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Incidence of plasmids in multiply-resistant salmonella isolates from diarrhoeal patients in Hong Kong from 1973–82

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SUMMARY

Plasmids present in multiply-resistant salmonella strains including *Salmonella typhimurium*, *S. johannesburg*, *S. wandsworth*, *S. derby*, *S. newport*, *S. london* and *S. choleraesuis* causing diarrhoea in patients in Queen Mary Hospital in Hong Kong from 1973–82 were studied. In multiply-resistant *S. typhimurium*, plasmids belonging to groups F_1me, H_1 or H_2 and plasmids encoding trimethoprim-resistance which were compatible with standard plasmids of testable incompatibility groups were detected. In *S. johannesburg*, both the ASTCKSu- and ASCKSu-resistant strains which were predominant in two consecutive periods of an outbreak were found to harbour the same plasmid which belonged to the incompatibility group F_1me. *S. wandsworth* strains isolated from a hospital outbreak in 1980 harboured an identical R-plasmid belonging to group N. A few strains of the other salmonellae showing resistance to multiple antibiotics were found to harbour R-plasmids belonging to groups H_1, H_2 and F_1me. The only salmonella of the enteric fever group resistant to ampicillin, chloramphenicol and trimethoprim was an *S. paratyphi B* strain. The resistances were encoded on a plasmid of an unknown incompatibility group. The occurrence and distribution of plasmids in these salmonellae isolated within the 10-year period are discussed.

INTRODUCTION

Salmonella sp. are an important cause of gastroenteric infections in Hong Kong. *S. typhimurium* and *S. johannesburg* are two of the frequent gastroenteric salmonellae isolated from diarrhoeal patients during the period 1973–82 (Ling, Chau & Rowe, 1987).

Besides being the most common serotype of gastroenteric salmonellae causing human infection, *S. typhimurium* also shows the highest percentage of multiply-resistant strains including resistance to chloramphenicol and ampicillin (Anderson, 1968; Neu *et al.* 1975; Sharma *et al.* 1979; Panhotra *et al.* 1981*a*, *b*; Koshi, 1981; Bhatia, Vaze & Agarwal, 1981). Plasmid studies have thus been concentrated on this organism, although other serotypes such as *S. wien*, *S. newport* and *S. oranienburg* causing outbreaks have also been examined (McConnell *et al.* 1979; Threlfall, Bhat & Sharma, 1982; Rangnekar, Banker & Jhala, 1982*a*, *b*). Several

plasmid types have been identified in *S. typhimurium*, but only a few are widely distributed, such as group F_1me (Threlfall, Carr & Anderson, 1976), group H_1 (Makino *et al.* 1981; Niida *et al.* 1983; Rangnekar, Banker & Jhala, 1983), groups H_2, F_{II}, I_1, I_2, C, N or P (Bezanson & Lior, 1980; Threlfall *et al.* 1980; Makino *et al.* 1981; Palomares & Perea, 1982; Rangnekar, Banker & Jhala, 1983).

In recent years, an increasing incidence of trimethoprim-resistance has been noticed in the enterobacteriaceae (Towner *et al.* 1980) and in *S. typhimurium* strains in Britain (Ward, Rowe & Threlfall, 1982). Such resistance has also been found to be mediated by plasmids (Towner *et al.* 1980; Threlfall *et al.* 1980, 1983; Richards & Datta, 1981).

In Hong Kong, a comparatively large percentage of *S. typhimurium* and *S. johannesburg* and a small proportion of other gastroenteric salmonellae were multiply-resistant to high concentrations of antibiotics which is highly suggestive of plasmid-mediated resistance (Ling, Chau & Rowe, 1987). However, the incidence of plasmids in salmonella isolates of Hong Kong has not been reported previously. This paper aims to study the plasmids in multiply-resistant *S. typhimurium* and *S. johannesburg* to elucidate the epidemiological features of these two organisms. Plasmid studies were also carried out in multiply-resistant strains of other salmonellae to determine whether some of the plasmids in *S. typhimurium* and *S. johannesburg* had become widespread.

MATERIALS AND METHODS

Salmonellae isolated from diarrhoeal patients of Queen Mary Hospital were used in this study. They were tested for resistance to ampicillin (A), streptomycin (S), tetracycline (T), chloramphenicol (C), kanamycin (K), sulphonamide (Su) and trimethoprim (Tm) by the agar dilution method (Ling, Chau & Rowe, 1987). For determination of minimal inhibitory concentrations (MICs), serial twofold dilutions of each antibiotic were prepared and added to appropriate volumes of agar to give the final concentrations required. *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 were used as the controls. The minimal inhibitory concentration of an antibiotic against a bacterial strain was taken as the lowest concentration which inhibited visible growth on the inoculation spot.

Strains which were multiply-resistant were selected for plasmid study by the method of Anderson & Threlfall (1974), i.e. on the basis of the transferability of plasmids and mobilization of resistance(s) when transfer was not detected, incompatibility testing, and fertility inhibition character.

The presence of plasmids in bacterial strains was also detected by agarose gel electrophoresis (Meyers *et al.* 1976) of plasmid DNA extracted according to the method of Hansen & Olsen (1978). An electrophoresis cell (Protean Dual Slab Cell with cooling, BioRad Laboratories, Richmond, California) in a Tris-borate-EDTA buffer system (Greene *et al.* 1974) was used.

RESULTS

Salmonella typhimurium

The MICs (minimal inhibitory concentration) of ampicillin against resistant *S. typhimurium* strains varied between 320 and > 5120 mg/l, while that of chloramphenicol, tetracycline, kanamycin and streptomycin ranged between 320–640 mg/l, 80–160 mg/l, 64– > 5120 mg/l and 160–640 mg/l respectively. The MICs of sulphadiazine, trimethoprim and co-trimoxazole (trimethoprim/sulphamethoxazole) against resistant strains were > 10240 mg/l, > 1024 mg/l and > 256/5120 mg/l, respectively. The MICs of these antibiotics against *E. coli* K12 transconjugants of their corresponding *S. typhimurium* donors were usually similar to or two to fourfold lower than those against their respective salmonella donors (Table 1).

Thirty-five multiply-resistant isolates were selected for plasmid study. Four types of plasmids were identified: groups H_1 and F_1me (both with autotransferring and non-autotransferring members), group H_2, and a group of trimethoprim-resistant plasmids, the incompatibility group of which could not be determined because of its compatibility with all the available standard plasmids (Table 2).

Group H_1 plasmids (Anderson, 1975) were detected sporadically in 17 of the tested *S. typhimurium* strains which were isolated from 1972–78. All these strains belonged to phage type 193 except one which was of phage type RDNC. The plasmids could be conveniently divided into two types with respect to the antibiotic resistances encoded: one encoding resistance to ampicillin while the other encoded resistance to chloramphenicol. The ampicillin-resistant plasmids also encoded resistance to tetracycline or kanamycin and were found in 16 *S. typhimurium* strains tested. The chloramphenicol-resistant plasmid (STCSu-plasmid) was found in only one strain, the resistances encoded being identical to that commonly found in chloramphenicol-resistant *S. typhi* strains isolated elsewhere. The ampicillin with tetracyline or kanamycin resistances in 14 of these strains were non-autotransferring but could be mobilized by the X transfer factor. The resistances were coded on large plasmids of about 138 to > 143·7 Mdal. (Fig. 1).

Group F_1me plasmids (Anderson *et al.* 1977) were detected in six *S. typhimurium* strains isolated between 1975–80. Three F_1me carrying strains isolated in 1975 and 1977, were of phage type 104a while the remaining three strains all isolated in 1980 were untypable. All the six tested strains were resistant to ASTCSu and the resistances were encoded by the F_1me plasmid. Four of these strains harboured autotransferring plasmids, while the other two, both isolated in 1975, harboured non-autotransferring plasmids. Both these latter plasmids could be mobilized by the X transfer factor and one of them could also be mobilized by the △ transfer factor. All these plasmids were *fi*$^+$. Plasmid DNAs extracted from these strains showed multiple bands (Fig. 2). Definite identification of the F_1me plasmid where multiple plasmid bands were present was not possible, but it was probably the larger plasmid band. Two of these plasmids had a molecular size of 86 Mdal. and four of them, 110 Mdal.

A group H_2 plasmid, encoding resistance to CKSu was present in all three CKSu-resistant *S. typhimurium* strains of phage type 193 isolated in 1980. The

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Table 1. *Range of minimal inhibitory concentration (MIC) of eight antibiotics against multiply-resistant strains of gastroenteric salmonella serotypes and their respective* E. coli *transconjugants*

	MIC (mg/l) range of							
Organism	Amp	Sm	Tc	Cm	Km	Su	Tm	Sxt
S. typhimurium	320- > 5120	160–640	40–160	320–640	640- > 5120	> = 10240	> 1024	> 256/5120
Transconjugants	320-5120	80–160	40–160	160–640	320- > 5120	1280- > 10240	> 1024	64/1280- > 256/5120
S. derby	320- > 5120	80–320	40–80	640	2560- > 5120	> 10240	> 1024	> 256/5120
Transconjugants	160–2560	—	40–80	320–640	640–2560	1280- > 10240	> 1024	64/1280
S. johannesburg	2560- > 5120	40–160	320–640	320–640	2560- > 5120	> 10240	8–16	2/40–4/80
Transconjugants	640–2560	20–40	80–160	80–160	640–2560	> = 10240	< 2	< 1/20
S. newport	640–1280	80–320	—	—	> 5120	> 10240	> 1024	> 256/5120
Transconjugants	640	80–160	—	—	1280	1280- > 10240	> 1024	> 256/5120
S. london	1280	160–320	80–160	320	1280- > 5120	> = 10240	—	—
Transconjugants	160–320	40–80	80	320	1280–2560	> = 10240	—	—
S. choleraesuis	320–1280	160–320	80–160	320	> 5120	> = 10240	—	—
Transconjugants	160	80	80	320	1280	1280	—	—

Abbreviations: Amp = ampicillin; Sm = streptomycin; Tc = tetracycline; Cm = chloramphenicol; Km = kanamycin; Su = sulphadiazine; Tm = trimethoprim; Sxt = co-trimoxazole (trimethoprim/sulphamethoxazole = 1/20)

Table 2. *Distribution of R-plasmids in multiply-resistant* S. typhimurium *strains*

Plasmid incompatibility group	Resistance pattern	No. of strains tested	Phage type of strains	Year of isolation	Resistances: Autotransferrable	Resistances: Mobilized by X	Resistances: Mobilized by Δ	Plasmid: Resistances encoded	Plasmid: fi character	Plasmid: Molecular size (Mdal.)
H_1	AST Su	6	193	1973, 1977 1978	—	AT	SSu	AT	fi⁻	> 143·7
		2	193	1978	AT	—	SSu	AT	fi⁻	> 143·7
	AT	2	193	1973	—	AT	—	AT	fi⁻	> 143·7
	AK	1	193	1973	—	AK	—	AK	fi⁻	> 143·7
	ATK	2	193	1973, 1974	—	ATK	—	ATK	fi⁻	> 143·7
	ATK	2	193	1973, 1974	—	ATK	—	ATK	fi⁻	> 143·7
	AST KSu	3	193	1973	—	ATK	SSu	ATK	fi⁻	> 143·7
	STC Su	1	RDNC	1977	STCSu	—	—	STCSu	fi⁻	> 143·7
F_1me	ASTC Su	1	104a	1975	—	ASTCSu	—	ASTCSu	fi⁺	110
		1	104a	1975	—	ASTCSu	ASTCSu	ASTCSu	fi⁺	86
		3	UT	1980	ASTCSu	—	—	ASTCSu	fi⁺	110
		1	104a	1977	ASTCSu	—	—	ASTCSu	fi⁺	86
H_2	CKSu	1	193	1980	—	CKSu	CKSu	CKSu	fi⁻	> 143·7
		2	193	1980	CKSu	—	—	CKSu	fi⁻	> 143·7
Non-groupable	SC SuTm	1	22	1980	—	SCSuTm	SSu	SCSuTm	fi⁻	64
	ASTCKSuTm	1	UT	1979	—	ASTCKSuTm	SSu	ASTCKSuTm	fi⁻	> 143·7
		6	138,22,UT	1981	ASTCKSuTm	—	—	ASTCKSuTm	fi⁻	> 143·7
	AST KSuTm	1	22	1980	ASTKSuTm	—	—	ASTKSuTm	fi⁻	> 143·7

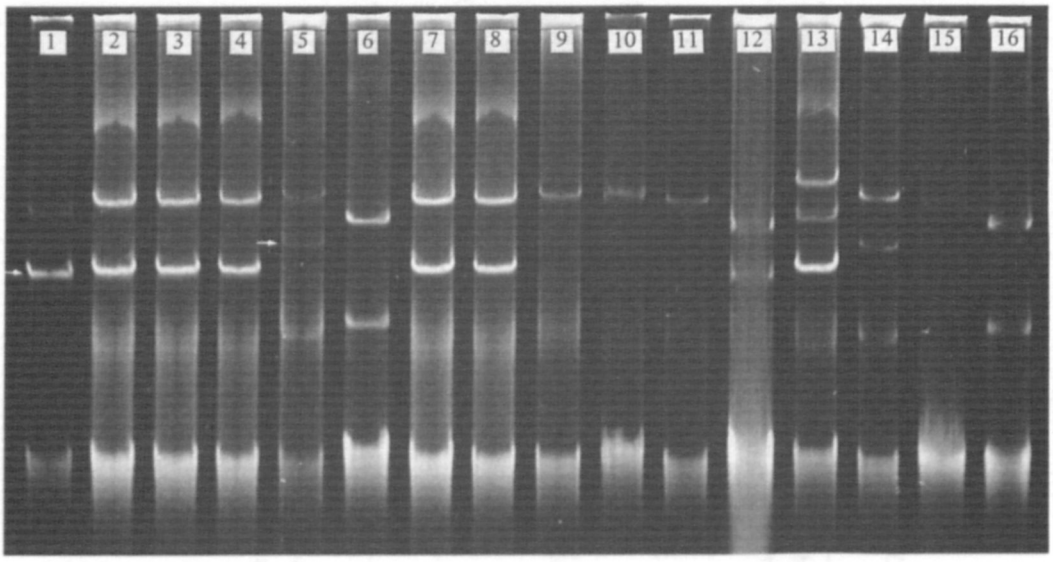

Fig. 1. Agarose gel electrophoresis of groups H_1 and H_2 plasmids extracted from *E. coli* transconjugants of multiply-resistant *S. typhimurium* strains. Group H_1 plasmids in lanes: 1, AK-resistant with the X transfer factor (arrow); 2, ATK-resistant with the X transfer factor; 3, 4, 7 and 8, ATK-resistant from ASTKSu-resistant donor and with the X transfer factor; 5, AT-resistant from ASTSu-resistant donor and with the △ transfer factor (arrow); 9 and 10, AT-resistant from ASTSu-resistant donor; 11, STCSu-resistant. The AT-, ATK- or STCSu-plasmid showed a similar band. Group H_2 plasmids in lanes: 13, CKSu-resistant with the X transfer factor; 14 and 15, CKSu-resistant. Size reference plasmids are contained in lanes: 6, S-a (25·9 MDa.), TP129 (77·6 MDa.); 12 and 16, RP1 (37·8 MDa.), TP125 (64·0 MDa.), TP116 (143·7 MDa.).

H_2 plasmid in one of the strains was non-autotransferring but could be mobilized by both the X and △ transfer factors while that in the other two strains was autotransferring, the transfer being temperature-sensitive. The plasmids in all these strains were *fi*$^-$. Agarose gel electrophoresis of plasmid DNAs extracted from *E. coli* transconjugants which had received the H_2 plasmids are shown in Fig. 1. One of the autotransferring CKSu-plasmid showed two bands, with the uppermost band corresponding to the one band from the autotransferring CKSu-plasmid. This single band most probably corresponded to the H_2 plasmid and had a molecular size $> 143{\cdot}7$ Mdal. Where multiple plasmid bands were observed, the larger band with a similar molecular weight $> 143{\cdot}7$ Mdal. was tentatively identified as an H_2 plasmid.

Nine trimethoprim-resistant *S. typhimurium* strains were studied. These strains belonged to phage types 22 and 138 while some were untypable. Two of the strains tested (ASTCKSuTm and SCSuTm) harboured non-autotransferring plasmids which could be mobilized by the X transfer factor, while the plasmids in the remaining strains were autotransferring. These trimethoprim-resistant plasmids, which were *fi*$^-$, were compatible with all the reference plasmids of known incompatibility groups. There was a common plasmid band amongst these strains which was tentatively assigned as the multiply-resistant plasmid (Fig. 3). The SCSuTm-plasmid had a molecular size of 64 Mdal. while the other plasmids encoding trimethoprim-resistance had molecular sizes $> 143{\cdot}7$ Mdal.

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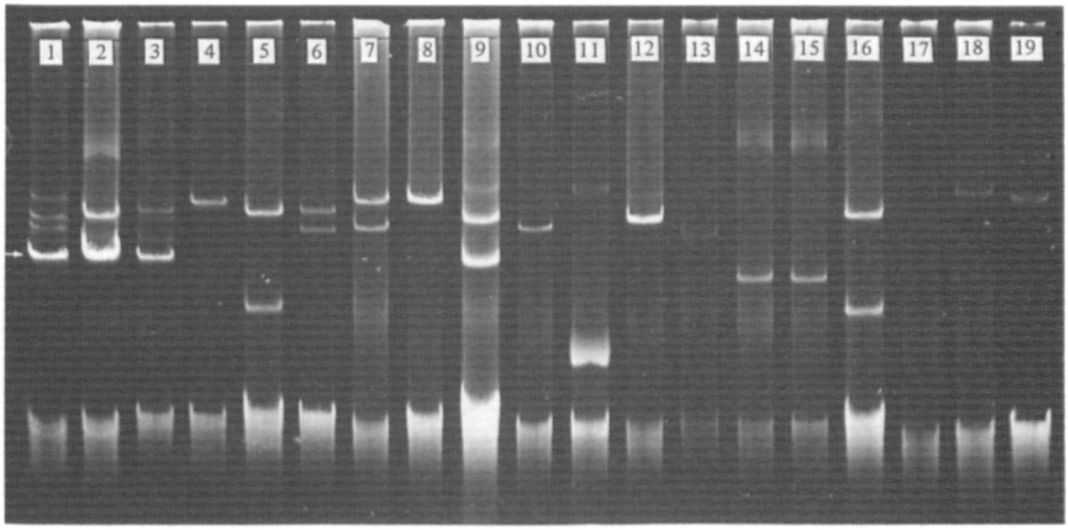

Fig. 2. Agarose gel electrophoresis of group F_1me plasmids extracted from *E. coli* transconjugants of multiply-resistant *S. typhimurium* and plasmids of other salmonella strains. Group F_1me plasmids of *S. typhimurium* in lanes: 1, ASTCSu-resistant and, 2, ASu-resistant, both were from the same ASTCSu-resistant *S. typhimurium* donor and with the X transfer factor (arrow); 3, ASTCSu-resistant, with the X factor; 4, 6, 7 and 8, ASTCSu-resistant. In lane 1, the second band from the top of the gel corresponds to the ASu-plasmid in lane 2, the upper band is most probably the ASTCSu-plasmid. In Lanes 3, 6, 7 and 8, the upper plasmid band is probably the ASTCSu-plasmid. Lanes 10 to 12 contain plasmids of *S. derby*: 10, ASKSuTm-resistant; 11, SCKSu-resistant from ASTCKSu-resistant donor; 12, ASTCSu-resistant. Lanes 13 to 15 contain plasmids of *S. newport*: 13, ASKSuTm-resistant; 14 and 15, ASTK-resistant. Lanes 17 and 18 contain plasmids of *S. london*: 17, ASTCKSu-resistant; 18, ASTCSu-resistant. The ASTK-plasmid of *S. newport* (lanes 14 and 15), the ASTCKSu- and ASTCSu-plasmid of *S. london* (lanes 17 and 18) showed similar plasmid bands. Lane 19 contains AT-resistant plasmid of ASTSu-resistant *S. choleraesuis*. Size reference plasmids are contained in lanes: 5 and 16, S-a (25·9 MDa.), TP129 (77·6 MDa.); 9, RP1 (37·8 MDa.), TP125 (64·0 MDa.), TP116 (143·7 MDa.).

Salmonella johannesburg

The MICs of ampicillin, streptomycin, chloramphenicol, kanamycin and sulphadiazine against 40 multiply-resistant *S. johannesburg* strains isolated throughout the epidemic were similar, being 2560– > 5120 mg/l, 40–160 mg/l, 320–640 mg/l, 2560– > 5120 mg/l and > 10240 mg/l respectively (Table 1). The MICs of tetracycline against ASTCKSu-resistant *S. johannesburg* strains were 320–640 mg/l. The MICs of trimethoprim against all the strains were < 2 mg/l except for four strains which had elevated MICs of 8–16 mg/l. The MICs of co-trimoxazole ranged from 2/40 to 4/80 against these four strains. The MIC range of the drugs against the corresponding *E. coli* transconjugants was similar, being only two or fourfold lower than that against their resistant donors.

A total of 24 multiply-resistant *S. johannesburg* strains with various resistance patterns were selected for plasmid study. All these strains were shown to harbour an F_1me plasmid of size around 110 Mdal. (Chau *et al.* 1982).

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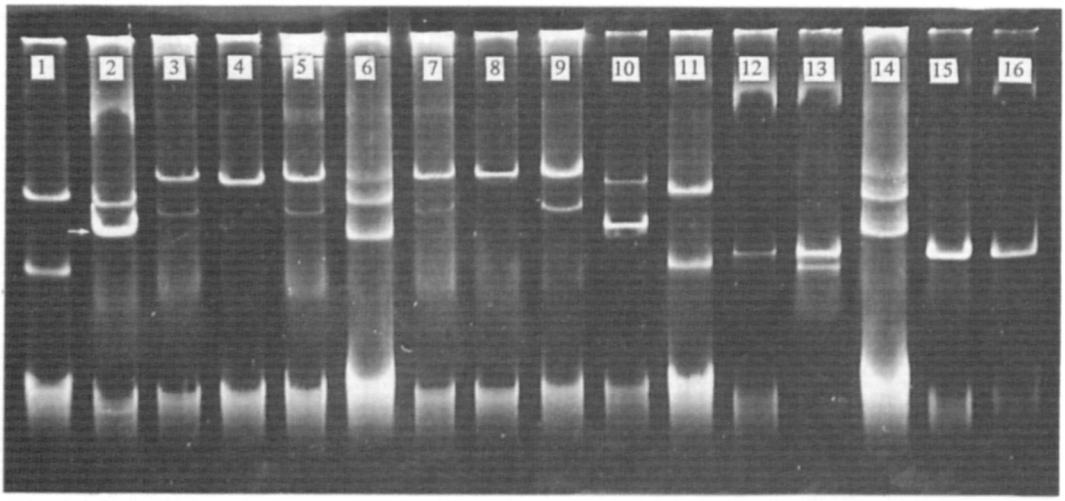

Fig. 3. Agarose gel electrophoresis of trimethoprim-resistant plasmids of *S. typhimurium* strains and plasmids of *S. wandsworth* strains extracted from *E. coli* transconjugants. Trimethoprim-resistant plasmids of *S. typhimurium* in lanes: 2, SCSuTm-resistant with the X transfer factor (arrow); 3, 5, 7, 8 and 9, ASTCKSuTm-resistant; 4, ASTKSuTm-resistant; 10, ASTCKSuTm-resistant with the X transfer factor. The upper band in lanes 3, 5, 7, 8 and 9 is most probably the trimethoprim-resistant plasmid while the lower band in lanes 3, 5 and 7 is the linear form of the upper plasmid band. It is similar in size although the one in lane 10 is slightly smaller probably because it is transfer-defective. Lanes 3, 5, 7 and 9 have similar plasmid contents, indicating that the strains from which they were found were probably related. Plasmids of *S. wandsworth* strains in lanes: 12, 13, 15 and 16: ASK-resistant. One similar plasmid band is observed in all the strains. The faint lower band in lane 13 is probably the linear form of the upper plasmid band. Size reference plasmids are contained in lanes: 1 and 11, S-a (25·9 MDa.), TP129 (77·6 MDa.); 6 and 14, RP1 (37·8 MDa.), TP125 (64·0 MDa.), TP116 (143·7 MDa.).

Salmonella wandsworth

All the ten *S. wandsworth* isolates resistant to ASK were studied. The plasmid conferring resistance to these antibiotics in all the strains belonged to group N, were *fi*$^-$ and were of 30·5 Mdal molecular size (Fig. 3). Subsequent sensitive isolates from the same patient from whom ASK-resistant *S. wandsworth* had been initially isolated showed no plasmid band, indicating that the sensitive isolates may have been derived from resistant ones by losing the plasmid.

Other gastroenteric salmonellae

The MICs of ampicillin, streptomycin, tetracycline, chloramphenicol, kanamycin, sulphonamide and trimethoprim against resistant strains of other salmonellae were also high and were indicative of plasmid-mediated resistances (Table 1).

Although *S. derby* was the most frequently isolated salmonella serotype in Hong Kong, the incidence of multiply-resistant isolates was low. Three multiply-resistant strains (ASKSuTm, ASTCSu and ASTCKSu) detected during 1973–82 were selected for plasmid study (Table 3). The plasmids encoding resistance to ASK-SuTm and ASTCSu were autotransferring, were *fi*$^+$ and *fi*$^+$ respectively, and

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Table 3. *Distribution of R-plasmids in multiply-resistant salmonellae of the gastroenteric group*

Salmonella serotype	Resistance pattern	No. of strains tested	Year of isolation	Plasmid				
				Autotransferable	Mobilized by		Incompatibility group	Molecular size (Mdal.)
					X	Δ		
S. derby	AS KSuTm	1	1979	ASKSuTm	—	—	F_1me	63
	ASTC Su	1	1980	ASTCSu	—	—	F_1me	58
	ASTCKSu	1	1980	SCKSu	—	—	H_2	> 143·7
S. newport	AST K	2	1976	ASTK	—	—	F_1me	36
	AS KSuTm	1	1977	ASKSuTm	—	—	F_1me	49
S. london	ASTC Su	1	1980	ASTCSu	—	—	H_1	> 143·7
	ASTCKSu	1	1980	ASTCKSu	—	—	H_1	> 143·7
S. choleraesuis	AST Su	2	1979, 1980	AT	—	SSu	H_1	> 143·7

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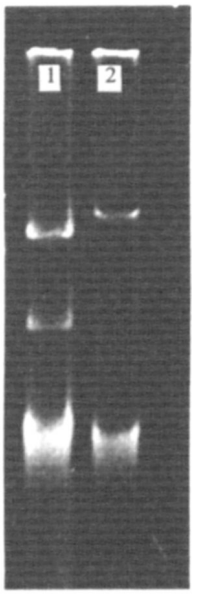

Fig. 4. Agarose gel electrophoresis of plasmid of *S. paratyphi B* strain extracted from *E. coli* transconjugants. Lane 2 contains the plasmid in ATCKSu-resistant *S. paratyphi B*. Size reference plasmids are contained in lane 1: S-a (25·9 MDa.), TP129 (77·6 MDa.).

belonged to group F_1me. The ASKSuTm-plasmid had a molecular size of 58 Mdal. and the ASTCSu-plasmid, 63 Mdal. (Fig. 2). The A- and SCKSu-determinant were transferred as separate units from the ASTCKSu-resistant strain while the T-determinant was non-autotransferable. The SCKSu-plasmid was fi^- and belonged to group H_2. It also had a molecular size > 143·7 Mdal. (Fig. 2).

Three multiply-resistant *S. newport* strains, two resistant to ASTK and one to ASKSuTm, were detected during the period of study (Table 3). All these strains could transfer their resistances which were encoded on an fi^+ group F_1me plasmid. The ASTK-plasmid and the ASKSuTm-plasmid had molecular sizes of 36 and 49 Mdal. respectively (Fig. 2).

The ASTCSu- and ASTCKSu-resistant strains, both isolated in 1980, were the only ampicillin- and chloramphenicol-resistant *S. london* strains detected throughout the period of study (Table 3). The ASTCSu- and ASTCKSu-plasmid in these strains could be transferred and the resistances were encoded on an fi^- group H_1 plasmid with molecular size > 143·7 Mdal. (Fig. 2).

Only two A-resistant *S. choleraesuis* strains (ASTSu) were detected throughout the period of study, one isolated in 1979 and another in 1980 (Table 3). The AT-determinant could be transferred and was conferred by an fi^- group H_1 plasmid of molecular size > 143·7 Mdal. (Fig. 2), while the SSu-resistance was non-autotransferring but could be mobilized by the transfer factors.

Salmonellae of the enteric fever group

All the salmonellae of the enteric fever group were sensitive to ampicillin, chloramphenicol or trimethoprim except for one *S. paratyphi B* strain isolated in 1982 with resistance pattern ATCKSuTm. The minimal inhibitory concentrations (MICs) of ampicillin, chloramphenicol, kanamycin, sulphadiazine, trimethoprim

and co-trimoxazole against this strain were all high, being 2560 mg/l, 320 mg/l, 2560 mg/l > 10240 mg/l, > 1024 mg/l and > 256/5120 mg/l respectively. These multiple resistances could be transferred *in toto* to *E. coli* K12 recipients and were compatible with standard plasmids of groups F_I, F_{IV}, MP10, I_1, I_2, B, N, P, T and J. Incompatibility testing with other standard plasmids could not be performed because of overlap of resistances with the plasmid in question. Agarose gel electrophoresis of the extracted plasmid DNA showed that it had a molecular size > 143/7 Mdal. (Fig. 4). The K-resistance was very rapidly lost from the ATCKSuTm-plasmid so that no colony was found resistant to kanamycin after overnight incubation in broth cultures, while the other resistance markers were very stable and were not deleted singly although the whole plasmid could be lost at a rate of 1%.

DISCUSSION

The present investigation is concerned with the study of antibiotic resistance of an important group of organisms causing diarrhoeal diseases in a specific locality over a substantial period of 10 years. The extensiveness of this study provides a better perspective of the natural distribution of antibiotic resistances and the plasmids coding for these resistances. The results confirm the ubiquitous occurrence of a large variety of R-plasmids in salmonellae and reveal that spread of these plasmids was confined to only certain organisms. Although a large variety of salmonella serotypes were present, only *S. typhimurium* had a high percentage of multiply-resistant and plasmid-harbouring strains, whereas in other salmonellae such multiple resistances and R-plasmids were rare.

In Hong Kong, four different types of plasmids were detected within *S. typhimurium* strains, namely H_1, H_2, F_1me and non-groupable Tm-resistant plasmids. All these plasmids were detected sporadically during the period of study without any obvious clustering, although H_2 and the non-groupable plasmids were only detected from 1979 onwards.

The group H_1 plasmids of *S. typhimurium* encoding resistances to AT, AK or ATK were similar in resistance pattern and incompatibility grouping to those found in *S. typhimurium* strains isolated in Singapore, Malaysia and the United Kingdom (Anderson, 1975) except that in the Hong Kong strains, they were non-autotransferable. This is the first non-autotransferring group H_1 plasmid to have been detected. In Hong Kong in 1978, two similar H_1 plasmids conferring resistance to the same spectrum of antibiotics were isolated from *S. typhimurium*, but these were autotransferring. The autotransferring and non-autotransferring H_1 plasmids were probably related. In the course of evolution, the transfer factor may become attached to or deleted from the plasmid. All these H_1 plasmid-harbouring strains from Hong Kong and elsewhere (Anderson, 1975) belonged to phage type 193. It is possible that these strains originated from the same clone.

Another important feature was the presence in only one *S. typhimurium* strain of a group H_1 plasmid encoding resistance to STCSu, a similar resistance pattern to that detected in many chloramphenicol-resistant *S. typhi* strains (Bissett, Abbott & Wood, 1974; Anderson, 1975).

Group H_2 plasmid has also been reported in other parts of the world, however, it has never been widely distributed (Anderson *et al.* 1977). In *S. typhimurium* in

Hong Kong, the situation is similar in that the occurrence of this plasmid was limited and sporadic.

Plasmids coding for Tm-resistance in Hong Kong has increased in frequency in *S. typhimurium* since it first appeared in 1979 in one of the strains. These plasmids were compatible with all the testable standard plasmids. Threlfall and his colleagues (1980) also reported the initial rare occurrence of Tm-resistant *S. typhimurium* which then became widespread in cattle in 1979 and subsequently in man.

Two transposons, Tn7 and Tn402, encoding Tm-resistance were known and have been found in various plasmids (Richards & Datta, 1981). Thus, detection of Tm-resistant plasmids of different incompatibility groups would not be surprising since the Tm-resistance, being located on a transposon, could be readily translocated from one genetic entity to another. Examples included groups I_1, H_2 and F_1me (Threlfall *et al.* 1980, Frost *et al.* 1982). Whether the Tm-resistant plasmids from Hong Kong belonged to a new incompatibility group requires further studies.

Since *S. typhimurium* has a well-developed phage typing scheme, these multiply-resistant strains were also phage-typed in order to study their epidemiology. They belonged to eight different phage types (Ling, Chau & Rowe, 1987). Apart from phage type 193 which was most prevalent before 1975 and types 100 and RDNC which were detected once only, the other phage types were of similar frequency of occurrence.

Thus, with results of plasmid analysis and phage typing studies, it could be concluded that multiply-resistant *S. typhimurium* strains of Hong Kong were probably different from each other and had originated from different clones except for the H_1-harbouring strains. It seems that these strains were from the same clone and were established before 1977.

In contrast to the situation seen in *S. typhimurium*, only one type of plasmid (group F_1me) was found in *S. johannesburg* and almost all the strains harboured this plasmid. Similarly, in *S. wandsworth*, only one type of plasmid (group N) was found in all the strains isolated during a hospital outbreak. In other salmonellae, R-plasmids coding for multiple antibiotic resistances were found only sporadically and belonged to a variety of groups. In other parts of the world, plasmid harbouring salmonellae other than *S. typhimurium* are also rare and infrequent so that there are few studies on R-plasmids in these salmonellae. However, some widespread epidemics have been investigated in detail. Outbreaks of *S. wien*, *S. newport* and *S. oranienburg* have been shown to involve epidemic strains containing plasmids conferring multiple resistances (McConnell *et al.* 1979; Threlfall, Bhat & Sharma, 1982; Rangnekar *et al.* 1982*a*). Recently, there have been two studies on plasmids of some rarely isolated salmonella serotypes, one was by Vicente & de Almeida (1984) and another by Saxena and his co-workers (1984).

It is worth noting that multiple resistance to five or more antibiotics including chloramphenicol and F_1me plasmids were only detected in salmonellae from 1975 onwards, i.e. only after *S. johannesburg* had become widespread in this locality. These plasmids differed from each other and from those found in *S. johannesburg* with respect to resistance pattern and molecular size. With respect to molecular size, the plasmids in *S. typhimurium* strains isolated in 1975 were similar to those

in *S. johannesburg* and showed much less variation than those detected in *S. newport* or *S. derby* strains isolated in 1976–80. The plasmids detected in this latter group of salmonellae were much smaller than those in *S. johannesburg* and were much more variable in molecular size. It is possible that F_1*me* plasmids were introduced to Hong Kong by *S. johannesburg*. After initial introduction, the plasmid evolved separately, one in *S. johannesburg* and another in other salmonellae. In *S. johannesburg*, the plasmid which was inherently unstable evolved to a more stable one through the deletion of a tetracycline-resistance most probably a transposable element (Chau *et al.* 1982), while in other salmonellae the plasmid underwent rapid genetic change resulting in a large portion of the plasmid being deleted.

In contrast to the group F_1*me* plasmids, all the group H_1 and H_2 plasmids detected in different salmonellae were similar in genetic properties and large molecular size (> 143·7 Mdal.) although some had lost the ability to transfer.

Although plasmids are found in other salmonellae and *S. typhi* is endemic locally, the typhoid bacilli remains largely sensitive to antibiotics. Both the F_1*me* plasmid from *S. johannesburg* and the H_1 plasmid from *S. typhi* isolated in Indonesia (Ling & Chau, 1983) could be easily transferred into a local *S. typhi* strain, however these plasmids were unstable in the new host (results not shown). This may explain why the local *S. typhi* strains have not become infected with R-plasmids from other salmonellae. The multiply-resistant *S. paratyphi B* strain was the only enteric fever group of salmonellae isolated so far in Hong Kong which was resistant to ampicillin, chloramphenicol and trimethoprim.

In contrast to the situation encountered in *S. johannesburg* in Hong Kong and in other salmonellae in other parts of the world, F_1*me* plasmid-harbouring *S. typhimurium* did not seem to spread in Hong Kong, as its occurrence was sporadic and infrequent. As F_1*me* plasmid was known for its widespread occurrence in *S. typhimurium* in the United Kingdom and *S. wien* in North Africa (Anderson *et al.* 1977; McConnell *et al.* 1979), it seemed that this plasmid did not have an advantage over other plasmids in maintaining its occurrence in the local *S. typhimurium* strains and probably did not confer particular advantage to the spread of this organism. In contrast, the groups H_1 and H_2 plasmids comprised of a homogenous population with respect to size and genetic properties although of different host origin. Presumably these plasmids were relatively stable inherently so that genetic change, even if it did occur, was not significant and noticeable.

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