Plasmid profiles of antibiotic-resistant *Shigella dysenteriae* types 2, 3, 4, 6 and 7 isolated in Ethiopia during 1976–85

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SUMMARY

Plasmid profile analysis by agarose gel electrophoresis was carried out on 37 drug-resistant strains of *Shigella dysenteriae* types 2, 3, 4, 6 and 7. These strains were collected between 1976 and 1985 in Addis Ababa, Ethiopia.

The plasmid profile of *S. dysenteriae* type 2 strains with R-type CSSuT did not show middle-sized plasmids likely to code for CSSuT resistance. All strains contained a large plasmid of about 120 megadaltons (MDa), and a cryptic plasmid of about 2·2 MDa. The plasmid profiles of *S. dysenteriae* type 3 with R-types ACSSuT, SSuT and SSu showed a 4·2 MDa SSu-determinant, which was demonstrated in *Escherichia coli* K12 recipients resulting from triparental crosses. The ACT determinant in *S. dysenteriae* type 3 with R-type ACSSuT is probably chromosomally mediated. Cryptic plasmids of about 3·0 and 2·2 MDa were found in all *S. dysenteriae* type 3 isolates. The 4·2 MDa plasmid featured prominently in the plasmid profiles of *S. dysenteriae* types 4, 6 and 7 with R-types SSuT and SSu. However, this plasmid was not mobilizable by triparental crosses. There was a relative paucity of transferable plasmids in non-Shiga bacillus isolates. However, incompatibility group N plasmids, coding for tetracycline resistance, were detected.

INTRODUCTION

A plasmid is a replicon that is stably inherited in an extra-chromosomal state. These genetic elements can mediate, among other things, resistance to antibiotics. In fact, plasmid encoded drug resistance has now become the single most important mechanism by which bacteria exchange genetic information and become resistant to useful drugs [1]. Studies by Möller and colleagues [2] have shown that most naturally occurring strains of Enterobacteriaceae contain plasmids of different molecular weights. The separation of these plasmids by agarose gel electrophoresis shows strain-specific patterns (finger-prints) and these have been used to investigate the epidemiology of bacterial infections [3, 4]. Shigellosis is a major health problem in developing countries where conditions favouring endemicity still exist. In most of these countries, there is a continuous

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transmission with sporadic occurrence of outbreaks or epidemics of serious proportions [5]. Considering the magnitude of the problem, however, laboratory investigation of the aetiological agent has been very limited. From Ethiopia, there are some reports on the prevalence of various serotypes [6, 7] and their drug resistance patterns [8, 9]. Reports on drug transfer studies appeared only recently [10, 11] and studies on plasmid profiles are only now being attempted.

The plasmid profile analysis of *Shigella dysenteriae* type 1 (Shiga bacillus) of African and Asian strains has been described [12–15]. However, there is no report on the plasmid profile of non-Shiga members of the same sub-group. These strains are important in endemic infections in Ethiopia [7]. The purpose of the present study was to evaluate the plasmid profile of antibiotic-resistant non-Shiga strains isolated in Addis Ababa, Ethiopia. An attempt was also made to characterize the R-plasmids responsible for drug resistance.

MATERIALS AND METHODS

Strains used

The organisms were isolated at the National Research Institute of Health (Addis Ababa, Ethiopia) from stools or rectal swabs of patients suffering from an acute diarrhoea. These were individuals between whom there was no recognizable epidemiological link; from different parts of town at different times. A total of 99 isolates belonging to *S. dysenteriae* types 2, 3, 4, 6 and 7 were collected between 1976 and 1985 and stored at -70 °C in trypticase soy yeast broth with 25% glycerol (v/v). A total of 37 randomly selected strains was used for the plasmid profile analysis.

Genetic transfer studies

Direct transfer of plasmids was examined by the methods of Anderson and Threlfall [16]. Broth cultures of donor shigella strains and the recipient strain (*E. coli* K12, F⁻, Lac⁺, Nalʳ, 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 exconjugant colonies were counted. From each selective plate, 5–10 colonies were resistance typed by the agar dilution method. Transfer frequency was expressed as the proportion of resistant progeny per recipient cell.

Non-conjugative plasmids were mobilized by triparental crosses [6] with the FI⁺, group FII plasmid X and the F⁻, group I₁ plasmid Δ (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 and 0·5 ml of intermediate host (containing X or Δ) were incubated for 18 h at 37 °C before the addition of 1 ml of the final recipient (*E. coli* K12).

Plasmid extraction

Plasmid DNA was extracted according to the mini-preparation of Birnboim and Doly [17]. Eppendorf type 1·5 ml polypropylene tubes and a bench-top centrifuge (Anderman type 5412) capable of generating 8–10000 g were used.

Agarose gel electrophoresis

Plasmid DNA from *S. dysenteriae* types 2, 3, 4, 6 and 7 or their *E. coli* derivatives were run on a horizontal gel apparatus (BRL, Model H4) for 3·5 h at 150 volts, using 0·7 % agarose (w/v) (Sigma). A low-salt buffer system (40 mM Tris-Acetate and 2 mM disodium EDTA, pH 8) was used. Gels were soaked in an aqueous solution of 0·5 μg/ml ethidium bromide for 1 h. Finally, plasmids were visualized on a model 61 UV-Transilluminator (Ultra-Violet Products, San Gabriel, California, USA) and photographed with an MP4 Land camera (Polaroid Corporation, Cambridge, Massachusetts, USA), using type 57 Land film and a number 9 Wratten filter (Eastman Kodak, Rochester, N.Y., USA).

Molecular weight determination

Molecular weights were determined by reference to plasmids carried in standard strains. The sizes of unknown plasmids were determined by extrapolations with reference to the well characterized plasmids (98, 42, 23·9 and 4·6 MDa) carried by *E. coli* 39R861 (NCTC 50192). *E. coli* V517 (NCTC 50193) and *E. coli* with plasmids TP116 and TP124 (Plasmid Reference Centre, Stanford, California, USA) were used to check the suitability of the mini-preparation for the detection of large and small plasmids.

Incompatibility testing

Escherichia coli transconjugants [16] carrying single plasmids were mated with standard strains carrying reference plasmids of the following incompatibility (Inc) 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, FI_{me} and MP10. The standard organisms were from the National Collection of Type Cultures (UK). Inc testing was performed by the method of Anderson and co-workers [16, 18].

RESULTS

The plasmid profiles of antibiotic-resistant *S. dysenteriae* type 2 is shown in Table 1. All strains contained the 120 and 2·2 MDa plasmids. Plasmids pYH19a (30 MDa, Inc N coding for tetracycline resistance) and pYH20 (58 MDa, coding for KSSuT resistance) were found in two isolates. Strains with CSSuT resistance did not contain middle-sized plasmids.

Table 2 shows plasmid profiles of *S. dysenteriae* type 3. The strains showed similar patterns of carriage of cryptic plasmids. Plasmids of about 3·0 and 2·2 MDa were found in all isolates. All strains with R-type ACSSuT, SSuT and SSu contained a 4·2 MDa plasmid. These SSu-plasmids were also demonstrated in *E. coli* recipients resulting from triparental crosses. The SSu-resistance plasmids

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Table 1. *Plasmid profiles of antibiotic-resistant* Shigella dysenteriae *type 2 isolates from Ethiopia*

No.	Strain	Year of isolation	R-type	Plasmid profile (size in megadaltons)				
(1)	A2-69	1978	CSSuT	120	—	—	—	2·3
(2)	A2-161	1978	CSSuT	120	—	—	3·7	2·3
(3)	A2-209	1979	CSSuT	—	—	—	—	2·3
(4)	A2-371	1980	CSSuT	120	—	—	—	—
(5)	A2-421	1980	CSSuT	120	—	—	—	2·3
(6)	A2-458	1980	CSSuT	120	—	—	—	2·3
(7)	A2-710	1983	CSSuT	120	—	5·3	3·7	2·3
(8)	A2-727	1983	CSSuT	120	—	—	—	2·3
(9)	A2-ZZ	1977	SSuT	120	30*	8·3	4·3†	2·2
(10)	A2-94	1978	KSSuT	120	58‡	—	—	2·3
(11)	A2-176	1978	SSu	120	—	—	4·3	2·2

* Transferable plasmid (pYH19a, 30 MDa, Inc N coding for ACT resistance).
† SSu-determinant, mobilizable by pYH19a.
‡ Transferable plasmid (pYH20, 58 MDa, unclassified coding for KSSuT resistance).
C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphadiazine; T, tetracycline.

transferred at a frequency of 10^{-3}–10^{-4}. Tetracycline resistance in strains with R-type SSuT was coded by pYH19b (27 MDa, Inc N).

Plasmid profiles of *S. dysenteriae* types 4, 6 and 7 are shown in Table 3. In strains with R-types SSuT and SSu, the 4·2 MDa plasmid featured prominently in their plasmid profiles. A single transmissible plasmid (pYH21, Inc N coding for SSuT resistance) was found in strain A4-27-76.

DISCUSSION

It has been reported earlier that R-type CSSuT in *S. dysenteriae* type 2 and R-type ACST in type 3 could not be transferred to *Escherichia coli* recipient either directly or by mobilization [10]. In this study, the plasmid profiles of strains with R-type CSSuT did not show middle-sized plasmids likely to code for drug resistance (Table 1), and these determinants are probably chromosomally mediated. However, there were plasmids of about 50–60 MDa in those strains with R-type ACST, and it was not proved that these were cryptic; the existence of R-types that can be mobilized with transfer factors other than X and Δ cannot be ruled out [19].

Of special interest is the plasmid profile of *S. dysenteriae* type 3 with R-type ACSSuT (Table 2). These strains contained the 4·2 MDa plasmid which was demonstrable in the plasmid profiles of *E. coli* K12 recipients resulting from triparental crosses. The plasmid profiles of most of these isolates contained plasmids of about 70 MDa in size. In one strain (A3-527-81), we were able to mobilize this plasmid along with the 4·2 MDa plasmid in a Δ-mediated transfer. The *E. coli* recipient showed the SSu-resistance phenotype only, and it was concluded that the 70 MDa plasmid does not code for ACT resistance. It seems that ACT resistance in strains with R-type ACSSuT is chromosomally mediated.

Table 2. *Plasmid profiles of antibiotic-resistant* Shigella dysenteriae *type 3 strains from Ethiopia*

No.	Strain	Year of isolation	R-type	Plasmid profile* (size in megadaltons)									
(1)	A3-527	1981	ACSSuT*	120	70	—	—	4·2	2·9	2·7	2·1	1·9	1·8
(2)	A3-738	1983	ACSSuT	—	—	—	—	4·2	2·9	2·7	2·1	1·9	1·8
(3)	A3-819	1984	ACSSuT	—	70	50	—	4·2	2·9	2·7	2·1	—	1·8
(4)	A3-821	1984	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	2·0	—
(5)	A3-822	1984	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	2·0	—
(6)	A3-824	1984	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	2·0	—
(7)	A3-872	1984	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	1·9	—
(8)	A3-875	1984	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	1·9	—
(9)	A3-945	1985	ACSSuT	120	72	—	—	4·3	3·0	—	2·2	1·9	—
(10)	A3-962	1985	ACSSuT	120	72	63	56	4·2	2·9	2·6	2·1	—	1·8
(11)	A3-699	1985	ACST	—	60	50	—	—	2·8	2·6	2·0	1·9	1·7
(12)	A3-923	1985	ACST	—	—	46	—	—	2·8	2·6	2·0	—	—
(13)	A3-440	1980	SSuT	—	64	43	27†	4·2	2·8	2·7	2·1	1·9	—
(14)	A3-172	1978	SSuT	—	70	—	27†	4·2	3·0	—	2·2	—	1·8
(15)	A3-262	1979	SSu	—	—	—	—	4·2	3·0	—	2·2	1·9	—
(16)	A3-530	1981	SSu	—	—	—	—	4·2	3·0	—	2·2	1·9	—

* The SSu-determinant in all strains with R-type ACSSuT, SSuT and SSu was mobilizable by transfer factors X and Δ (Enteric Reference Laboratory numbers 48R626 & R1641, respectively).

† Conjugative plasmid pYH19b (27 MDa, Inc N, coding for ACT resistance).

A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulphadiazine; T, tetracycline.

Table 3. *Plasmid profiles of antibiotic-resistant* Shigella dysenteriae *types 4, 6 and 7 from Ethiopia*

No.	Strain	Year of isolation	R-type	Plasmid profile* (size in megadaltons)									
(1)	A4-27	1976	SSuT	—	72†	—	—	—	4·2	2·9	—	2·2	2·0	1·8
(2)	A4-68	1978	SSu	—	—	—	—	—	4·2	2·9	—	2·2	2·0	1·8
(3)	A4-171	1978	SSu	—	—	—	—	—	4·2	2·9	2·3	2·2	2·0	1·8
(4)	A4-593	1982	SSu	—	—	—	—	—	4·2	2·9	—	2·2	2·0	1·8
(5)	A6-25	1976	SSu	—	—	—	5·0	4·2	2·9	—	2·1	—	—
(6)	A6-26	1976	SSu	—	—	—	5·0	4·2	2·9	2·7	2·1	—	—
(7)	A6-922	1985	SSu	—	—	—	5·0	4·2	2·9	—	2·1	—	—
(8)	A7-50	1978	SSu	120	62	40	—	4·2	—	—	2·2	—	1·7
(9)	A7-406	1980	SSu	120	—	—	—	4·2	—	—	2·2	—	1·7
(10)	A7-981	1985	CSSuT	120	—	—	—	—	—	—	2·2	—	1·7

* The 4·2 MDa plasmids were non-mobilizable by tri-parental crosses.

† Conjugative plasmid pYH21 (72 MDa, Inc N, coding for SSuT resistance).

C, chloramphenicol; S, streptomycin; Su, sulphadiazine; T, tetracycline.

Earlier studies from Ethiopia have shown that non-Shiga bacillus isolates usually showed R-types with an SSu-resistance component [9]. The present plasmid profile study was instructive in that it was possible to demonstrate an approximately 4·2 MDa plasmid in all non-Shiga serotypes whose R-type shows an SSu-resistance component. It is now well established that, at least for Shiga bacillus from Africa, plasmids of about 4·2 MDa code for SSu-resistance [13; unpublished data]. Historically, a group of small, non-autotransferable plasmids were frequently observed in a wide range of Gram-negative bacteria [20–22]. Some of these plasmids conferred resistance to streptomycin and sulphonamides. According to WHO [23] the dissemination of these plasmids constitutes a global epidemic of a plasmid. It is difficult to account for the wide distribution of these non-autotransferable plasmids. However, it was postulated that the plasmids were transferred by specific phages [24].

It has been reported that African and Asian strains of Shiga bacillus show multiple species of small cryptic plasmids [13, 15]. The present study showed that, other than *S. dysenteriae* type 2 which usually contained a single cryptic plasmid, non-Shiga isolates contained 3–6 cryptic plasmids less than 10 MDa in size. A plasmid of about 2·2 to 2·3 MDa characterized *S. dysenteriae* types 2, 3, 4, 6 and 7; and a plasmid of about 2·8–3·0 MDa was found in types 3, 4 and 6. A large plasmid of about 120 MDa was found in non-Shiga isolates in this study, as was reported for Shiga bacillus of African origin [13]. This plasmid is thought to be responsible for bacterial invasiveness [25]. The 6·0 MDa plasmid which characterizes Shiga bacillus [13, 15, 26] was not found in any non-Shiga isolates. It seems that the 6·0 MDa plasmid is truly serotype-specific of the Shiga bacillus.

An earlier report from Ethiopia has shown the relative paucity of conjugative plasmids in non-Shiga isolates [10]. In this study, an attempt was made to characterize these plasmids. Tetracycline resistance was coded by pYH19a (30 MDa, Inc N) in a strain of *S. dysenteriae* type 2 with R-type SSuT. In two strains of type 3, with the same R-type, tetracycline resistance was coded by pYH19b (27 MDa, Inc N). To our knowledge, these small plasmids have not been reported from African strains. Plasmid pYH20 (58 MDa, unclassified) coded for KSSuT in a strain of *S. dysenteriae* type 3, and pYH21 (72 MDa, Inc N) coded for SSuT resistance in type 4. Ours is the first report of plasmid profile in non-Shiga isolates, and it is hoped that this base-line study will be useful for future surveillance of these shigella serotypes.

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