Epidemiol. Infect. (1993). **110**. 437–446

Molecular studies of plasmids of multiply-resistant *Shigella* spp. in Hong Kong

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(*Accepted 25 January 1993*)

SUMMARY

One hundred and two *Shigella* spp. isolated in two hospitals in Hong Kong were analysed for antibiotic resistances, resistance plasmids and plasmid profiles. Three quarters of the isolates were *S. flexneri*. All isolates harboured plasmids, up to a maximum of ten within one strain. Plasmids of 220 kb encoding resistances to tetracycline, chloramphenicol and sulphonamide and probably also associated with invasiveness in the Sereny test were found in 80 strains and were transferable in 18% of cases. Resistance plasmids of 92 and 99 kb were found in 27 and 15 strains respectively and encoded resistances to ampicillin, tetracycline, chloramphenicol, kanamycin, sulphonamide, trimethoprim, cotrimoxazole and gentamicin; these plasmids were usually transferable. Four plasmids of 3·9, 2·8, 2·2 and 1·8 kb were commonly found in *S. flexneri* strains, but were rare in other species. In contrast, there was no predominant plasmid profile in *S. sonnei*. *S. flexneri* is endemic in Hong Kong and these plasmid studies suggest that the strains in circulation are derived from only a few clones.

INTRODUCTION

Infection with *Shigella* spp. is endemic in Hong Kong. *Shigella flexneri* infection is particularly common, often causing dysentery in children severe enough to warrant hospitalization. We have recently shown that multiple antibiotic resistance is common amongst Hong Kong strains of shigella [1]. An analysis of their resistance plasmids was made to examine the resistance mechanism in these organisms and the relatedness between them. We now report the results of a study of the plasmids of these organisms which suggests that the *S. flexneri* isolates are derived from only a few clones.

MATERIALS AND METHODS

Bacterial strains

We studied 102 shigella strains isolated from patients presenting at the Princess Margaret Hospital (June 1986–March 1987) and from the Prince of Wales Hospital (May 1984–March 1987) [1].

Antimicrobial susceptibility tests

The susceptibility of these organisms to ampicillin (A, 16 mg/l), streptomycin (S, 16 mg/l), tetracycline (T, 8 mg/l), chloramphenicol (C, 16 mg/l), kanamycin (K, 8 mg/l), sulphamethoxazole (Su, 128 mg/l), trimethoprim (Tm, 2 mg/l), cotrimoxazole (Sxt, 40 mg/l), gentamicin (G, 4 mg/l), cephalothin (Cth, 16 mg/l), cefamandole (Ma, 16 mg/l) and cefoperazone (Cp, 16 mg/l) was determined by the agar dilution method as previously described [1].

Genetic studies of plasmids

Multiply-resistant strains were tested for resistance transfer or mobilization by the method of Anderson and Threlfall [2] using a standard nalidixic acid-resistant *Escherichia coli* recipient, 14R525 (provided by Dr B. Rowe, Colindale, UK). Plasmids, including high-molecular-weight ones, which could not be transferred or mobilized were transformed according to the method of Hanahan [3].

Molecular studies of plasmids

Crude plasmids in the parent strains and in their transconjugants were extracted according to the method of Kado and Liu [4] and analysed by agarose gel electrophoresis performed with a horizontal gel apparatus (GNA-200, Pharmacia Fine Chemicals AB, Uppsala, Sweden) as described previously [5]. The following strains containing standard plasmids were used as molecular weight markers: 28R823 (221 kb), 40R448 (120 kb), 40R268 (99 kb), RT641 (92 kb), 34R193 (49 kb) and 40R25 (7·2 kb) (provided by Dr B. Rowe); and WG4483 (4·2, 3·5 kb) (provided by Dr W. B. Grubb, Western Australia). Plasmid sizes were estimated using a computer program [6].

Purified plasmids were obtained with QIAGEN anion-exchange mini-columns (DIAGEN GmbH, Dusseldorf, Germany) following the manufacturer's instructions or with modification when the plasmid yield was low. This included increasing the inoculum of the bacterial strains for extraction of plasmids. The purified plasmid preparations were then digested with *Eco*R I or *Hin*d III (Pharmacia) according to the manufacturer's instructions and the resulting restriction fragments were again visualized by agarose gel electrophoresis.

Sereny test

Shigella strains with or without a high-molecular-weight plasmid were subjected to the Sereny test [7] to determine if they were invasive in the guinea-pig eye.

RESULTS

Transferability of resistance plasmids

The 102 isolates examined comprised 77 *S. flexneri*, 24 *S. sonnei* and 1 *S. dysenteriae*. They contained 26 distinct resistance patterns. The commonest

Table 1. *Distribution of resistance patterns in* Shigella *spp.*

No of resistances	Resistance pattern	Resistances	
		Non-transferable	Transferable
1	S	4	
2	ST	2	1
	T Su	2	
3	ST Su	4	4
	Other	2	
4	STC Su	5	14
	ASTC	16	4
	Other	1	2
5	ASTC Su	5	4
	Other	3	1
6	STC SuTmSxt		1
	AST SuTmSxt		1
7	ASTC SuTmSxt		3
	Other	1	4
8	ASTC SuTmSxtG	1	10
	Other		1
9	ASTCKSuTmSxtG		5
11	ASTC SuTmSxtGCthMaCp		1
	Total number of strains	46	56

pattern was ASTC in 20 isolates, but this was transferable to *E. coli* in only 4 isolates (Table 1). The next most frequent patterns were STCSu, which was found in 19 isolates (14 transferable), and ASTCSuTmSxtG, found in 11 isolates (10 transferable). Fifty-six isolates (55%) could transfer their resistances in all or part, including most of those resistant to six or more antibiotics (26 out of 28 isolates).

Plasmid profiles

All strains contained at least 1 and up to 10 plasmids. Isolates containing 4 or 5 plasmids were most abundant (28–33 strains), followed by those containing 3 or 6 plasmids (10–12 strains). There was 1 strain each with 9 or 10 plasmids. All but 6 strains contained plasmids greater than 7·7 kb in size. Some plasmids were common: a high-molecular-weight plasmid (220 kb) with migration similar to the 220 kb standard plasmid was found in 80 isolates, the 92 kb one in 27, and the 99 kb one in 15. Another large plasmid (183 kb) was found in 13 *S. flexneri* isolates but was not seen in other species. Except for 3 *S. sonnei* isolates, the 3·9, 2·8, 2·2 and 1·8 kb plasmids were found only in *S. flexneri* (Figs 1, 2). Twenty-nine *S. flexneri* isolates contained all 4 of these plasmids while 68 contained only the 3·9 and 2·8 kb plasmids. There were 42 other plasmids each of which was found in 1–5 isolates.

There were 68 plasmid types, 2 of which were found in only 1 isolate each. Nine plasmid types were found in more than 1 isolate (Table 2). The most common was type 1 which was found in 16 isolates, while types 2–9 were found in 2–6 isolates. All except type 9, which was found in 2 *S. sonnei* isolates, were found in *S. flexneri*. There was little relationship between plasmid profile and antibiotic resistance pattern.

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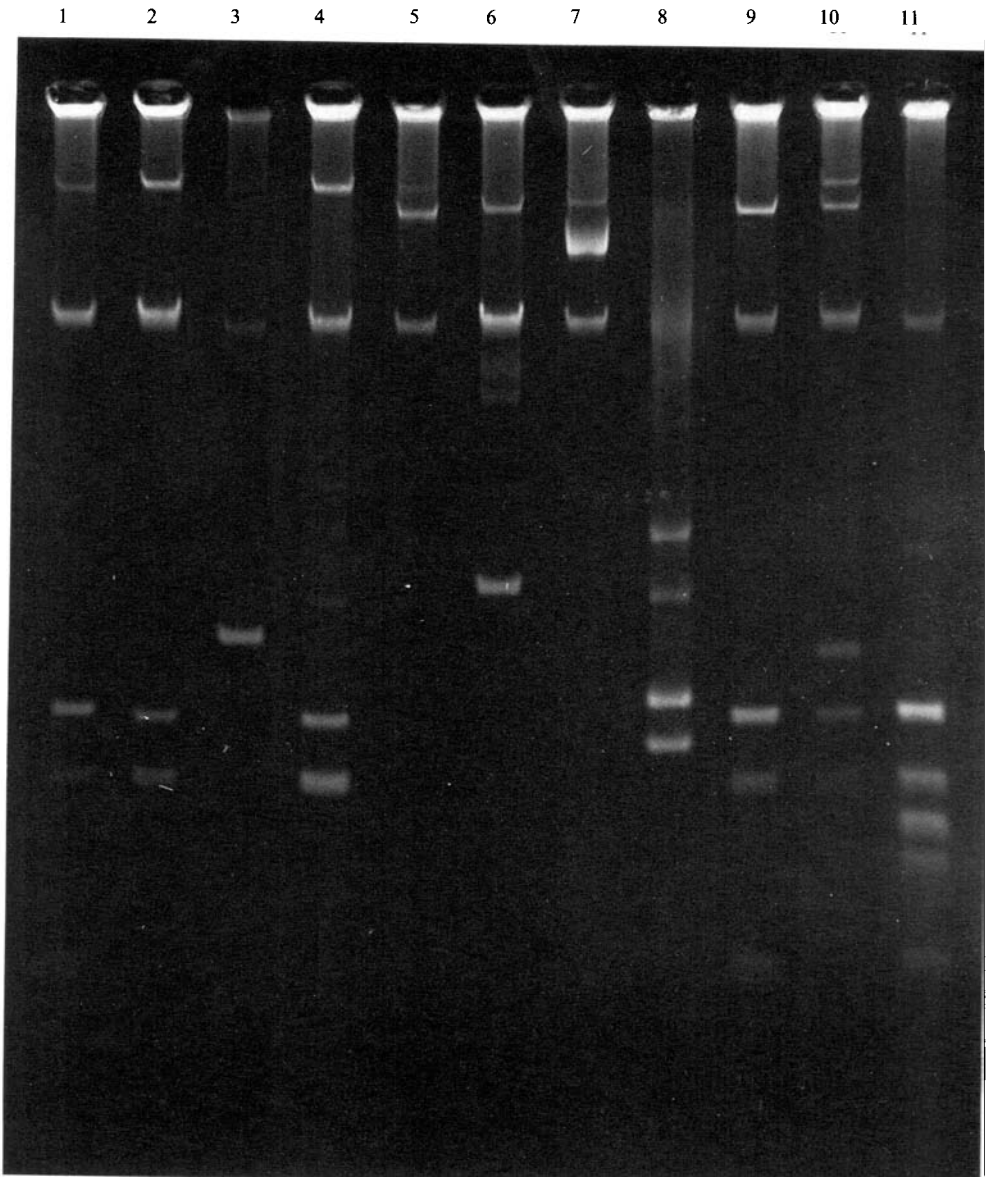

Fig. 1. Agarose gel electrophoresis of plasmid DNA of *Shigella flexneri*. Lanes 1–4 and 9–11 contained plasmid DNA from different *S. flexneri*. Plasmid size markers were in lanes 5 (221, 92 kb); 6 (99, 7·2 kb); 7 (120, 49 kb); and 8 (4·2, 3·5 kb).

Genetic studies of plasmids

Fifty-six of the 102 shigella isolates contained transferable plasmids (Table 1): 4 strains contained a 99 kb plasmid which could be transformed to an *E. coli* recipient. None of the 220 kb plasmid could be transformed. Kanamycin resistance was transferable in only 2 isolates.

Table 3 shows resistance plasmids in isolates with 3 of the more common resistance patterns. Although a 220 kb plasmid was found in 80 isolates, it was

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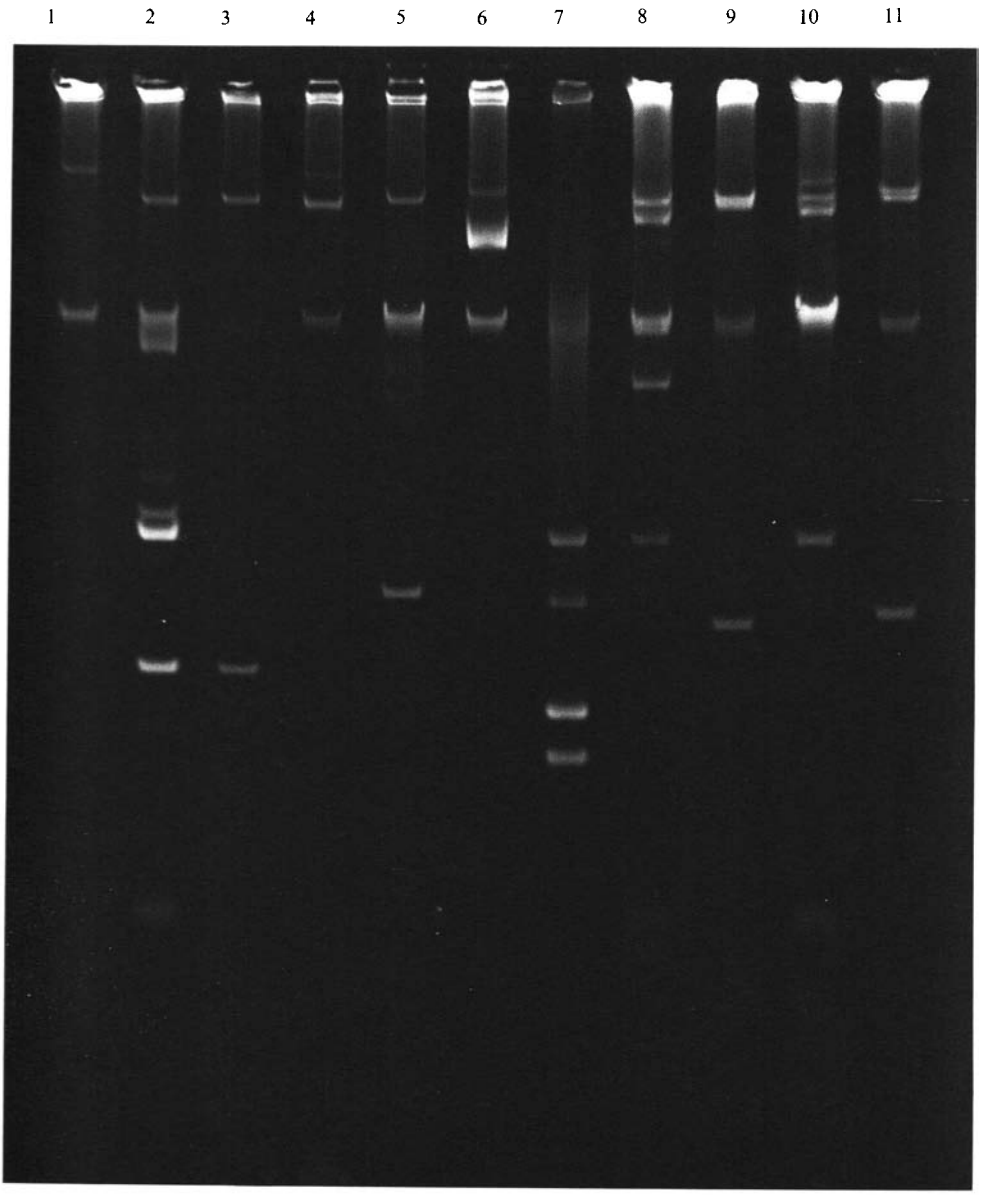

Fig. 2. Agarose gel electrophoresis of plasmid DNA of *Shigella dysenteriae* and *S. sonnei*. Lane 1 contained plasmid DNA from *S. dysenteriae*, lanes 2–3 and 8–11 contained plasmid DNA from *S. sonnei*. Plasmid size markers were in lanes 4 (221, 92 kb); 5 (99, 7·2 kb); 6 (120, 49 kb); and 7 (4·2, 3·5 kb).

transferable in only 14 of them. Eight of these plasmids decreased in size after transfer. The 220 kb plasmids usually encoded tetracycline, chloramphenicol and sulphonamide resistances. Tetracycline resistance was also encoded on plasmids of 49 and 92 kb. Strains which harboured a non-transferable 220 kb plasmid often contained transferable resistances to ampicillin, chloramphenicol, sulphonamide, trimethoprim, gentamicin (and occasionally tetracycline and kanamycin) encoded

Table 2. *Plasmid types found in two or more shigella isolates*

	Plasmid (molecular size (kb))											No of strains	
Type	220	183	120	92	7·2	6·1	5·0	3·9	2·8	2·2	1·8	0·8	*S. flexneri*	*S. sonnei*
1	+							+	+	+	+		16	0
2	+							+	+				6	0
3	+	+						+	+				5	0
4							+						4	0
5	+	+				+		+	+				3	0
6	+			+				+	+				3	0
7	+			+			+	+	+				2	0
8	+							+	+	+	+	+	2	0
9			+	+	+							+	0	2

on 99 or 92 kb plasmids. Streptomycin resistance was sometimes encoded by a 3·9 kb plasmid which was transferable in only 2 isolates. Ten isolates harboured 2 transferable plasmids.

Four transferable and 21 non-transferable plasmids of 221 kb were subjected to digestion by restriction endonucleases *Eco*R I and *Hin*d III. The 4 transferable plasmids showed 2 different restriction patterns (Fig. 3*a*). Fourteen of the non-transferable plasmids produced identical restriction fragments while the rest produced different fragments. All eleven 92 kb and five 99 kb transferable plasmids similarly digested produced different restriction patterns (Fig. 3*b*).

Sereny test

Shigella isolates which contained a 220 kb plasmid produced inflammation in the guinea-pig eye within 48 h at an inoculum of 10^8–10^9 while those which did not contain this plasmid produced no effect.

DISCUSSION

This study showed that shigella isolates in Hong Kong commonly harboured multiple plasmids, up to a maximum of ten within one strain, but in only 54% of these isolates were the plasmids transferable. This figure is much lower than the 73% reported by Chun and colleagues [8] but is slightly higher than the 45% reported by Frost and Rowe [9]. There were three main transferable plasmids: one of 220 kb which usually encoded resistance to tetracycline, chloramphenicol and sulphonamide, and plasmids of 92 and 99 kb, which encoded resistance to ampicillin, chloramphenicol, sulphonamide, trimethoprim, cotrimoxazole and gentamicin. However, there was an inconsistent relationship between resistance pattern and plasmid profile.

Several studies have reported a relationship between the presence of a c. 220 kb plasmid and virulence [10, 11]. We found that Hong Kong strains containing a 220 kb plasmid were invasive in the Sereny test while those lacking the plasmid were non-invasive.

Most of the other plasmids were less than 8 kb in size. Plasmids of 3·9 and 2·8 kb were found in 68 *S. flexneri*, and these together with plasmids of 2·2 and 1·8 kb

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Table 3. *Transferable resistance plasmids in some isolates with more common resistance patterns*

Resistance pattern	Number of strains of *S. flexneri*	Number of strains of *S. sonnei*	Plasmids (molecular size (kb))	Resistance plasmids: kb	Resistance plasmids: Resistances transferred
STCSu	4	0	220, 3·9, 2·8	220	TCSu(3)+
	2	0	220, 92, 3·9, 2·8	92	TC
	1	0	220, 49, 3·9, 2·8	220	TSu
	5	0	220, 183, 3·9, 2·8	220	TCSu
	1	0	220, 183, 6·1, 3·9, 2·8	220	TCSu
	1	0	220, 137, 3·9, 2·8	220	TCSu
ASTCSuTmSxtG	0	1	92, 6·1, 1·8	92	T
	1	0	220, 3·9, 2·8	173	TCSu
	1	0	220, 108, 11·7, 3·9, 2·8, 2·2, 1·8	108	ASuTmSxtG
	0	1	220, 99, 49, 8, 7, 1·5, 1·4	99	ACSuTmSxtG
				49	T
	1	0	220, 120, 5·7, 39, 2·8, 0·8	220	TSu
				120	ASSuTmSxtG
	0	1	220, 8·2, 7·2, 1·4, 0·8	92	ACSuTmSxtG
	1	0	220, 79, 10·9, 7·2, 3·9, 0·8	99	ACSuTmSxtG
	1	0	220, 92, 10·9, 8·7, 7, 39	92	ACSuTmSxtG
ASTCKSuTmSxtG	1	0	220, 49, 5·7, 39, 2·8, 2·2, 1·8, 0·8	220	ASTCKSuG
	0	1	99, 5·7, 1·3	92	ACSuTmSxtG
	0	1	220, 92, 18, 7·2, 0·8, 0·7	220	ACKSuTmSxt
	0	1	220, 99, 92, 49, 8·2, 7·2, 0·5	92	ACSuG
				49	T

+ Number of strains in which the 220 kb plasmid was reduced in size after transfer.

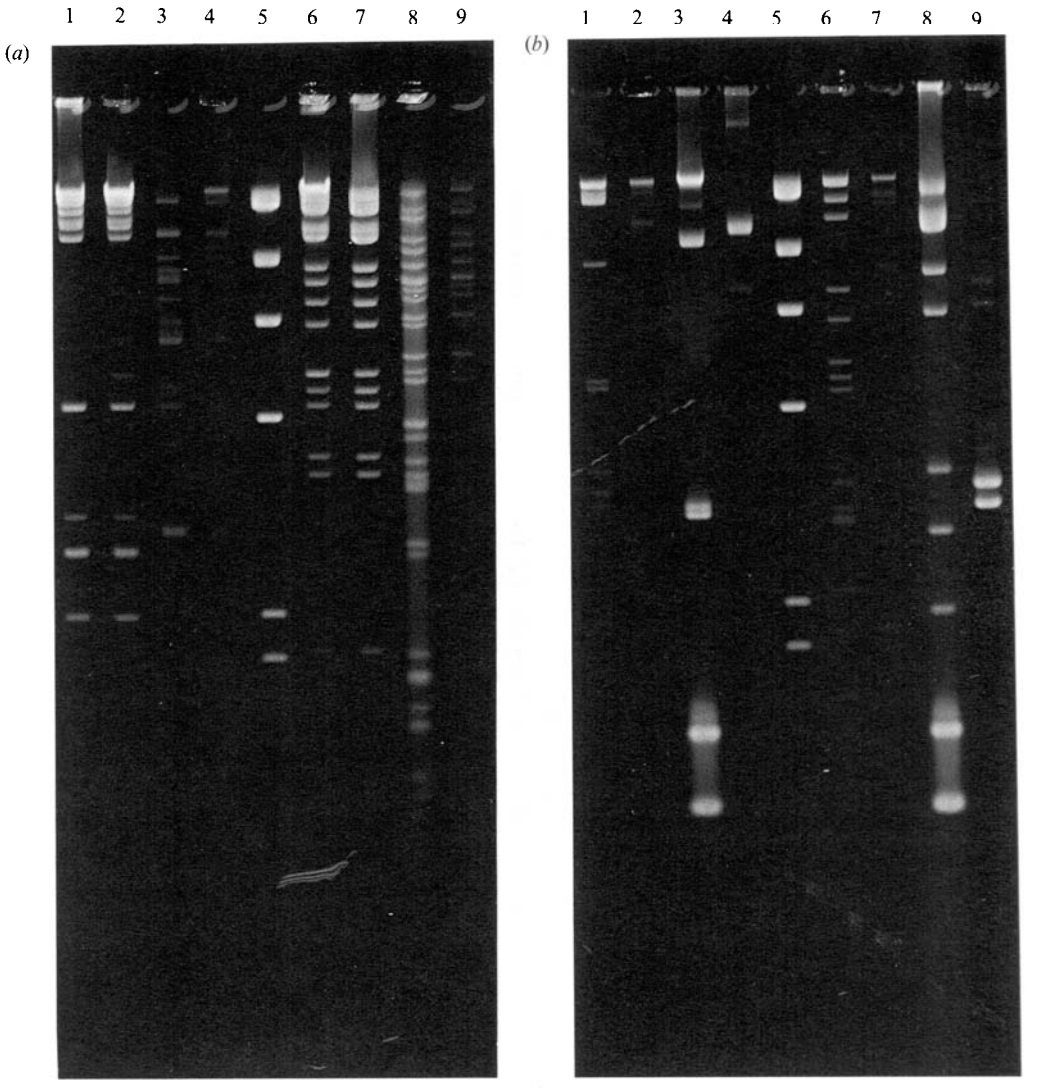

Fig. 3. (*a*) Restriction enzyme digested fragments of the 220 kb plasmid of *S. flexneri*. Lanes 1–4 contained the plasmid from four different strains digested with *Hin*d III. Lanes 6–9 contained *Eco*R I digests of the same plasmids as in lanes 1–4. Lane 5 contained *Hin*d III-digested lambda. (*b*) Restriction enzyme digested fragments of the 99 and 92 kb plasmids of *S. flexneri* and *S. sonnei*. Lanes 1 and 2 contained *Hin*d III digests of the 99 kb plasmid from *S. flexneri* and *S. sonnei* respectively, and lanes 3 and 4 contained *Hin*d III digests of the 92 kb plasmid from *S. flexneri* and *S. sonnei* respectively. Lanes 6–9 contained *Eco*R I digests of the same plasmids as in lanes 1–4. Lane 5 contained *Hin*d III-digested lambda.

were found in a further 29 isolates of this species. These four common plasmids suggested that the Hong Kong population of *S. flexneri* is fairly homogeneous is derived from just a few circulating clones. Similar observations have been reported by other workers in Sicily, Dhaka, Tokyo and Ethiopia [12–15]. In contrast, Hong Kong isolates of *S. sonnei* yielded a variety of plasmid profiles. indicating wide genetic diversity in this species. This is a similar finding to that

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observed by Jamieson and colleagues in Auckland [16] and Albert and colleagues in Central Australia [17].

Restriction endonuclease digestion of some 220 kb plasmids produced similar or identical fragments while some produced different fragments. This suggests that there is a 220 kb plasmid which is endemic in strains of *S. flexneri* and *S. sonnei*. In contrast, the 92 and 99 kb plasmids yielded different restriction patterns, indicating that they are quite different plasmids.

In summary, 220 kb plasmids encoding multiple antibiotic resistance and probably the virulence characteristic of invasiveness were commonly found in shigella isolates in Hong Kong. Four plasmids of 3·9, 2·8, 2·2 and 1·8 kb were found only in *S. flexneri*, suggesting that there was a clonal spread of this organism. In contrast, there appeared to be many different strains of *S. sonnei* in circulation in Hong Kong.

ACKNOWLEDGEMENTS

We thank Ms Y.-W. Hui and Mr T. H. S. Woo for technical assistance. This study was supported by a grant from the University and Polytechnic Grants Committee.

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