Antimicrobial Resistance and R Factors in *Salmonella* Isolated in California (1971-1972)

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The antimicrobial resistance of 2,246 strains of *Salmonella* isolated from humans in California was determined. Resistance to one or more of the 12 antimicrobial agents tested was found in 32% of the isolates. *Salmonella typhimurium* strains represented 31% of the serotypes isolated; 49% of these strains were resistant. Fifty-one percent of *S. heidelberg*, 42.5% of *S. newport*, and 40% of *S. saint paul* strains were resistant. Seventy-seven percent of all resistant serotypes were resistant to two or more of the antimicrobial agents tested. R factors were demonstrated in 70% of the multiply resistant strains. Resistance of the *Salmonella* strains to one or more of the 12 antimicrobials tested and the frequency of resistance to tetracycline and ampicillin were significantly greater than resistance reported in other studies performed in this country. Chloramphenicol resistance was encountered in 33 (1.5%) of the *Salmonella* isolates; 19 of these were *S. typhi* demonstrating a pattern of resistance to streptomycin, sulfisoxazole, tetracycline, and chloramphenicol and carrying an R factor capable of transferring the complete pattern of resistance.

R factors are episomes that confer upon the host organism the ability to transfer to susceptible organisms genes which mediate antimicrobial resistance. The increasing antimicrobial resistance of pathogenic bacteria and the presence of resistance transfer factors (R factors) in these organisms concerns both clinicians and medical microbiologists. Several surveys of salmonellae isolated in this country have been made to determine the impact of the presence of R factors on the control of infectious diseases (4, 9, 10, 12, 14, 17, 18, 24). These studies were performed with relatively small numbers of isolates and, therefore, conclusions concerning the frequency of resistance and the prevalence of R factors in many of the serotypes have not been possible. In the survey reported here resistance to 12 antimicrobial agents was determined on 2,246 isolates of salmonellae from humans in California that were submitted to the State Microbial Diseases Laboratory during a 12-month period, and the presence of R factors was determined in those strains with resistance to two or more of the antimicrobial agents. This represents the largest study of antimicrobial resistance of salmonellae in the United States to date and establishes a valid baseline of frequency of antimicrobial resistance and prevalence of R factors in the *Salmonella* serotypes most frequently isolated in California.

MATERIALS AND METHODS

The strains of salmonellae tested were submitted to the California State Department of Health Microbial Diseases Laboratory in compliance with a California regulation requiring submission of all human *Salmonella* isolates for serotyping. The majority of the strains tested were received between 1 November 1971 and 31 October 1972. Information on the age of the patient, sex, source of isolation, therapy, and symptoms were obtained when available. All salmonellae received were tested, but duplicate specimens from the same patient were excluded from the statistical analysis; the population analyzed consisted of 2,246 strains.

Antimicrobial susceptibility tests. The method of Bauer et al. (2) was used to determine antimicrobial susceptibility. The following disks were used (μ g size given): ampicillin, 10; cephalothin, 30; chloramphenicol, 30; colistin, 10; gentamicin, 10; kanamycin, 30; nalidixic acid, 30; neomycin, 30; nitrofurantoin, 300; streptomycin, 10; sulfisoxazole, 300; and tetracycline, 30. Zone sizes were interpreted according to the recommendations of Bauer et al. (2) for all antimicrobial agents except tetracycline. For this antibiotic the recommendations of Schroeder et al. (18) were followed, i.e., zone sizes of 11 mm or less indicating resistance and zones of 16 mm or more indicating susceptibility. For the statistical analysis of data, strains demonstrating "intermediate resistance" were considered susceptible to the antimicrobial agent in question.

Assay of transferable drug resistance. The recipient organism used was an *Escherichia coli* K-12, F⁻,

nalidixic acid-resistant strain (RC 709) supplied to us through the kindness of John Walton. The donor organisms were *Salmonella* strains with resistance to two or more of the antimicrobials tested. Overnight cultures of donor and recipient organisms were diluted 100-fold in Mueller Hinton (MH) broth to a total volume of 20 ml and incubated on a rotator at 35 C for 3.5 h to produce organisms in the log phase of growth. Mating mixtures were prepared by adding 0.25 ml of donor salmonellae to 1.0 ml of recipient *E. coli* cultures to produce a total volume mating mixture of 1.25 ml. Each mixture was incubated without aeration for 24 h at 35 C. The following were used as selection media: (i) MacConkey medium with 50 µg of nalidixic acid per ml plus 4 µg of tetracycline per ml and (ii) MacConkey medium with 50 µg of nalidixic acid per ml plus 10 µg of ampicillin per ml. Occasionally, when the antibiogram of the donor salmonellae indicated, MacConkey medium with 50 µg of nalidixic acid per ml plus 10 µg of streptomycin or kanamycin per ml was used. A 0.1-ml amount of undiluted mating mixture was plated on appropriate selection plates, and 0.05 ml of 10-fold dilutions of the mixture was plated on quadrants of similar selection plates to determine the efficiency of mating. Selection plates were incubated overnight at 35 C. Control cultures of the recipient *E. coli* and the donor salmonella strains were tested individually in a manner similar to that for the mating cultures to determine the efficiency of the selection plates and to act as controls on the media with and without incorporated drugs. The antimicrobial resistance of the recombinants was determined to all antimicrobials present in the antibiogram of the *Salmonella* parent strain by replica plating techniques (13). Five *Salmonella* strains showing resistance to nalidixic acid have not as yet been tested for R factors.

RESULTS

A total of 79 different serotypes was represented in the 2,246 *Salmonella* strains examined. The distribution of the 10 most frequently isolated serotypes in the study is presented in Table 1 where it is compared with the distribution of the same serotypes in the United States during 1971 (3). *Salmonella typhimurium* was the most frequently isolated serotype in both surveys, and all but one serotype occur in the top ten of both lists. A total of 31.6% of all *Salmonella* isolates were resistant to 1 or more of the 12 antimicrobial agents tested. Forty percent or more of the strains of *S. typhimurium*, *S. heidelberg*, *S. newport*, and *S. saint paul* were resistant, and 36.5% of *S. typhi* strains were resistant; on the other hand, only 1.5% of isolates of *S. infantis* and none of *S. enteritidis* isolates showed resistance (Table 2). Table 3 lists the percentage resistance of all serotypes and of the 10 most frequently isolated serotypes to each of the antimicrobial agents tested. The most commonly found resistance

was to streptomycin (26.0%), followed by tetracycline (20.3%), sulfisoxazole (19.5%), and ampicillin (18.5%). Thirty-three (1.5%) of the isolates were resistant to chloramphenicol; 19 of these were *S. typhi* strains. Nineteen of the 33 were *S. typhi* carrying the pattern of resistance to streptomycin, sulfisoxazole, tetracycline, and chloramphenicol, and the degraded Vi phage pattern associated with the so-called "Mexican" strain of *S. typhi* (8, 20). Each of these strains carried an R factor capable of transferring the complete pattern of resistance. The antibiograms of the remaining 14 chloramphenicol-resistant isolates are shown in Table 4. One strain of *S. java* and a strain of *S. panama* were resistant to streptomycin, sulfisoxazole, tetracycline, and chloramphenicol, the patterns of the "Mexican" strain of *S. typhi*; these strains also carried an R factor capable of transferring the complete pattern.

Table 5 lists antibiotic resistance in the present study in comparison with a report by Schroeder et al. (18), of 400 selected salmonellae received by the Center for Disease Control in 1967, and reports by Winshell et al. (24) and Cherubin et al. (4) on salmonellae isolated in the Northeastern United States in 1968-1969 and 1970, respectively. These studies were selected for comparison because they also used the Bauer-Kirby technique for determination of antimicrobial susceptibility. California isolates were significantly more resistant to all antimicrobials ($P < 0.001$) as well as to individual antimicrobials (Table 5).

A large percentage of the resistant strains (77%) were multiply resistant, i.e., resistant to two or more of the antimicrobial agents tested (Table 6). Figure 1 shows the cumulative percentage of multiple resistance for all serotypes and for the four individual serotypes having a sufficient number of resistant strains to permit a reliable comparison of percentages. A high percentage of resistant strains of *S. newport*, *S. typhimurium*, and *S. heidelberg* (94, 90, and 86%, respectively) were resistant to two or more antimicrobial agents. Resistance to five or more drugs was observed in 58% of the *S. typhimurium* resistant strains, in 69% of the *S. newport* resistant strains, but in only 6% of the *S. heidelberg* resistant strains. Of the 115 *S. typhi* strains, 42 (37%) were resistant to one or more drug; 23 (55%) of the 42 were resistant to streptomycin alone, whereas 19 (45%) carried a pattern of resistance to four drugs (streptomycin, sulfisoxazole, tetracycline, and chloramphenicol). No other resistance pattern was demonstrated in the *S. typhi* strains submitted.

One hundred and sixty-two strains were re-

TABLE 1. *Distribution of Salmonella serotypes in California and in the United States*

Serotype	California 1971-1972		United States ^a 1971	
	Rank	%	Rank	%
<i>S. typhimurium</i>	1	30.8	1	26.8
<i>S. heidelberg</i>	2	7.4	4	6.5
<i>S. infantis</i>	3	6.1	5	5.5
<i>S. newport</i>	4	5.4	3	6.7
<i>S. typhi</i>	5	5.1	10	2.3
<i>S. java</i>	6	3.1	9	2.3
<i>S. derby</i>	7	3.0	> 10	2.1
<i>S. thompson</i>	8	2.8	7	3.2
<i>S. saint paul</i>	9	2.7	6	3.6
<i>S. enteritidis</i>	10	2.5	2	8.8
Other serotypes		31.1		32.2
Total		100 (2,246) ^b		100 (25,694) ^b

^a Data from Center for Disease Control (3).

^b Figures in parenthesis indicate total numbers. *S. blockley* was ranked 8th in serotypes isolated from human sources in the United States in 1971. This serotype ranked 12th in the California study and represented 1.8% of the California strains studied.

TABLE 2. *Resistance of Salmonella serotypes to one or more antimicrobial agents*

Serotype	No. Tested	No. resistant	Percent resistant
<i>S. typhimurium</i>	691	337	48.8
<i>S. heidelberg</i>	166	84	50.6
<i>S. infantis</i>	137	2	1.5
<i>S. newport</i>	120	51	42.5
<i>S. typhi</i>	115	42	36.5
<i>S. java</i>	70	7	10.0
<i>S. derby</i>	68	16	23.5
<i>S. thompson</i>	64	4	6.3
<i>S. saint paul</i>	60	24	40.0
<i>S. enteritidis</i>	56	0	0
Other serotypes	699	142	20.3
Total serotypes	2,246	709	31.6

sistant to only one of the antimicrobial agents tested; 96 of these were resistant to streptomycin, 32 to tetracycline, 17 to ampicillin, 13 to sulfisoxazole, and 1 each to chloramphenicol, nitrofurantoin, cephalothin, and nalidixic acid.

Resistance transfer factors. Tests for the detection of R factors were performed on 505 strains that were resistant to two or more antimicrobial agents; 70% had the ability to transfer resistance. The prevalence of R factors among the multiply resistant strains of the 10 most frequently isolated serotypes is given in Table 7. Detection of R factors has been reported to be affected by the recipient strain used in the mating procedure (11, 22). Several different recipients were tested in this study to determine if more efficient transfer with *S. typhimurium* strains might be obtained. Recipient strains *E. coli* K-12 Am 20, F⁻, nalidixic

acid-resistant (obtained through the courtesy of Sidney Cohen), *S. typhimurium* LT2 JW526, nalidixic acid resistant, and *S. typhimurium* LT2 JW527, nalidixic acid resistant (obtained by courtesy of Bruce Stocker) were used. Results were similar to those obtained with the *E. coli* RC 709 recipient. The lower prevalence of R factors in *S. typhimurium* may result from the known instability of R factors in this serotype (21, 23). There were 58 different antibiograms represented in the resistant strains detected in this study; eight of these antibiograms were to a single antimicrobial agent. The antibiograms most frequently determined and the prevalence of R factors in isolates with these antibiograms are shown in Table 8. Strains showing these antibiograms represent 70% (495) of all the resistant strains in the study. The remaining 48 patterns were seen in 18 or fewer strains each.

Epidemiology. The seasonal distribution of *Salmonella* isolations in this study is representative of that reported for the nation as a whole (3), with the greatest number of isolations reported from July through October and the lowest number from January through April. Fifty-one percent of the strains were isolated from males and 49% from females. Prevalence of resistant strains was exactly the same (31.6%) for both sexes. Information on the age of patients was available for 79% (1,782) of the patients in this study. Over fifty percent (52%) of patients were 10 years of age or younger. There was no evidence of increased resistance of strains in any age group (Table 9). Patients from whom *S. typhimurium*, *S. heidelberg*, *S. newport*, or *S. infantis* were isolated showed age

TABLE 3. Percentage of resistance of *Salmonella* serotypes to individual antimicrobial agents

Antimicrobial agents	All serotypes	<i>S. typhimurium</i>	<i>S. heidelberg</i>	<i>S. infantis</i>	<i>S. newport</i>	<i>S. typhi</i>	<i>S. java</i>	<i>S. derby</i>	<i>S. thompson</i>	<i>S. saint paul</i>	<i>S. enteritidis</i>	Other serotypes
Streptomycin	26.0	42.8	41.0	1.5	38.3	36.5	8.6	17.6	4.7	31.7	0	13.0
Tetracycline	20.3	37.6	33.7	0.7	37.5	16.5	2.9	13.2	1.6	21.7	0	7.2
Sulfisoxazole	19.5	39.8	18.7	0.7	32.5	16.5	5.7	8.8	0	16.7	0	7.4
Ampicillin	18.5	39.7	14.5	0.7	37.5	0	7.1	5.9	0	21.7	0	7.2
Kanamycin	11.9	26.2	9.0	0.7	30.0	0	4.3	2.9	1.6	15.0	0	2.7
Neomycin	11.8	26.2	8.4	0.7	28.3	0	4.3	2.9	1.6	15.0	0	2.7
Cephalothin	3.6	8.0	1.8	0.7	4.2	0	0	0	0	10.0	0	1.6
Chloramphenicol	1.5	0	1.2	0.7	0.8	16.5	4.3	4.4	0	0	0	0.6
Nitrofurantoin ^a	0.3	0.6	0	0	0	0	0	0	0	0	0	0.1
Nalidixic Acid	0.2	0.1	0	0	0	0	0	0	0	1.7	0	0.4
Colistin	0	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0	0	0	0
Total no. of strains	2,246	691	166	137	120	115	70	68	64	60	56	699
Total percentage and (no.) of resistant strains	31.6 (709)	48.8 (337)	50.6 (84)	1.5 (2)	42.5 (51)	36.5 (42)	10.0 (7)	23.5 (16)	6.3 (4)	40.0 (24)	0	20.3 (142)

^a Based on tests performed on 1,830 of 2,246 *Salmonella* strains submitted.

distributions similar to those for all serotypes. One hundred and five isolates were from blood specimens, and 45 (43%) of these isolates were resistant to one or more of the antimicrobial agents tested. This is a higher proportion of resistant strains than was found in isolates from feces (31% resistant), urine (33%), or other sources (29%).

TABLE 4. Antibigrams of chloramphenicol-resistant strains of *Salmonella*^a

Serotype	Antibiogram	R factor present
<i>S. derby</i> ^b	SSuTCA	—
<i>S. derby</i> ^b	SSuTCA	—
<i>S. derby</i> ^b	SSuTCA	—
<i>S. heidelberg</i>	SSuKTCN	+
<i>S. heidelberg</i>	SSuKTCN	+
<i>S. infantis</i>	SSuKTCNCeA	+
<i>S. java</i>	SSuTC	+
<i>S. java</i>	SSuKCNA	—
<i>S. java</i>	SSuKCNA	+
<i>S. newport</i>	C	NT
<i>S. panama</i>	SSuTC	+
<i>S. senftenberg</i>	SSuTCCeA	—
<i>S. worthington</i>	NxSuTCCeA	NT
<i>S. worthington</i>	NxSSuKTCNCeA	NT

^a NT, Not tested; —, not present; +, present. Streptomycin (S), sulfisoxazole (Su), kanamycin (K), tetracycline (T), neomycin (N), cephalothin (Ce), ampicillin (A), chloramphenicol (C), and nalidixic acid (Nx).

^b These strains were isolated from 3 members of the same family.

DISCUSSION

The public health implications of antimicrobial resistance and prevalence of R factors in medically important bacteria has been of concern since the first observation of the transfer of drug resistance by bacterial episomes (1, 5–7, 15, 16, 19, 21). Several studies of the prevalence of antimicrobial resistance and R factors in *Salmonella* strains found in this country have been reported (4, 9, 10, 12, 14, 17, 18, 24). The majority of these studies (9, 10, 12, 14, 17) were performed before the wide-spread use of the standardized disk method for the determination of antimicrobial susceptibility (2). Results of the national survey of 1967 (18) and studies of strains isolated in the Northeastern United States in 1968–1969 (24) and in 1970 (4), where the Bauer-Kirby method was used, indicated no increase in resistance to tetracycline in *Salmonella* strains isolated in 1967 and 1970. However, the 1971–1972 California *Salmonella* strains show a highly significant difference in frequency of tetracycline resistance ($P < 0.001$). Frequency of ampicillin-resistant salmonellae varied from 8.0% in the 1967 national survey (18), to 13.5% in the 1968–1969 Northeastern U.S. survey (24), and to 9.5% in the 1970 Northeastern U.S. Survey (4). The frequency of ampicillin-resistant strains in the California survey (18.5%) was significantly greater than that seen in the other three studies ($P < 0.05$). No chloramphenicol resistant strains were found in these previous studies

TABLE 5. Comparison of antimicrobial resistance of salmonellae isolated in California with reports of other studies

Antimicrobials	Percentage resistant			
	California ^a 1971–1972	Northeastern U.S. 1970 (4)	Northeastern U.S. 1968–1969 (24)	U.S. 1967 (18)
Streptomycin	26.0	14.9	15.0	14.2
Tetracycline	20.3	11.7	8.2	12.5
Sulfisoxazole	19.5			11.5
Ampicillin	18.5	9.5	13.5	8.0
Kanamycin	11.9	1.9	3.3	1.2
Neomycin	11.8			1.2
Cephalothin	3.6	0.9	3.2	8.0
Chloramphenicol	1.5	0	0	0
Nitrofurantoin	0.3 ^b			2.5
Nalidixic acid	0.2			0
Colistin	0			0
Gentamicin	0			0
Total percentage and (no.) of resistant strains	31.6 (709)	17.4 (55)	21.6 (63)	22.2 (89)
Total no. of strains in study	2,246	315	292	400

^a Present Study.

^b Based on tests performed on 1,830 of 2,246 *Salmonella* strains submitted.

TABLE 6. *Salmonella* serotypes resistant to one or more of 12 antimicrobial agents tested

Serotype	No. of resistant strains	Multiply resistant ^a strains		No. of strains resistant to no. of antimicrobial agents indicated							
		No.	%	1	2	3	4	5	6	7	≥8
<i>S. typhimurium</i>	337	302	89.6	35	22	29	55	61	109	26	0
<i>S. heidelberg</i>	84	72	85.7	12	42	10	15	3	2	0	0
<i>S. infantis</i>	2	1	50.0	1	0	0	0	0	0	0	1
<i>S. newport</i>	51	48	94.1	3	1	8	4	7	25	3	0
<i>S. typhi</i>	42	19	45.2	23	0	0	19	0	0	0	0
<i>S. java</i>	7	5	71.4	2	0	1	1	1	2	0	0
<i>S. derby</i>	16	9	56.2	7	3	2	1	3	0	0	0
<i>S. thompson</i>	4	2	50.0	2	2	0	0	0	0	0	0
<i>S. saint paul</i>	24	20	83.3	4	9	3	0	3	1	4	0
<i>S. enteritidis</i>	0	0	0	0	0	0	0	0	0	0	0
Other serotypes	142	69	48.6	73	28	15	12	9	4	0	1
Total, all serotypes	709	547	77.1	162	107	68	107	87	143	33	2

^a Resistant to two or more of the antimicrobial agents tested.

FIG. 1. Cumulative percent resistance of four selected serotypes to any combination of 12 antimicrobial agents.

(4, 18, 24), whereas 33 (1.5%) of the California strains were chloramphenicol resistant. The previous investigators (4, 18, 24) did not include isolates of *S. typhi* in their studies, nor had the chloramphenicol-resistant "Mexican" strain been reported in the time period covered in their reports. However, 14 strains other than *S. typhi* were found to be chloramphenicol resistant in the present study. Although only seven were resistant to tetracycline and ampicillin, as well as chloramphenicol, and although three of these were isolates of *S. derby* from members of the same family, the difficulties that would face the clinician in choice of treatment

TABLE 7. Prevalence of R factors in resistant strains of *Salmonella* serotypes

Serotype	No. of strains tested	No. with R factor	Percentage with R factor
<i>S. typhimurium</i>	284	159	56.0
<i>S. heidelberg</i>	67	61	91.0
<i>S. infantis</i>	1	1	^a
<i>S. newport</i>	46	44	95.7
<i>S. typhi</i>	19	19	100.0
<i>S. java</i>	5	4	^a
<i>S. derby</i>	7	4	^a
<i>S. saint paul</i>	18	18	100.0
Other serotypes	58	45	77.6
Total	505	355	70.3

^a Percentage was not computed when the number of strains tested was fewer than 10.

of serious cases with similar resistance patterns are apparent and emphasize the need for continuing surveillance to determine the prevalence of this type of resistance.

The salmonella strains tested in the California study showed an overall antimicrobial resistance of 31.6% which is significantly greater than that reported in previous studies (4, 18, 24) by using a similar method of determining susceptibility ($P < 0.001$). Because we have no earlier California studies with which to compare our findings, we cannot say whether this represents an increase in the number of resistant strains in this state or a difference in the strains evaluated in California as compared with those analyzed elsewhere. It will be necessary to survey the antimicrobial susceptibility of strains from California for a further period of time to determine if an actual increase in

TABLE 8. Most frequent antibiograms encountered and the prevalence of R factors for each^a

Antibiogram	Number determined	R factors		
		No. tested	No. with R factor	Percentage with R factor
SSuKTNA	135	131	81	61.8
S	96	NT		
SSuTA	65	62	41	66.1
ST	51	45	40	88.9
SSuKNA	41	36	16	44.4
T	32	NT		
SSuKTNCeA	32	31	16	51.6
SSuTCeA	25	25	19	76.0
SSu	22	18	10	55.6
SSuTC	21	21	21	100.0

^a Streptomycin (S), sulfisoxazole (Su), kanamycin (K), tetracycline (T), neomycin (N), cephalothin (Ce), ampicillin (A), and chloramphenicol (C); NT, not tested.

resistance in salmonellae is occurring.

Other surveys (4, 14, 17, 18, 24) have reported a higher frequency of resistance in *S. typhimurium* strains than in other *Salmonella* serotypes. Our data do not corroborate these findings. The percentage of resistant strains of *S. typhimurium* was 48.8% versus 56.6% for *S. heidelberg*. Resistant strains of *S. newport* (42.5%) and *S. saint paul* (40.0%) were found at percentages approaching that of *S. typhimurium*. Much larger numbers of the most frequently isolated serotypes were tested in the California study than in the studies done elsewhere in this country. Therefore, percentages of resistant serotypes are more reliable in our study than in previous studies where very small numbers of serotypes other than *S. typhimurium* were tested.

It is of interest to note that R factors were demonstrated at a very high frequency in multiply resistant strains of *S. heidelberg* (91%), *S. newport* (95.7%), and *S. saint paul* (100%) (Table 7). It can be postulated that these serotypes obtained their increased resistance through the process of R factor transfers because of large numbers of resistant R factor-bearing organisms in the population and the selective pressures of exposure to antimicrobial agents of man and animals. The singular susceptibility to antimicrobials demonstrated by *S. infantis* and *S. enteritidis* strains isolated during this period may reflect reduced ability of these serotypes to act as recipients of R factors by virtue of their cell wall composition (22) or deoxyribonucleic acid restriction activities (21). These postulations will require further in vitro studies.

TABLE 9. Antimicrobial susceptibility by age of patient

Patient age in years	No. (%)	Percentage resistant	Percentage susceptible
<1	344 (19%)	29	71
1-10	592 (33%)	34	66
11-20	233 (13%)	35	65
>20	612 (34%)	31	69
Total	1,782 (100%)	32	68

Overall prevalence of R factors in the salmonellae in this survey was 70%, which is in agreement with other U.S. surveys of R factors in salmonellae (18, 19, 24). Multiply resistant strains of *S. typhimurium* in our survey, however, showed a lower prevalence of R factors than reported by others. Winshell et al. (24) reported that 19 of 30 (63.5%) strains of *S. typhimurium* transferred resistance. Gill and Hook (9) studied 20 strains of multiply resistant *S. typhimurium* and demonstrated R factors in 13 (65%) of these strains, whereas Schroeder et al. (18) reported that 28 of 36 (77.8%) *S. typhimurium* strains possessed R factors. We demonstrated R factors in 159 of 284 (56%) of the multiply resistant strains of *S. typhimurium*. This lower prevalence of R factors in *S. typhimurium* does not appear to be related to the recipient used in the study, because no difference in results occurred when other recipients were substituted. Demonstration of a lower number of *S. typhimurium* strains with R factors in our study may be related to segregation and loss of the transfer factor of the R factor, whereas the organism retains the r determinants that mediate resistance. Crosses with organisms containing only the transfer factor and subsequent transfer of the r determinants to sensitive recipient organisms would be necessary to support this hypothesis (1).

The results of this survey indicate that a high frequency of resistant salmonellae are being isolated in this state and that a large percentage of these organisms possess the ability to transfer their resistance patterns. The large percentage of *Salmonella* strains which are resistant to tetracycline and ampicillin, and the number of salmonellae with R factors which are resistant to chloramphenicol, are further indications of the problems which may increasingly confront the clinician in his management of patients with salmonellosis.

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LITERATURE CITED

1. Anderson, E. S. 1968. The ecology of transferable drug resistance in the enterobacteria. *Annu. Rev. Microbiol.* **22**:131-180.
2. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turk. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.* **45**:493-496.
3. Center for Disease Control. 1972. *Salmonella* surveillance annual summary, 1971. Center for Disease Control, Atlanta, Ga.
4. Cherubin, C. E., M. Szmunn, and J. Winter. 1972. Antibiotic resistance of *Salmonella*. *Northeastern United States-1970*. N.Y. State J. Med. **72**:369-372.
5. Davies, J. E., and R. Rownd. 1972. Transmissible multiple drug resistance in *Enterobacteriaceae*. *Science* **176**:758-768.
6. Dulanez, E. L., and A. I. Laskin (ed.). 1971. The problems of drug resistant pathogenic bacteria. *Ann. N.Y. Acad. Sci.* **182**:1-415.
7. Finland, M. 1972. Changing patterns of susceptibility of common bacterial pathogens to antimicrobial agents. *Ann. Intern. Med.* **76**:1009-1036.
8. Gangarosa, E. J., J. V. Bennett, C. Wyatt, P. E. Pierce, J. Olarte, P. M. Hernandez, V. Vasquez, and D. Bessudo M. 1972. An epidemic-associated episome? *J. Infect. Dis.* **126**:215-218.
9. Gill, F. A., and E. W. Hook. 1966. *Salmonella* strains with transferable antimicrobial resistance. *J. Amer. Med. Ass.* **198**:1267-1269.
10. Huey, C. R., and P. R. Edwards. 1958. Resistance of *Salmonella typhimurium* to tetracyclines. *Proc. Soc. Exp. Biol. Med.* **97**:550-551.
11. Jarolmen, H., and G. Kemp. 1969. Association of increased recipient ability for R factors and reduced virulence among variants of *Salmonella choleraesuis* var. *kunzendorf*. *J. Bacteriol.* **97**:962-963.
12. Kaye, D., J. C. Merselis, Jr., and E. W. Hook. 1963. Susceptibility of salmonella species to four antibiotics. *N. Engl. J. Med.* **269**:1084-1086.
13. Lederberg, J., and E. M. Lederberg. 1952. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63**:399-406.
14. McWhorter, A., M. C. Murrell, and P. R. Edwards. 1963. Resistance of salmonellae isolated in 1962 to chlortetracycline. *Appl. Microbiol.* **11**:368-370.
15. Mitsuhashi, S. 1970. The transferable drug-resistance factor "R." Tokyo University Press, Tokyo, Japan.
16. National Academy of Sciences. 1969. An evaluation of the *Salmonella* problem, p. 187-202. Publ. 1683. National Academy of Sciences, Washington, D.C.
17. Ramsey, C. H., and P. R. Edwards. 1961. Resistance of salmonellae isolated in 1959 and 1960 to tetracyclines and chloramphenicol. *Appl. Microbiol.* **9**:389-391.
18. Schroeder, S. A., P. M. Terry, and J. V. Bennett. 1968. Antibiotic resistance and transfer factor in *Salmonella*, United States 1967. *J. Amer. Med. Ass.* **205**:903-906.
19. Smith, D. H. 1969. The influence of drug resistant bacteria on the health of man, p. 334-345. *In* Symposium on the use of drugs in animal feeds. Publ. 1679. National Academy of Sciences, Washington, D.C.
20. Vasquez, V., E. Calderon, and R. S. Rodriguez. 1972. Chloramphenicol-resistant strains of *Salmonella typhosa*. *New Engl. J. Med.* **286**:1220.
21. Watanabe, T. 1971. Infectious drug resistance in bacteria, p. 43-98. *In* Current topics in Microbiology and Immunology, no. 56. Springer-Verlag, New York.
22. Watanabe, T., T. Arai, and T. Hattori. 1970. Effects of cell wall polysaccharide on the mating ability of *Salmonella typhimurium*. *Nature (London)* **225**:70-71.
23. Watanabe, T., and Y. Ogata. 1970. Genetic stability of various resistance factors in *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **102**:363-368.
24. Winshell, E. B., C. Cherubin, J. Winter, and H. C. Neu. 1970. Antibiotic resistance in *Salmonella* in the Eastern United States. *Antimicrob. Ag. Chemother.* 1969, p. 86-89.