyo) and peptone water were used. Peptone water consisted of 10 g of peptone, 5 g of sodium chloride, and 1,000 ml of distilled water. For the assay of sulfanilamide (SA) resistance or the selection of SA-resistant transconjugants, semisynthetic medium consisting of 1,000 ml of medium A (1), 2 g of Casamino Acids (Difco), 10 mg of tryptophan, 1 mg of nicotinic acid, 10 mg of thiamine, and 2 g of glucose was used. Bromothymol blue (BTB) lactose agar, containing each selective drug and NA (25 μg/ml), was used as the selective medium for R$^+$ ML1410 transconjugants. AL agar medium consisted of 1,000 ml of medium A (1), 10 g of lactose, 40 ml of 0.2% bromothymol blue, and 14 g of agar. AL agar containing each of the selective drugs was used for R-factor transfer from NA-resistant *Salmonella* strains to W3630 or from ML1410 R$^+$ to W3630.

Drugs. Chloramphenicol (CM), tetracycline (TC), dihydrostreptomycin (SM), SA, kanamycin (KM), paromomycin, fradiomycin, ampicillin (APC), cephaloridine, NA, colistin methansulfonate, and gentamicin C complex were used.

Determination of drug resistance and conjugal transfer of R factor. Determination of drug resistance and conjugal transfer of R factor were as described previously (10). Drug resistance of each strain was determined on agar plates containing serial twofold dilutions of each drug, and the degree of resistance was expressed as the maximum concentration of drug that allowed the same degree of bacterial growth as on the control plates without the drug. The criteria for drug resistance was determined by the distribution of maximum concentration levels of each drug to 100 *Salmonella* strains

used in this study. The criteria are as follows (in micrograms per milliliter): TC, 12.5; CM, 12.5; SM, 25; SA, 25; KM; 3.1; paromomycin, 3.1; fradiomycin, 3.1; APC, 12.5; cephaloridine, 6.25; NA, 12.5; colistin methansulfonate, 0.8; gentamicin C complex, 0.8.

The concentration of drugs used in the selective medium in the R-factor transfer experiments were (in micrograms per milliliter): TC, 25; CM, 25; SM, 12.5; SA, 100; KM, 25; APC, 25.

RESULTS

Demonstrated frequency of drug resistance. Of 1,980 *Salmonella* strains examined, SA resistance was demonstrated most frequently, followed by SM, TC, CM, KM, NA, and APC resistance in that order. Colistin methansulfonate- or gentamicin C complex-resistant strains were not demonstrated during this survey (Table 1). Resistance patterns of *Salmonella* strains to TC, CM, SM, and SA are shown in Table 2. Singly resistant strains were demonstrated most frequently (68.6%), and triply, doubly, and quadruply resistant strains were demonstrated, respectively, at frequencies of 15.9, 12.6, and 2.8%. Among triply resistant strains, most were TC,SM,SA resistant. Of the doubly resistant strains, SM,SA- and TC,SA-resistant strains were demonstrated at frequencies of 5.3 and 4.1%, respectively. Among singly resistant strains, SA-resistant strains were demonstrated most frequently (97.3%); the demonstrated frequency of strains with single TC or SM resistance was very low. Singly CM-resistant strains were not demonstrated during this survey.

The frequency of R transfer was highest (88.6%) from quadruply resistant strains, followed by triply (73.6%), doubly (39.9%), and singly resistant ones (2.2%) (these figures are in relation to TC, CM, SM, and SA only) (Table 3). Of 427 R factors obtained, the frequency of R(TC) factor was the highest, followed by R(TC,SM,SA), R(SM,SA), R(TC,SM), and R(TC,CM,SM,SA) factors in that order (Table 4). R factors with CM resistance were very few in number when compared with those in *Shigella* strains (10, 11). Of 105 SM,SA-resistant strains, 12.4% carried R(SM,SA) factor, and 51 R(SM,SA) factors were included among the 427 R factors obtained. Among 81 TC,SA-resistant strains, 61 strains (75.3%) could transfer R(TC) by conjugation, but their SA resistance was all nonconjugative. However, the gene governing the SA resistance of 61 strains was shown to be located on nonconjugative plasmids by spontaneous or artificial elimination by treatment with ethidium bromide or elevated temperature. The data and molecular studies of nontransferable plasmids encoding SA resistance will be described elsewhere. The majority of multiply resistant strains were found to carry more than two types of R factors in a cell. Therefore, the number of R factors demonstrated was more than the number of donor strains tested.

Demonstrated frequencies of KM-, APC-, and NA-resistant strains, with reference to the multiplicity of resistance to TC, CM, SM, and SA, are shown in Table 5. The KM- and NA-resistant strains were mostly demonstrated from quadruply, triply, and doubly resistant ones in relation to resistance to the four drugs. The APC-resistant strains were demonstrated from triply and quadruply resistant ones. Of 51 KM-resistant and 20 APC-resistant strains, 69 and 70% transferred, respectively, KM or APC resistance (Table 6). These facts indicate that most KM and APC resistances were determined by R factors. It should be noted that R factors with NA resistance were not demonstrated.

The relationship between the demonstrated frequency of R factors and the serogroup in *Salmonella* strains is shown in Table 7. The demonstrated frequency of R factors from serogroup B was the highest and that from other serogroups was low.

TABLE 1. *Demonstrated frequency of drug-resistant Salmonella strains*[a]

Drug	No. of resistant strains (%)
TC	402 (20.3)
CM	53 (2.7)
SM	412 (20.8)
SA	1,531 (77.3)
KM	51 (2.6)
PRM	50 (2.5)
FRM	43 (2.2)
APC	20 (1.0)
CER	24 (1.2)
NA	23 (1.2)
CL	0
GM	0

[a] Number of strains examined was 1,980. PRM, Paromomycin; FRM, fradiomycin; CER, cephaloridine; CL, colistin methanesulfonate; GM, gentamicin C complex.

DISCUSSION

Of 1,980 *Salmonella* strains isolated from humans, SA resistance (77.3%) was demonstrated most frequently, and TC- and SM-resistant strains were isolated at a frequency of about 20%. Only 2.7% of *Salmonella* strains were CM resistant. In *E. coli* strains isolated in 1971, the demonstrated frequencies of SA, SM, TC, and CM resistance were 60.4, 43.5, 30.5, and 20.4%, respectively (9). Among 2,688 *Shigella*

TABLE 2. *Drug resistance patterns of Salmonella strains isolated from 1965 to 1973*

Drug resistance patterns	No. of strains isolated in:										Total (%)	Isolation frequency among resistant strains (%)
	1955–1961	1962–1965	1966	1967	1968	1969	1970	1971	1972	1973		
Quadruple												
TC,CM,SM,SA							1	9	29	5	44 (2.2)	2.8
Triple												
TC,SM,SA			3	1	2	9	13	81	77	59	245 (12.4)	15.6
CM,SM,SA								1	2		3 (0.2)	0.2 (15.9)
TC,CM,SA							1		1		2 (0.1)	0.1
Double												
SM,SA			3	6	1	9	7	47	21	11	105 (5.3)	6.7
TC,SA		4	6	1	1	3	2	27	11	26	81 (4.1)	5.2 (12.6)
TC,SM					1	1	1	4	1		8 (0.4)	0.5
CM,SA								4			4 (0.2)	0.3
Single												
SA	8	57	107	147	98	130	113	211	99	77	1,047 (52.9)	66.8
TC		1	1	1	1		1	5	2	10	22 (1.1)	1.4 (68.6)
SM			1	1		1	1		3		7 (0.4)	0.4
Susceptible	36	113	57	43	16	27	35	46	16	23	412 (20.8)	

TABLE 3. *Demonstrated frequency of R factors from drug-resistant Salmonella strains*

Bacterial strains with resistance to:	No. of strains tested	No. of R^+ strains (%)
Quadruple		
TC,CM,SM,SA	44	39 (88.6)
Triple		
TC,SM,SA	245	181 (73.9)
CM,SM,SA	3	2[a] (73.6)
TC,CM,SA	2	1
Double		
SM,SA	105	13 (12.4)
TC,SA	81	61 (75.3) (39.9)
TC,SM	8	5
CM,SA	4	0
Single		
SA	1,047	9 (0.9)
TC	22	15 (68.2) (2.2)
SM	7	0

[a] Percentage was not computed when the number of strains was less than 10.

TABLE 4. *Demonstration of R factors from Salmonella strains and their resistance patterns*[a]

Resistance patterns transferred	No. of R factors obtained (%)
TC,CM,SM,SA	39 (9.1)
TC,SM,SA	53 (12.4)
CM,SM,SA	2 (0.5)
TC,CM,SA	2 (0.5)
SM,SA	51 (11.9)
TC,SM	40 (9.4)
CM,SM	2 (0.5)
TC,CM	1 (0.2)
TC	215 (50.4)
SM	13 (3.0)
SA	9 (2.1)

[a] Number of resistant strains examined was 1,568.

TABLE 5. *Demonstrated frequency of KM-, APC-, and NA-resistant Salmonella strains with reference to resistance to TC, CM, SM, and SA*

Resistance patterns[a]	No. of strains tested	No. of strains resistant to (%):		
		KM	APC	NA
Quadruple	44	2 (4.5)	2 (4.5)	3 (6.8)
Triple	250	31 (12.4)	15 (6.0)	10 (4.0)
Double	198	14 (7.1)	2 (1.0)	7 (3.5)
Single	1,076	3 (0.3)	1 (0.1)	3 (0.3)
Susceptible	412	1 (0.2)		

[a] In relation to resistance to TC, CM, SM, and SA.

strains isolated from 1968 to 1970, the demonstrated frequencies of SA, SM, CM, and TC resistance were 93.0, 80.2, 78.1, and 71.2%, respectively (11). The demonstrated frequencies of strains resistant to the four drugs were highest in *Shigella* strains, followed by those in *E. coli* and *Salmonella* strains in that order. High frequencies of resistant *Shigella* strains may perhaps be explained by the fact that

TABLE 6. *Resistance patterns of R factors carrying KM or APC resistance*[a]

R factor carrying:	Resistance patterns	No. of R factor isolated
KM resistance	TC,SM,SA,APC,KM	6
	TC,SM,SA,KM	11
	TC,SM,KM	3
	SM,SA,APC,KM	2
	SM,APC,KM	4
	TC,KM	8
	KM	1
APC resistance	TC,SM,SA,KM,APC	6
	SM,SA,KM,APC	2
	CM,SM,SA,APC	2
	SM,KM,APC	4

[a] Results are based on surveys of 51 strains with KM resistance and 20 strains with APC resistance.

TABLE 7. *Demonstrated frequency of R factors from various serogroups in Salmonella strains*

Serogroup	No. of strains isolated (%)	No. of R^+ strains (%)
A	12 (0.6)	1 (8.3)
B	687 (34.7)	249 (36.2)
C	426 (21.5)	22 (5.2)
D	423 (21.4)	35 (8.3)
E	399 (20.2)	12 (3.0)
Others	33 (1.7)	7 (21.2)

Shigella infections are spread from human to human, especially from carriers who have been treated with drugs. By contrast, *Salmonella* infections may be mostly derived from food, especially meat from animal *Salmonella* carriers.

The demonstrated frequency of CM resistance in *Salmonella* strains was extremely low (2.7%) compared with that in *E. coli* (20.4%) and in *Shigella* (78.1%) strains. But the demonstrated frequency of R(TC) factor was very high in the *Salmonella* group.

We have reported that the resistance patterns of R factors from *Shigella* and *E. coli* (7.9%; 7, 9–11) were similar to each other but distinct from those from *Proteus* (8) or *Pseudomonas* (3) strains. The R factors from *Salmonella* showed another distinct resistance pattern compared with those of the above strains (12).

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