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Antimicrobial Resistance and Incompatibility Groups of R Plasmids in *Salmonella typhimurium* Isolated from Human Sources in Bombay from 1978 to 1980

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Salmonella typhimurium isolated in Bombay from fecal samples of 145 patients suffering from gastroenteritis (group 1) and from the cerebrospinal fluid, feces, or blood of 42 patients with systemic salmonellosis (group 2) were examined for the antimicrobial resistance and incompatibility groups of their R plasmids. Multiple drug resistance was encountered in 88.9% of the isolates from group 1 and in all the isolates from group 2. The resistance found was mainly against ampicillin, chloramphenicol, kanamycin, streptomycin, sulfonamides, and tetracycline. In addition to these resistances, a number of isolates were also resistant to sulfamethoxazole-trimethoprim and gentamicin. The overall isolation frequency of strains resistant to these last drugs was significantly higher in group 2. The drug resistance in 95.3% of the isolates from group 1 and in all the isolates from group 2 was plasmid mediated. Incompatibility grouping of the R plasmids and phage typing of the isolates indicated that a clone of *S. typhimurium* with phage type pattern 66/122/untypable carrying Tra$^-$ IncF1*me*, Tra$^-$ Inc1, and Tra$^-$ Inc2 plasmids was most prevalent in Bombay from 1978 to 1980, and examples of this clone, especially those resistant to sulfamethoxazole-trimethoprim and gentamicin, were most often responsible for severe septicemic infection. A majority of the remaining *S. typhimurium* isolates were untypable and harbored plasmids of groups IncC, IncF1*me*, IncFII, IncH1, IncH2, IncI1 and IncI2; these isolates were rarely associated wtih systemic infection.

Since 1962, R plasmids have been noticed in *Salmonella typhimurium* isolated in various regions of the world (2, 11, 13, 17, 19, 20), and a geographical predominance of resistance transfer systems characterized mainly by their incompatibility groups has been identified (2). In India, sporadic cases of salmonellosis due to drug-resistant *S. typhimurium* have been recorded in Vellore (16) since 1968, but in the last few years plasmid-bearing, multidrug-resistant strains have emerged in various parts of the country (21). Incompatibility between plasmids indicates that they are phylogenetically related (12). Not only is this property used for plasmid classification, but it is also useful in conjunction with phage typing of *S. typhimurium* for clone identification (2, 19). Such an investigation (19) made it apparent that, during the period 1977 to 1979, a clone of *S. typhimurium* with the phage type pattern 66/122/untypable (UT) was responsible for a series of outbreaks of gastroenteritis often leading to severe systemic complications in infants and children in the Middle East and in Southeast Asia, including several states of India. Phage typing studies (19) have shown that phage types 66 and 122 are closely related, differing only in the degree of lysis obtained with the *S. typhimurium* typing phages. Strains of type 122 can spontaneously give rise to type 66 strains owing to the loss of a temperate phage. The spontaneous conversion of type 66 or 122 into UT strains is also influenced by temperate phages. These data, together with the presence in each of these phage types of non-self-transmissible (Tra$^-$) R plasmids belonging to the same three incompatibility groups (IncF1*me*, Inc1, and Inc2), suggest that strains of type 66/122/UT have a clonal origin (19). This clone has caused hospital outbreaks by cross-infection between patients in Bombay (19). However, during the period 1978 to 1980, a number of patients who seemed to have contracted *S. typhimurium* infection in Bombay were also recorded. A preliminary study at our laboratory indicated that a noticeable proportion of the isolates harbored transfer-proficient (Tra$^+$) R plasmids. In view of these observations, an investigation was undertaken to find the drug resistance and incompatibility

groups of R plasmids in *S. typhimurium* strains isolated from patients who had contracted infection in Bombay from 1978 to 1980.

MATERIALS AND METHODS

Test cultures. *S. typhimurium* isolates were taken in various hospitals and other medical institutions in Bombay between January 1978 and October 1980. Isolates were made from fecal samples from 145 patients suffering from gastroenteritis only (group 1) and from the cerebrospinal fluid, feces, or blood of 42 patients with gastroenteritis and systemic complications (group 2). More than 60% of the patients were infants less than 2 years old, and the mortality rate was especially high in these patients after systemic infection and meningitis. Nearly 80% of the patients were from families dwelling in crowded old buildings and slum areas. All the patients showed symptoms of gastroenteritis with or without pyrexia when they were admitted to the hospitals. The *Salmonella* cultures were serotyped and phage typed by the Salmonella Phage Typing Centre, New Delhi, and the Central Public Health Laboratory, London.

Test for drug resistance. The antibiogram of each isolate was examined by the disk diffusion test (8) with ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), sulfamethoxazole (300 μg), tetracycline (30 μg), and trimethoprim (10 μg). *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571 were used as the susceptible controls. Disks of gentamicin were obtained from C. E. Fulford Co. Disks of sulfamethoxazole and trimethoprim were from Burroughs Wellcome Co., Research Triangle Park, N.C. Disks of all other drugs were provided by Span Diagnostics. Tests for sulfamethoxazole and trimethoprim were made on Wellcotest agar (Burroughs Wellcome Co.) seeded with 7.5% lysed horse blood (9); tests for other drugs were conducted on Wellcotest agar. The minimal inhibitory concentrations of the drugs were determined by incorporating pure drug powders (obtained from different manufacturers) in Wellcotest agar in concentrations ranging from 2 to 1,200 μg/ml (10).

Transfer of drug resistance. Isolates found to be resistant to one or more drugs were examined for their ability to transfer their drug resistance to *E. coli* K-12 J53-1 F^-, *met pro*, Nal^r, in primary crosses and to *E. coli* K-12 C600 Rif^r (F^-, *lac leu thi thr*, Rif^r) in secondary crosses. Conjugation was conducted in Penassay broth (Difco Laboratories) at 28 or 37°C for 18 h, and transconjugants were selected on MacConkey agar (without bile salt) containing drugs corresponding to the resistance carried by the *S. typhimurium* donor strain and to the chromosomal resistance of the recipient to nalidixic acid or rifampin (4). Mobilization of non-self-transmissible resistance was examined in triparental crosses (3) with *E. coli* K-12 J53 (F^-, *met pro*, Nal^s) bearing transfer factors X (IncFII) and Δ (IncI1). All the *E. coli* K-12 strains (7) were kindly provided by K. B. Sharma, New Delhi.

Incompatibility grouping. The R plasmids in *S. typhimurium* were classified into incompatibility groups (14) by examining their ability to coexist with the standard R plasmids of various incompatibility groups as described earlier (18).

TABLE 1. R plasmids and their incompatibility groups in *S. typhimurium* isolates

Group	Source	Yr of isolation	No. of drug-resistant isolates	No. of R^+ isolates[a]	No. with R plasmids[b]		No. of R plasmids from each incompatibility group:									
					Tra^+	Tra^-	Tra^+						Tra^-			
							C	F1*me*	FII	H1	H2	I1	I2	F1*me*	1	2
1	Feces	1978	18	18 (100)	7 (13.7)	44 (86.3)	0	7	0	0	0	0	0	11	15	18
		1979	58	54 (93.1)	35 (35.4)	64 (64.6)	3	17	0	2	2	11	0	27	8	29
		1980	53	51 (96.2)	51 (60.7)	33 (39.3)	3	16	3	4	0	23	2	17	5	11
2	Blood or cerebrospinal fluid	1978–1980	42	42 (100)	12 (11.9)	89 (88.1)	0	6	0	0	0	6	0	36	19	34
	Feces	1978–1980	16	16 (100)	6 (14.3)	36 (85.7)	0	2	0	0	0	4	0	14	9	13

[a] Numbers in parentheses show percentages of drug-resistant isolates that contained R plasmids.
[b] Numbers in parentheses show percentages of R plasmids that were Tra^+ or Tra^-, as indicated.

TABLE 2. Drug resistance patterns encoded by R plasmids in each incompatibility group of *S. typhimurium*

Incompatibility group	Group 1		Group 2	
	Drug resistance pattern	No. of R plasmids	Drug resistance pattern	No. of R plasmids
IncC	Ap Cm Km Sm Su Tc	6		
Tra$^-$, IncF1*me*	Ap Cm Km Sm Su Tc	24	Ap Cm Gm Km Sm Su Tc Tp	40
	Ap Cm Gm Km Sm Su Tc Tp	17	Ap Cm Km Sm Su Tc	8
	Ap Cm Km Sm Su Tc Tp	10	Ap Cm Sm Su Tc	2
	Ap Cm Gm Km Sm Tc	3		
	Ap Cm Sm Su Tc Tp	1		
Tra$^+$ IncF1*me*	Ap Cm Km Sm Su Tc	30	Ap Cm Sm Su Tc	4
	Cm Km Sm Su Tc	4	Ap Cm Gm Km Sm Su Tc Tp	2
	Ap Cm Km Sm Su Tc Tp	2	Ap Cm Km Sm Su Tc Tp	2
	Ap Sm Tc	1		
	Cm Km Tc	1		
	Ap Km Sm Su Tc	1		
	Ap Cm Gm Km Sm Su Tc Tp	1		
IncH1	Ap Cm Sm Su Tc	6		
IncH2	Ap Cm Sm Su Tc	2		
IncI1	Ap Cm Km Sm Su Tc	15	Ap Cm Km Sm Su Tc	10
	Ap Cm Gm Km Sm Su Tc Tp	8		
	Cm Km Sm Su Tc	3		
	Ap Cm Sm Su Tc Tp	2		
	Ap Cm Sm Su Tc	2		
	Cm Km Sm Su Tc Tp	1		
	Cm Gm Km Su Tc Tp	1		
	Ap Cm Su Tc Tp	1		
	Cm Gm Km Sm Su Tc Tp	1		
IncI2	Ap Cm Sm Su Tc	2		
Inc1	Ap	22	Ap	23
	Ap Km	6	Ap Km	5
Inc2	Sm Su	58	Sm Su	47

RESULTS

Incidence and level of drug resistance in isolates. Multiple drug resistance was recorded in 88.9% of the isolates from group 1 and in all the isolates from group 2. In group 1 patients, the occurrence of isolates resistant to commonly employed drugs like ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline was uniformly high (82.8 to 100%) during each of the three years from 1978 to 1980. The incidence of isolates resistant to gentamicin and sulfamethoxazole-trimethoprim was significantly higher ($P < 0.05$ by the chi-square test) in 1978 than in the subsequent two years. The systemic isolates of *S. typhimurium* from group 2 patients were invariably resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline, and the overall incidence of resistance to gentamicin and sulfamethoxazole-trimethoprim was higher ($P < 0.05$ by the chi-square test) in this group. Irrespective of their source and the year of isolation, the isolates were highly resistant to the various antimicrobial agents. The minimal inhibitory concentrations (in micrograms per milliliter) for each drug against the resistant isolates were: ampicillin, 200 to >1,200; chloramphenicol, 400 to >1,200; gentamicin, 100 to 600; kanamycin, 400 to >1,200; streptomycin, 200 to >1,200; sulfamethoxazole, 400 to >1,200; tetracycline, 200 to >1,200; and trimethoprim, 400 to >1,200.

R plasmid incompatibility groups in the isolates. Most of the drug-resistant isolates from group 1 carried R plasmids; only a minority (4.7%) of the isolates which were doubly (Sm Su) or triply (Ap Sm Su) resistant did not transfer their resistances either directly or in triparental crosses (Table 1). Tra$^-$ plasmids were prevalent in 1978 and 1979, but in 1980 there was a marked increase in the isolation frequency of strains bearing Tra$^+$ plasmids. The strains isolated in 1978 harbored plasmids predominantly

of the IncF1*me*, Incl (Ap or Ap Km), and Inc2 (Sm Su) groups, and only two strains from the feces of group 2 patients contained Tra$^+$ IncI1 plasmids. Diverse incompatibility groups of R plasmids were found in group 1 isolates, with an emergence of IncI1 plasmids during 1979 and 1980. All the *S. typhimurium* isolates from group 2 harbored R plasmids; however, their plasmid content was limited to Tra$^+$, Tra$^-$ IncF1*me*, Tra$^+$ IncI1, and Tra$^-$ Incl and Inc2 plasmids. IncC, IncFII, IncH1, IncH2, and IncI2 plasmids, which were found in group 1 isolates, were not encountered in any of the group 2 isolates.

Nearly 50% of the fecal isolates from group 1 patients carried Ap Cm Km Sm Su Tc plasmids; isolates from group 2 patients were predominantly Ap Cm Gm Km Sm Su Tc Tp (Table 2).

R plasmid incompatibility groups in various phage types of *S. typhimurium*. Data on phage typing of 184 of these *S. typhimurium* isolates revealed that UT strains were prevalent (61.9%) in Bombay between 1978 and 1980, followed by strains of phage type 66/122 (35.3%). Except for three strains which were found to be drug susceptible, all the isolates of type 66/122 invariably harboured Tra$^-$ IncF1*me*, Tra$^-$ Incl, or Tra$^-$ Inc2 plasmids. A significant proportion (31.3%) of UT strains showed an R plasmid content which was similar to that of type 66/122 isolates. The type 66/122 and UT strains isolated from group 2 patients invariably harbored Tra$^-$ plasmids of groups IncF1*me*, Incl, and Inc2, and only a few of these isolates also harbored Tra$^+$ IncF1*me* and IncI1 plasmids.

A few strains of two other phage types, 208 and 193, were also encountered. Both the strains of type 208, although isolated in 1978 and 1979, contained Tra$^-$ IncF1*me* (Ap Cm Km Sm Su Tc), Tra$^-$ Incl, and Tra$^-$ Inc2 plasmids. Three strains of type 193 were found in 1979, and all of them contained only IncI1 (Ap Cm Km Sm Su Tc) plasmids.

DISCUSSION

In the last decade, multidrug-resistant *S. typhimurium* has emerged, particularly in countries where this *Salmonella* serotype is one of the most common causes of salmonellosis (2, 13, 17, 19–21). In India, a few sporadic isolates of drug-resistant *S. typhimurium* have been encountered every year since one was found in 1968 in Vellore (16), but from 1977 onwards a remarkable increase has occurred in the isolation frequency of such strains all over the country (21). Nearly 90% of the strains encountered from clinical sources at Bombay since 1978 were found to be multidrug resistant. In fact, at Bombay the isolation frequency of multidrug-resistant *S. typhimurium* has increased since the emergence in 1977 of the *S. typhimurium* clone of phage type 66/122/UT, characterized by its phage type pattern and the presence of Tra$^-$ IncF1*me*, Tra$^-$ Incl, or Tra$^-$ Inc2 R plasmids. This clone caused hospital outbreaks in Bombay and also at Delhi, Ludhiana, and Trivandrum from 1977 to 1979 (19). From the present data on phage typing and R plasmid incompatibility groups, it is evident that this clone was also responsible for community outbreaks in Bombay. Furthermore, a majority of these strains were responsible for severe septicemic infection resulting in high mortality, particularly in infants at Bombay. The presence of Tra$^-$ IncF1*me*, Tra$^-$ Incl, or Tra$^-$ Inc2 plasmids with a predominant resistance pattern (Ap Cm Gm Km Sm Su Tc Tp) was a salient feature of these strains. Since the exclusive occurrence of plasmids of other incompatibility groups was rarely recorded in *S. typhimurium* strains causing severe systemic infection, it is reasonable to believe that these Tra$^-$ plasmids in association with strains of phage type 66/122/UT confer enhanced virulence.

Besides the clone of *S. typhimurium* phage type 66/122/UT, which was most prevalent, sporadic *S. typhimurium* isolates of phage types 208 and 193 and a number of UT strains were also encountered in cases of gastroenteritis in the present study. All the patients had contracted the infection in the community itself, and nearly 80% of them belonged to families of low socioeconomic strata residing in crowded old buildings and slum areas, where sanitation and personal hygiene are expected to be poor. The plasmid contents of the *S. typhimurium* strains of phage types 208 and 193 were identical with those of corresponding isolates (2) from Middle Eastern countries and Britain (type 208) and from France and Britain (type 193). These strains are relatively rare in India and must have been introduced from abroad. A majority of the UT strains carried Tra$^+$ R plasmids belonging to different incompatibility groups, and at least some of these plasmids have been encountered in typable strains of *S. typhimurium* from other countries (2). Also, the ability of R plasmids and temperate phages to render typable strains UT is well known (19, 22). Nevertheless, the origin of these UT strains deserves further study. The genetic properties of R plasmids in *S. typhimurium* closely resemble those of the R plasmids occurring in intestinal *E. coli* of people in Bombay (18), and hence it is likely that they might have acquired these plasmids from *E. coli*. The acquisition of Tra$^+$ IncI1 plasmids by members of the clone 66/122/UT strongly supports this proposition.

Antimicrobial agents are not recommended in the treatment of *Salmonella* gastroenteritis in humans (6, 20), but ampicillin, chloramphenicol,

sulfamethoxazole-trimethoprim, and gentamicin are the drugs of choice in the treatment of systemic salmonellosis (20). Resistance to ampicillin and chloramphenicol has been encountered in *S. typhimurium* isolates in earlier surveys, but almost all the strains were susceptible to sulfamethoxazole-trimethoprim and gentamicin (1, 5, 13, 16, 17, 20, 21). In the last few years, however, *S. typhimurium* strains resistant to sulfamethoxazole-trimethoprim and to gentamicin have emerged (16, 19, this study). This situation points towards serious problems in the treatment of systemic infections caused by these organisms.

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