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Plasmid profiles of drug resistant *Shigella boydii* types 1–5, 8, 10, 12–14 from Ethiopia (1974–85)

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SUMMARY

Plasmid profile analysis by agarose gel electrophoresis was performed on 42 drug resistant strains of *Shigella boydii* serotypes 1–5, 8, 10, 12–14, collected between 1974 and 1985 from endemic cases of shigellosis in Ethiopia, and their *Escherichia coli* K12 transconjugants. Resistance factors (R factors) were further characterized by incompatibility testing.

Patterns of small plasmids, less than 15 kb, were similar within each of the individual *S. boydii* serotypes. Plasmids of about 3·3–3·7 kb were found in all strains of serotypes 2 and 4. Plasmids of about 4·3–4·6 kb were found in about 86% of strains. Serotypes 1, 2 and 3 were characterized by plasmids of about 5·6–5·7 kb. The 6·4–6·7 kb plasmid was found consistently in serotypes 1, 2, 3, 5, 8, 12 and 13 which were resistant to SSu or had an SSu resistance component in their phenotypes. Large plasmids (155–186 kb) were found in most *S. boydii* strains.

Conjugative drug resistance plasmids, most often coding for three or less drugs, were found in about 26% of drug resistant strains. R-factors, coding for AT resistance (in types 2 and 8), and ASSuT resistance (in type 4), were compatible with all reference plasmids tested. Plasmids belonging to incompatibility groups X and N were found in serotypes 5 and 10, respectively.

INTRODUCTION

Shigellosis is hyperendemic in developing countries, where conditions favouring its transmission still exist. These countries often suffer from severe outbreaks or epidemics of serious proportions. Nevertheless, laboratory investigation of the aetiological agents has not been adequate. Limited reports are available in Ethiopia on the prevalence of various serotypes [1, 2], and their drug resistance patterns [3, 4]. Reports of R-plasmids, studies on plasmid profiles or R-plasmid characterization have concentrated on *S. dysenteriae* and *S. flexneri* [5–8]. These species comprise more than 80% of the total shigella isolates in Ethiopia, while *S. boydii* contributes only 13–18% [9, 10]. However, although *Shigellae* are excellent plasmid hosts, the studies on *S. dysenteriae* and *S. flexneri* demonstrated similarities and differences between them and between their serotypes.

Previous reports in Ethiopia have shown the relative paucity of drug resistance in *S. boydii* strains [4]. Studies of plasmid profiles in *S boydii* are extremely rare. The study reported in the present communication was undertaken, therefore, to determine the plasmid profile patterns of *S. boydii* strains from Ethiopia and to see whether the limited resistance in this shigella serogroup is plasmid encoded or not.

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MATERIALS AND METHODS

Shigella strains used

Shigella boydii recovered from cases of endemic shigellosis referred to the National Research Institute of Health were used. These were individual isolates without any recognizable epidemiological link, coming from different parts of town at different times. A total of 136 strains was collected between 1974 and 1985, and stored at −70 °C in trypticase soy yeast broth with 25% (v/v) glycerol. A total of 42 randomly selected strains, representing ten *S. boydii* serotypes and collected between 1974 and 1985, were then subjected to plasmid profile analysis and R-plasmid characterization.

Antibiotic susceptibility testing

Sensitivity tests were done according to Bauer and colleagues [11]. The results were recorded as sensitive, intermediate or resistant. Antimicrobial agents tested included: ampicillin (A), chloramphenicol (C), gentamicin (G), kanamycin (K), polymyxin B (Px), streptomycin (S), sulphadiazine (Su), tetracycline (T), and trimethoprim (Tp). The 'i' with these abbreviations refers to partial (intermediate) resistance.

Genetic drug resistance transfer

Direct transfer of plasmids was examined by the method of Anderson and Threlfall [12]. Broth cultures of donor shigella strains and the recipient strain (*Escherichia coli* K12, F^-, Lac^+, nal^r, prototrophic) were grown to exponential phase with continuous agitation at 37 °C. Equal volumes (0·5 ml) of the cultures were mixed and incubated overnight at 28 and 37 °C. After incubation, serial tenfold dilutions were prepared in phosphate buffer and 0·01 ml volumes were spread with a calibrated loop on MacConkey agar containing antimicrobial agents. Appropriate dilutions were spread on MacConkey agar without antibiotics to obtain colony counts of each parent. The plates of selective media were incubated overnight at 37 °C, and transconjugant colonies were counted. From each selective plate, 5–10 colonies were resistance typed by agar dilution methods.

An attempt was made to mobilize non-conjugative plasmids by triparental crosses [12] with the Fi^+, group FII plasmid X and the Fi^-, group I, plasmid △ (Enteric Reference Laboratory nos. 48R626 and RT641, respectively). The procedure was similar to that used to detect direct transfer except that 0·5 ml of donor (containing X or △) and 0·5 ml of wild strain were incubated for 18 h at 37 °C before the addition of the final recipient (*E. coli* K12).

Plasmid extraction

Plasmid DNA from wild-type *S. boydii* isolates and *E. coli* K12 recipients [12] was extracted according to the method of Birnboim and Doly [13]. Eppendorf type 1·5 ml polypropylene tubes and a bench-top centrifuge (Anderman 5412), capable of generating 8–10000 g, were used. All chemicals used were Analar standard or equivalent, and were from BDH (Poole, Dorset, UK) or Sigma (St Louis, MO, USA).

Agarose gel electrophoresis

Agarose (0·7%) concentration was heated and dissolved in TE buffer (40 mM Tris-acetate, 2 mM disodium EDTA, pH 8·0). The agarose was allowed to solidify at room temperature in a horizontal gel apparatus (BRL, Model H4). About 35 μl of plasmid DNA from strains of *S. flexneri* and *E. coli* K12 transconjugants was mixed with 6 μl of tracking dye [14] (0·1% bromocresol purple and 50% glycerol), and exposed to a constant voltage of 150 V for 3·5 h. Gels were then soaked in an aqueous solution of 0·5 μg/ml ethidium bromide for 1 h. Finally, plasmids were visualized on a Blak-Ray model 61 ultraviolet transilluminator (Ultraviolet Products, San Gabriel, CA, USA) and photographed with MP4 land camera (Polaroid Corporation, Cambridge, MA, USA) using type 57 land film and a number 9 orange wratten filter (Eastman Kodak Co., Rochester, NY, USA) [15].

Molecular weight determination

Molecular weights of plasmids were determined in relation to the mobility of reference plasmids carried in *E. coli* 39R861 (NCTC no. 50192, harbouring plasmids of molecular weight 152, 65, 37 and 7·1 kb). *Escherichia coli* V517 [16] with plasmids of 55·5, 7·4, 5·7, 5·3, 4·0, 3·1 and 2·8 kb; and *E. coli* with plasmids TP 116 (222 kb) and TP 124 (186 kb) (Plasmid Reference Centre, Stanford, CA, USA) were also used. These strains were routinely included to check the suitability of the mini-preparation of Birnboim and Doly [13] for the detection of large and small plasmids.

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Table 1. *Plasmid profiles of* Shigella boydii

	Strain*/year	R-type†	Plasmid profiles, size in kilobases‡														
1	C1-388/80	SSuT	—	*112*	—	—	—	*7·6*	—	5·6	—	4·7	—	—	2·9	2·8	—
2	C1-874/84	SSuT	186	—	25	15	8·7	7·6	6·4	—	—	—	—	—	2·9	—	—
3	C1-178/78	AST	186	—	60	—	—	7·6	—	5·6	—	4·7	—	—	2·9	2·8	—
4	C1-903/84	S	—	—	—	—	—	7·6	—	5·6	—	4·7	—	—	2·9	2·8	—
5	C1-719/83	T	186	—	60	—	—	7·6	—	5·6	—	4·7	—	—	2·9	2·8	—
6	C2-461/80	ASSuT	—	—	*74*	—	—	—	6·4	5·7	—	4·3	3·3	—	—	—	—
7	C2-187/78	SSuT	170	—	—	—	—	7·1	6·4	5·7	—	4·3	3·3	—	—	—	—
8	C2-787/83	SSuT	186	—	—	—	—	—	6·4	5·7	—	4·3	3·3	—	—	—	—
9	C2-839/84	AT	170	—	*74*	—	—	—	—	5·7	—	4·3	3·3	—	—	—	—
10	C2-620/84	SSu	—	—	—	—	—	—	6·4	5·7	—	4·3	3·3	—	—	—	—
11	C2-775/83	SSu	—	—	—	—	—	—	6·4	5·7	—	4·3	3·3	—	—	—	—
12	C2-926/85	T	—	—	—	—	—	—	—	5·7	—	4·3	3·3	—	—	—	—
13	C3-777/83	SSu	186	—	—	—	—	7·6	6·7	5·6	—	4·7	—	3·1	2·9	2·8	—
14	C3-963/85	SSu	—	—	—	—	—	7·6	6·7	5·6	—	4·7	—	3·1	2·9	2·8	—
15	C4-528/81	ACSSuT	140	—	—	—	—	7·1	—	—	—	4·7	3·4	—	—	—	2·5
16	C4-783/83	ASSuT	155	*93*	—	—	—	7·1	—	—	—	4·7	3·4	—	—	—	2·5
17	C4-679/84	ACST	155	—	—	—	—	—	—	—	—	4·7	3·4	—	—	—	—
18	C4-907/84	ACST	155	—	—	—	—	—	—	—	—	4·7	3·4	—	—	—	2·5
19	C4-956/85	ACST	155	—	—	—	—	7·1	—	—	—	4·7	3·4	—	—	—	2·5
20	C4-704/83	ACST	—	—	—	—	—	7·1	—	—	—	4·7	3·4	—	—	—	2·5
21	C4-223/79	AKiS	170	*105*	—	—	10	—	—	—	—	4·7	3·4	—	—	—	2·5
22	C4-896/84	SSuT	170	—	*23*	13	9·3	—	—	—	—	4·7	3·4	—	—	2·8	—
23	C4-293/79	iST	170	105	—	—	10	—	—	—	—	4·7	3·7	—	—	—	2·5
24	C4-224/79	Su	—	105	—	—	10	—	—	—	—	4·7	3·7	—	—	—	2·5
25	C5-240/74	ACSSuT	—	—	*62*	—	—	—	*6·7*	—	*5·3*	4·0	—	—	2·9	—	—
26	C5-240/79	SSu	155	—	—	—	—	—	—	—	—	—	—	—	—	—	—
27	C5-246/79	S	155	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	C8-337/80	ASSuT	155	*87*	—	—	—	—	6·4	—	—	4·7	3·3	3·1	—	2·8	—
29	C8-444/80	SSuT	155	105	—	—	—	—	—	—	5·3	4·7	—	—	—	2·8	—
30	C8-469/80	SSuT	155	105	—	—	—	7·4	—	—	5·3	4·7	—	—	—	2·8	—
31	C8-779/83	SSuT	—	—	—	11·2	—	7·4	—	—	5·3	4·7	—	—	—	2·8	—
32	C8-775/83	iSSuT	—	—	*57*	*11·2*	—	*7·4*	—	5·6	—	4·7	—	—	—	—	—
33	C8-360/80	AT	186	—	*74*	—	—	*7·4*	—	5·6	5·1	4·7	—	—	2·9	—	—
34	C8-033/76	iST	—	99	—	—	—	7·4	—	5·6	5·1	4·7	—	—	2·9	—	—
35	C10-020/76	SSuT	—	*112*	—	—	—	—	—	—	—	4·5	4·0	—	2·9	2·8	—
36	C10-252/79	SSuT	170	*112*	—	—	—	—	—	—	5·4	4·5	3·3	—	—	2·8	—
37	C10-358/80	SSu	—	—	—	—	—	—	—	—	—	4·5	3·3	—	—	2·8	—
38	C12-229/78	iSSu	186	122	—	—	—	—	6·7	—	—	4·7	3·7	—	2·9	—	—
39	C13-983/85	SSu	186	93	78	—	—	—	6·7	5·6	5·1	4·7	—	—	2·9	—	—
40	C14-817/84	CSSuTTm	186	—	—	—	—	—	—	—	—	4·1	3·6	—	—	—	—
41	C14-801/83	ACSSuT	—	—	—	—	—	—	—	—	—	4·5	4·0	—	2·9	2·8	—
42	C14-334/80	SSu	—	—	—	—	—	—	—	—	—	4·3	—	—	—	—	—

Key: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphadiazine; T, tetracycline; Tm, trimethoprim; 'i' refers to partial resistance.
* C serotype strain/year. † R type, Resistance type. ‡ Transferable plasmids in italics.

Table 2. Shigella boydii: *resistance plasmids transferred to* Escherichia coli *K12* hosts*

No.	Strain/year	R-type†	*Escherichia coli* K12 hosts R-type	Plasmid(s)‡ Size (kb)			Plasmid name (Inc group)
1	C1-388/80	SSuT	SSuT	112	7·6		—
2	C2-461/80	ASSuT	AT	74			pYH22a (UNC)§
3	C2-839/84	AT	AT	74			pYH22b (UNC)
4	C4-783/83	ASSuT	ASSuT	93			pYH23 (UNC)
5	C4-233/79	AKiS	AK	105			pYH24 (NT)¶
6	C5-240/74	ACSSuT	ACSSuT	62	6·7	5·3	—
			ACT	62			pYH25 (X)
7	C8-755/83	iSSuT	iSSuT	57	11·2	7·4	—
			SuT	57	11·2	7·4	—
8	C8-337/80	ASSuT	AT	87			pYH26 (UNC)
9	C8-360/80	AT	AT	74	7·4		—
10	C10-020/76	SSuT	SSuT	112			pYH21b (N)
11	C10-252/79	SSuT	SSuT	112			pYH21c (N)

* *Escherichia coli* K12, F^-, Lac^+, Nx^r, prototrophic; Enteric Reference Laboratory number 14R525.
† Resistance type.
‡ Plasmids were correlated with parent plasmid profile.
§ Compatible with all reference plasmids tested (unclassified).
¶ Lack of a suitable marker (not tested).

R-plasmid incompatibility testing

Escherichia coli K12 carrying single plasmids and derived by mating experiments [12] with *S. boydii* isolates were, in turn, mated with bacterial strains (NCTC) carrying reference plasmids of the following incompatibility groups: B, C, D, FI, FII, FIII, FIV, FV/OF, H_1, H_2, H_3, I_1, I_2, J, K, M, N, P, T, U, W, X, FIme and MP10. Incompatibility testing was undertaken by the method of Grindley and co-workers [17] and Anderson and Threlfall [12].

Plasmid designation

Plasmid designation was according to the recommendations of Novick and co-workers [18].

RESULTS

The results of plasmid profile studies in the ten different *S. boydii* serotypes from Ethiopia are shown in Table 1. It is interesting to note that small plasmids less than 8 kb occur in 40 of the 42 strains examined. Strains belonging to the same serotype tended to have similar patterns of plasmid carriage. Plasmids of 4·3–4·6 kb were found in 36 of 42 (85·7%) of strains tested. *Shigella boydii* types 1, 2, 3, 7, 8, 12 and 13 with SSu-resistance component in their R-types showed plasmids of about 6·4–6·7 kb. Plasmids of about 5·6–5·7 kb characterized *S. boydii* types 1–3, and a plasmid of about 3·3–3·7 was found in all strains of types 2 and 4. Large plasmids of about 155–186 kb were found in representatives of the *S. boydii* serotypes tested but not all strains.

The characteristics of R-plasmids of *S. boydii* that could be transferred to *E. coli* K12 are shown in Table 2. Conjugative plasmids were found in 11 of 42 (26·2%) strains tested. Most of these R-plasmids coded for resistance to few (3 or fewer) drugs. Plasmids coding for AT and ASSuT were compatible with all reference plasmids tested. Incompatibility groups X and N were found in types 5 and 10, respectively.

Transferable drug resistance was detected in strains of serotypes 1, 2, 4, 5, 8 and 10. Attempts to mobilize other resistance plasmids were unsuccessful.

DISCUSSION

It has been reported that African and Asian strains of *Shigella dysenteriae* type 1 show multiple species of small cryptic plasmids [19, 20]. Examination of strains of *S. dysenteriae* and *S. flexneri* from Ethiopia illustrated the prominence of such plasmids [5–8]. A study of 12 strains of *S. boydii* isolated in USA showed plasmids of molecular weight 20, 18, 16·2, 12,

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5·5, 4·1, 3·8, 2·5, 1·5 and 1·4 kb. No relationship to serotype was reported [21]. The result of the present survey of *S. boydii* confirmed the ubiquitous distribution of these plasmids. It is interesting to note that the type and number of these plasmids seemed in this study to be related to specific serotypes (Table 1). Plasmids of about 4·3–4·6 kb were found in 36 of 42 (85·7%) strains tested. In addition, plasmids of about 5·6–5·7 kb were found in serotypes 1–3, and plasmids of about 3·3–3·7 kb were found in all isolates of types 2 and 4. There is remarkable uniformity of plasmid profile through the period of study within the serotypes. This finding must be tempered by recognition of the small numbers of strains. However, within serotypes 1, 2, 4 and 8, common patterns are discernible coupled to a limited variability that may be useful for epidemiological investigation of these strains.

Shigella boydii types 1, 2, 3, 7, 8, 12 and 13 with SSu-resistance component in their R-types showed plasmids of about 6·4–6·7 kb. It is now well established that, at least for the Shiga bacillus from Africa, plasmids of about 6·5 kb code for SSu resistance [19]. Historically, a group of small, non-autotransferable plasmids were frequently observed in a wide range of Gram-negative bacteria [22, 23]. Many of these conferred resistance to streptomycin and sulphonamides – two of the earliest drugs to enter clinical practice. According to the World Health Organization [24], the dissemination of these plasmids was characterized as a 'global epidemic'.

Large plasmids (155–186 kb) were found in all *S. boydii* serotypes. Circumstantial evidence, which included the results obtained by the DNA hybridization technique, suggested that a large plasmid in *S. boydii* is responsible for bacterial invasiveness [25, 26]. Studies also gave direct evidence that a plasmid of about 186 kb is responsible for epithelial cell penetration in *S. boydii* and further investigations have implicated a 31 kb plasmid borne fragment [14, 27]. This finding may explain the diversity of molecular size among large plasmids from virulent *Shigellae*.

Earlier studies from Ethiopia showed that over 50% of *S. boydii* strains were sensitive to commonly available drugs [4]. The present study also showed the relative paucity of conjugative plasmids in *S. boydii*. Plasmids coding for AT resistance in *S. boydii* types 2 and 8, and a plasmid coding for ASSuT resistance in type 4 were compatible with all reference plasmids at our disposal. Plasmids pYH21b and pYH21c (112 kb, Inc N coding for SSuT resistance) were found in serotype 10. A single Inc X plasmid coding for ACT resistance was found in *S. boydii* type 5 isolated in 1974.

Attempts to mobilize non-conjugative plasmids were unsuccessful. In previous studies on *S. dysenteriae*, from Ethiopia, SSu determinants in serotype 3 could be mobilized. However, in these same studies CSSu determinants could not be mobilised [5]. On this basis it might have been expected that the SSu determinants found in *S. boydii* serotypes 3, 12, 13 and 14 could have been mobilized but this was not the case.

It is hoped that the present data will be useful for future surveillance of this comparatively rare *Shigella* species.

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