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# Molecular epidemiology of plasmid patterns in *Shigella flexneri* types 1–6

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## SUMMARY

A total of 123 drug-resistant and drug-sensitive *Shigella flexneri* types 1–6, and their *Escherichia coli* K12 transconjugants were used for plasmid profile analysis by agarose gel electrophoresis. Resistance factors (R-factors) were further characterized by incompatibility testing.

The overall distribution of small plasmids in *S. flexneri* showed that a cryptic plasmid of about 4·6 Kb was found in all serotypes, and a plasmid of about 4·2 Kb was found in serotypes 1–4. *Shigella flexneri* types 2, 4 and 6 showed a 6·5 Kb plasmid which correlated with SSu-resistance. All *S. flexneri* serotypes harboured large plasmids of about 217 Kb. Plasmid profile analysis of *S. flexneri* in Ethiopia showed a high degree of uniformity within individual serotypes. However, there was a limited variability which, at times, could be useful for epidemiological investigation. *Shigella flexneri* serotypes 1–6 harboured resistance plasmids with diverse molecular weights but mostly belonging to incompatibility groups N and X.

## INTRODUCTION

*Shigella flexneri* is the dominant serogroup in developing countries [1, 2]. In Ethiopia, *S. flexneri* and *S. dysenteriae* comprise over 80% of total Shigella isolates [3], and the prevalence of *S. flexneri* alone has been reported to be between 50% [3] and 70% [4]. Nevertheless, laboratory investigation of this important aetiological agent has not been adequate. Limited reports are now available in Ethiopia regarding the prevalence of various serotypes [3, 5] and their drug resistance patterns [4, 6]. Reports of R-factors have appeared only recently [7, 8] and studies on plasmid profile and R-plasmid characterization have never been attempted.

The plasmid profile analysis of *S. dysenteriae* type 1 (Shiga bacillus) of African and Asian origin has been carried out by various authors [9,–11]. To our knowledge, reports of plasmid profile patterns of *S. flexneri* are comparatively rare. The purpose of the present communication was, therefore, to determine the plasmid profile pattern of *S. flexneri* 1–6, with special reference to R-plasmids. The

results indicated the uniformity of plasmid profiles within specific serotypes, and the diversity of R-plasmids in *S. flexneri* isolates from Ethiopia.

## MATERIALS AND METHODS

### *Shigella strains used*

*Shigella flexneri* 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 492 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 123 randomly selected strains, representing the six *Sh. flexneri* 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 [12]. 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 [13]. 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 types by agar dilution methods.

Non-conjugative plasmids were mobilized by triparental crosses [13] with the $Fi^+$, group FII plasmid X and the $Fi^-$, group $I_1$ 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 and 0·5 ml of intermediate host (containing X or △) 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. flexneri* isolates and *E. coli* K12 recipients [13] was extracted according to the method of Birnboim and Doly [14]. 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 $\mu$l of plasmid DNA from strains of *S. flexneri* and *E. coli* K12 transconjugants was mixed with 6 $\mu$l of tracking dye [15] (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 $\mu$g/ml ethidium bromide for 1 h. Finally, plasmids were visualized on a Blak-Ray model 61 ultraviolet transilluminator (Ultraviolet Products, San Gabriel, California, USA) and photographed with MP4 land camera (Polaroid Corporation, Cambridge, Massachusetts, USA) using type 57 land film and a number 9 orange wratten filter (Eastman Kodak Co., Rochester, NY, USA) [16].

*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 [17] 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, California, USA) were routinely included to check the suitability of the mini-preparation of Birnboim and Doly [14] for the detection of large and small plasmids.

*R-plasmid incompatibility testing*

*Escherichia coli* K12 carrying single plasmids and derived by mating experiments [13] with *S. flexneri* 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 [18] and Anderson and Threlfall [13].

*Plasmid designation*

Plasmid designation was according to the recommendations of Novick and co-workers [19].

## RESULTS

The results of plasmid profile studies in *S. flexneri* type 1 are shown in Table 1. It is interesting to note the ubiquity and uniformity of small plasmids, less that 15·5 Kb, in all *S. flexneri* type 1 isolates, irrespective of drug resistance pattern and year of isolation. Strains with resistance types such as SuT, iSSuT and Su were invariably non-conjugative. The SSu determinant, in some strains of *S. flexneri* type 1, was mobilized by transfer factors; the size of these SSu-resistance plasmids was about 6·5–6·7 Kb (data not shown in Table).

Table 2 shows the plasmid profile of the dominant *S. flexneri* type 2 in Ethiopia. It carried a smaller number of cryptic plasmids than type 1. The plasmid profile of *S. flexneri* type 2 with resistance type ACSSuT and ACiSSuT was very interesting. Most of the strains failed to transfer their resistance partly or *in toto*.

Table 1. *Plasmid profiles of* Shigella flexneri *type 1*

| No. | Strain/year | Resistance type | | Plasmid profiles* (Size in kilobases) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B1-666/82 | ACKSSuT | — | *127* | | | | 17 | | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 2 | B1-392/80 | ACSSuTTm | — | | | *73* | | 18·6 | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 3 | B1-113/78 | ACSSuTTm | — | *125* | | | *57* | 18·6 | | 4·6 | 4·2 | 3·7 | 3·4 | | | 2·5 |
| 4 | B1-423/80 | ACSSuT | 217 | | *104* | | | 18·6 | | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 5 | B1-986/85 | ACSSuT | — | | | | *62* | | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·6 |
| 6 | B1-374/80 | ACiSSuT | — | | *84* | | | 12·4 | | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 7 | B1-375/80 | ACiSSuT | — | | | *76* | | 12·7 | | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 8 | B1-276/79 | ACiSSuT | — | | | *76* | | 12·7 | | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 9 | B1-079/78 | SSuT | — | | *85* | | | | | 4·8 | 4·5 | 3·9 | 3·4 | | | 2·5 |
| 10 | B1-160/78 | SSuT | — | | | | | 18·6 | | 4·6 | 4·3 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 11 | B1-253/79 | SSuT | — | | | | | 18·6 | 7·9 | | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 12 | B1-385/80 | SSuT | — | | | | | 18·6 | | 4·6 | 4·3 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 13 | B1-640/82 | SSuT | — | | *102* | | | 12·4 | | 4·6 | | 3·7 | 3·4 | 3·1 | 2·9 | 2·5 |
| 14 | B1-210/79 | ACT | — | | | | 62 | | | 4·6 | | 3·7 | 3·4 | | | 2·5 |
| 15 | B1-912/85 | ACT(iS)† | — | | | | | | | 4·6 | | 3·7 | 3·4 | | 2·9 | 2·5 |
| 16 | B1-441/80 | SuT | — | | | | | 18·6 | 7·9 | 4·6 | 4·3 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 17 | B1-135/78 | SuT | — | | | | | 18·6 | 7·9 | 4·6 | 4·3 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 18 | B1-120/78 | SuT | — | | | | 43 | 18·6 | 7·9 | 4·6 | 4·3 | 3·7 | 3·4 | 3·4 | | 2·5 |
| 19 | B1-283/79 | SuT | — | | | | | 18·6 | 7·9 | 4·6 | 4·3 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 20 | B1-377/80 | SuT(iS) | 217 | | | | | 18·6 | 7·9 | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 21 | B1-413/80 | SuT(iS) | — | | | | | 18·6 | 7·9 | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 22 | B1-634/82 | SuT(iS) | 217 | | | | | 18·6 | 7·9 | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 23 | B1-507/81 | SuT | — | | | | | 18·6 | 7·9 | 4·6 | 4·2 | 3·7 | 3·4 | 3·1 | | 2·5 |
| 24 | B1-163/78 | Su(iS) | — | | | | 23 | 12·4 | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 25 | B1-541/81 | Su | — | | | | 23 | 12·4 | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 26 | B1-032/76 | Sensitive | — | | | | | | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |
| 27 | B1-044/77 | Sensitive | — | | | | | | 7·9 | 4·6 | 4·3 | 3·9 | 3·4 | 3·1 | | 2·5 |

* Transferable plasmids are italicized.

† iS = partial resistance to streptomycin.

A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphadiazine; T, tetracycline; Tm, trimethoprim.

Table 2. *Plasmid profile of* Shigella flexneri *type 2*

| No. | Strain/year | Resistance type | | Plasmid profile* (Size in kilobases) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B2-884/84 | ACKSSuTTm | — | | *93* | | | 13 | *6·7* | | 4·8 | 4·3 | 3·7 | | |
| 2 | B2-782/83 | AiCSSuTTm† | — | | | *78* | | 13 | 6·7 | | 4·8 | 4·3 | 3·7 | | |
| 3 | B2-557/81 | KSSuT | — | | | *87* | | 15 | 7·3 | 5·4 | 4·8 | 4·5 | | 3·1 | 2·8 |
| 4 | B2-459/80 | ACSSuT | — | *152* | 98 | | | | | | 4·6 | 4·2 | 3·6 | | |
| 5 | B2-529/81 | ACSSuT | — | | *115* | *81* | | | 7·8 | | 4·6 | | 3·9 | | |
| 6 | B2-680/82 | ACSSuT | — | | | | | 12 | 6·5 | | 4·6 | | 3·7 | | |
| 7 | B2-744/83 | ACSSuT | — | | *108* | | | 12 | 6·5 | | 4·6 | | 3·7 | | |
| 8 | B2-707/83 | ACSSuT | — | | | | | 12 | 6·5 | | 4·6 | | 3·7 | | |
| 9 | B2-842/84 | ACSSuT | — | | | *65* | | | 6·5 | 5·6 | | 4·3 | 3·9 | 3·1 | |
| 10 | B2-867/84 | ACSSuT | 217 | | | | | 13 | 6·5 | | 4·6 | 4·3 | 3·9 | | |
| 11 | B2-950/85 | ACSSuT | — | | | | 17 | 12·5 | 6·7 | 6·5 | 4·6 | 4·2 | 3·6 | | |
| 12 | B2-501/81 | AiCSSuT | — | | | | | 12·5 | 6·5 | | 4·6 | 4·2 | 3·6 | | 2·8 |
| 13 | B2-623/82 | AiCSSuT | — | | | | | 12·5 | 6·5 | | 4·6 | 4·2 | 3·6 | | |
| 14 | B2-726/83 | AiCSSuT | — | | 105 | | | 13 | 6·5 | | 4·6 | 4·2 | 3·7 | | |
| 15 | B2-745/83 | AiCSSuT | — | 101 | | | 13 | 6·5 | | 4·6 | 4·2 | 3·7 | | | |
| 16 | B2-773/83 | AiCSSuT | — | | | | | 13 | 6·5 | | 4·6 | 4·2 | 3·7 | | |
| 17 | B2-910/85 | AiCSSuT | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 18 | B2-668/82 | ACST | — | | | | 33 | | | | 4·6 | 4·2 | 3·7 | | |
| 19 | B2-752/83 | ACST | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 20 | B2-880/84 | ACST | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 21 | B2-674/82 | AiCST | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 22 | B2-768/83 | AiCST | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 23 | B2-885/84 | AiCST | — | | | | | | | | 4·6 | 4·2 | 3·7 | | |
| 24 | B2-472/80 | AiCST | — | | | | | | | | 4·6 | 4·2 | 3·7 | 3·1 | |
| 25 | B2-524/81 | ASSuT | — | | | | | | 6·5 | | 4·6 | | 3·7 | | |
| 26 | B2-206/79 | CSSuT | — | | *108* | | | 8 | | | 4·6 | 4·2 | 3·7 | | |
| 27 | B2-980/82 | ACT | — | | *102* | | | | | | 4·6 | | 3·7 | | |
| 28 | B2-987/85 | ACT | 217 | 130 | | | | | | | 4·6 | | 3·7 | | |
| 29 | B2-N35/76 | SSuT | 217 | | *105* | | | | 6·7 | | 4·6 | 4·2 | 3·7 | | |
| 30 | B2-897/84 | ACS | 217 | | | | | | 6·7 | | 4·6 | 4·2 | 3·7 | | |
| 31 | B2-179/78 | SSuT | — | | *108* | | | | | | 4·6 | | 3·7 | | |
| 32 | B2-417/80 | SSuT | — | | *108* | | | | 6·5 | | 4·6 | | 3·7 | | |
| 33 | B2-544/81 | SSuT | — | 130 | | | 11·8 | 8·7 | | | 4·6 | 4·2 | 3·7 | | |
| 34 | B2-005/74 | Sensitive | — | | | | | | 6·8 | | 6·0 | 4·0 | | 3·1 | 2·8 |
| 35 | B2-066/78 | Sensitive | 217 | | | | | | | | | 4·2 | 3·7 | 3·1 | |
| 36 | B2-601/82 | Sensitive | — | | | | | | | | | 4·2 | 3·7 | | |

* Transferable plasmids are italicized.

† iC & iS, partial resistance to chloramphenicol and streptomycin, respectively.

Drug abbreviations as in Table 1.

Table 3. Shigella flexneri *types 1 and 2: resistance plasmids transferred to* Escherichia coli *K12 hosts**

| | | | *Escherichia coli* K12 hosts | | | |
|---|---|---|---|---|---|---|
| No. | Strain/year | Resistance type | R-type(s)† | Plasmid(s)‡ size in kilobases | Plasmid name | (Incompatibility) |
| 1 | B1-666/82 | ACKSSuT | ACKSSuT | 127 | pYH27a | (NT)§ |
| | | | ACSSuT | 127 | pYH28 | (FIme) |
| 2 | B1-392/80 | ACSSuTTm | ACSSuTTm | 73 | pYH29a | (N) |
| 3 | B1-113/78 | ACSSuTTm | ACSSuTTm | 125, 57 | — | |
| 4 | B1-423/80 | ACSSuT | ACSSuT | 104 | pYH30a | (N) |
| 5 | B1-986/85 | ACSSuT | ACSSuT | 62 | pYH31 | (X) |
| | | | ASuT | 62 | pYH32 | (UNC) ‖ |
| | | | ACSuT | 62 | pYH33 | (X) |
| | | | ACT | 62 | pYH34 | (X) |
| 6 | B1-374/80 | ACiSSuT | ACiSSuT | 84 | pYH35 | (N) |
| 7 | B1-374/80 | ACiSSuT | ACT | 76 | pYH37 | (UNC) |
| 8 | B1-276/79 | AiSSuT | AT | 76 | pYH38b | (UNC) |
| 9 | B1-079 | SSuT | SSuT | 85 | pYH41 | (UNC) |
| 10 | B1-640/82 | SSuT | SSuT | 102 | pYH42 | (N) |
| 11 | B1-210/79 | ACT | ACT | 62 | pYH43 | (X) |
| 12 | B2-884/84 | ACKSSuTTm | ACKSSuTTm | 93, 6·7 | — | |
| 13 | B2-782/83 | AiCSSuTTm | ASSuTTm | 78 | pYH45 | (N) |
| 14 | B2-557/81 | KSSuT | KSSuT | 87, 15, 7·3 | — | |
| 15 | B2-459/80 | ACSSuT | AiCSSuT | 152 | pYH47 | (FIme) |
| | | | T | 124 | pYH48 | (FIme) |
| 16 | B2-529/81 | ACSSuT | ACSSuT | 115, 81 | — | |
| 17 | B2-744/83 | ACSSuT | CSSuT | 109, 6·5 | — | |
| 18 | B2-842/84 | ACSSuT | ACT | 65 | pYH51 | (X) |
| 19 | B2-206/79 | CSSuT | CSSuT | 109 | pYH55 | ($I_1$) |
| 20 | B2-980/82 | ACT | T | 102 | pYH56 | (N) |
| 21 | B2-N35/76 | SSuT | SSuT | 105 | pYH58 | (N) |
| 22 | B2-179/78 | SSuT | SSuT | 109 | pYH60a | (N) |
| 23 | B2-417/80 | SSuT | SSuT | 109 | pYH60c | (N) |

* *Escherichia coli* K12, $F^-$, $Lac^+$, $Nx^r$, prototrophic; Enteric Reference Laboratory number 14R525.
† Resistance type.
‡ Plasmids were correlated with parent plasmid profile.
§ Lack of a suitable market (not tested).
‖ Compatible with all reference plasmids (unclassified).
Drug abbreviation as in Table 1.

Table 4. *Plasmid profiles of* Shigella flexneri *type 3*

| No. | Strain/year | Resistance type | | Plasmid profile* (Size in kilobases) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B3-430/80 | ACSSuT | — | | 93 | 48 | | | | 5·6 | | 4·3 | | 3·7 | 3·1 | 2·8 |
| 2 | B3-451/80 | ACSSuT | — | | *91*† | | | | | | | | | | | |
| 3 | B3-345/80 | SSuT | 217 | *112*† | | | | | | 5·4 | 4·8 | 4·5 | | 3·6 | 3·2 | |
| 4 | B3-367/80 | ACSSuT | — | | | | | | | 5·4 | 4·8 | 4·5 | | 3·6 | | |
| 5 | B3-893/84 | SSuT | — | | | | 26 | 13 | 10 | 5·7 | | 4·3 | 4·0 | 3·7 | | |
| 6 | B3-946/85 | SSuT | 217 | | | | | 13 | 10 | 5·7 | | 4·3 | 4·0 | 3·7 | | |
| 7 | B3-860/84 | iSSu‡ | — | | | | 19 | | 7·1 | | 4·6 | | | 3·7 | | |
| 8 | B3-568/81 | SuT | — | | | | 19 | | | | 4·6 | | | 3·7 | 3·4 | |
| 9 | B3-826/84 | Su | — | | | | | | | 5·6 | | 4·3 | 4·2 | 3·6 | 3·1 | |
| 10 | B3-345/80 | Sensitive | 217 | | | | | | | | 4·6 | | 4·2 | 3·6 | | |
| 11 | B3-442/80 | Sensitive | 217 | | | | | | | 5·4 | | 4·3 | | 3·4 | | |
| 12 | B3-603/82 | Sensitive | — | | | | | | | 5·4 | | 4·3 | 4·2 | | 3·1 | |

* Transmissible plasmids are italicized.

† pYH61 (91 Kb, Inc N, coding for ACSSuT resistance) and pYH60d (112 Kb, Inc, N, coding for SSuT resistance) were found from strains B3-451/80 and B3-345/80, respectively.

‡ iS, partial resistance to streptomycin.

Drug abbreviations as in Table 1.

Table 5. *Plasmid profile of* Shigella flexneri *types 4 and 5*

| No. | Strain/year | Resistance type | | Plasmid profile* (Size in kilobases) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B4-774/83 | ACKSSuT | — | *113* | | | | | | 5·4 | 4·3 | 3·7 | | | | |
| 2 | B4-T22/77 | ACSSuT | — | 125 | | | | 15·5 | | | 4·0 | 3·7 | 3·1 | 2·8 | | |
| 3 | B4-583/82 | AiCSSuTTm† | 217 | | | *71* | | 12 | 7·0 | 6·7 | 5·7 | 4·2 | 4·0 | 3·4 | 2·6 | 2·5 |
| 4 | B4-118/78 | ACSSuT | 217 | | *90* | | 19 | | | | 5·4 | 4·3 | 3·7 | | | |
| 5 | B4-569/81 | ACSSuT | 217 | | | *65* | 19 | | | | | 4·2 | | | 2·8 | |
| 6 | B4-771/83 | ACT | — | | | *60* | 18 | | 7·3 | | | 4·3 | 3·4 | 3·1 | | |
| 7 | B4-042/78 | ACiST | — | | | *54* | | | | | | 4·3 | | | 2·8 | |
| 8 | B4-189/79 | SSuT | — | | *90* | | | | 7·3 | 6·4 | | 4·6 | 3·7 | | 2·8 | 2·5 |
| 9 | B4-828/84 | SSuT | — | | | | 24 | | 7·3 | | | 4·6 | 3·7 | | | |
| 10 | B4-037/76 | iSSuT | — | | | | 18·6 | 14·9 | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 11 | B4-044/78 | iSSuT | 217 | | | | | | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 12 | B4-290/79 | iSSuT | 217 | | | | 18·6 | 14·9 | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 13 | B4-393/80 | iSSuT | 217 | | | | | | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 14 | B4-909/85 | iSSuT | — | | | | 18·6 | 14·9 | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 15 | B4-095/78 | SuT | — | | | | 18·6 | | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 16 | B4-220/79 | SuT | — | | | | 19 | | 7·4 | 6·7 | | 4·6 | 4·0 | | | |
| 17 | B4-493/81 | SuT | 217 | | | | 19 | | 7·4 | | | 4·6 | 3·9 | | | |
| 18 | B4-T14/74 | iST | 217 | | | *53* | | | | | | 4·6 | 3·9 | | 2·8 | 2·5 |
| 19 | B4-654/82 | SuT | 217 | | | | 19 | | 7·4 | 6·7 | | 4·6 | 3·9 | | | |
| 20 | B4-036/76 | SSu | — | | | | | | 7·4 | 6·7 | | 4·6 | 3·9 | | | |
| 21 | B4-577/81 | iSSu | — | | | | | | 7·1 | | | 4·6 | 3·9 | | | |
| 22 | B4-254/79 | Su | — | | | | | 12 | 7·1 | | | 4·6 | 3·9 | | | |
| 23 | B4-043/78 | Sensitive | 217 | | | | | | | | | 4·6 | 3·9 | | | |
| 24 | B4-219/79 | Sensitive | 217 | | | | | | 7·1 | | | 4·6 | 3·9 | | | |
| 25 | B5-500/81 | iST | — | | | | | | | | | 4·6 | 3·9 | | | |

* Transferable plasmids are italicized.
† iC & iS, partial resistance to chloramphenicol and streptomycin, respectively.
Drug abbreviations as in Table 1.

Table 6. *Plasmid profiles of* Shigella flexneri *type 6*

| No. | Strain/year | Resistance type | | Plasmid profile* (Size in kilobases) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | B6-769/83 | ACSSuT | — | | | | 6·5 | 4·6 | 3·4 | | 2·8 | |
| 2 | B6-782/83 | ACSSuT | — | | | | 6·5 | 4·6 | 3·4 | | 2·8 | |
| 3 | B6-788/83 | ACSSuT | — | *91* | | | 6·5 | 4·6 | 3·4 | | 2·8 | |
| 4 | B6-057/78 | SSuT | — | *87* | | | | 4·6 | 3·4 | | | |
| 5 | B6-539/81 | SSuT | — | 99 | | | 6·5 | 4·6 | 3·4 | | | 2·5 |
| 6 | B6-791/83 | SSuT | — | | 13 | 9·1 | 6·5 | 4·6 | 3·4 | | | |
| 7 | B6-863/84 | SSuT | — | | 15 | | 6·5 | 4·6 | 3·4 | | | 2·5 |
| 8 | B6-076/78 | SSu | 217 | | | | 6·4 | 4·3 | 3·4 | | | 2·5 |
| 9 | B6-349/80 | SSu | 217 | | | | 6·4 | 4·3 | 3·4 | | | 2·5 |
| 10 | B6-889/84 | SSu | 217 | | | | 6·5 | 4·3 | 3·4 | | | 2·5 |
| 11 | B6-019/75 | iSSu† | — | | | | 6·4 | 4·3 | 3·4 | 2·9 | | 2·6 |
| 12 | B6-00Z/77 | iSSu | 217 | | | | 6·4 | 4·3 | 3·4 | 2·9 | | 2·6 |
| 13 | B6-131/78 | iSSu | 217 | | | | 6·4 | 4·3 | 3·4 | | | 2·6 |
| 14 | B6-378/80 | iSSu | 217 | | | | 6·4 | 4·3 | 3·4 | | | 2·6 |
| 15 | B6-688/82 | iSSu | 217 | | | | 6·4 | 4·3 | 3·4 | | | 2·6 |
| 16 | B6-792/83 | iS | — 130 | | | | 6·4 | 4·5 | 3·3 | | 2·8 | |
| 17 | B6-833/84 | Su | — 130 | | | | 6·4 | 4·5 | 3·3 | | 2·8 | |
| 18 | B6-761/83 | iST | — 130 | *81* | | | | 4·5 | 3·3 | | 2·8 | |
| 19 | B6-562/81 | SuT | — 136 | *87* | | | 6·4 | 4·5 | 3·3 | | 2·8 | |
| 20 | B6-106/78 | Sensitive | 217 | | | | | 4·5 | 3·3 | | 2·8 | |
| 21 | B6-483/81 | Sensitive | 217 | | | | | 4·5 | 3·3 | | 2·8 | |
| 22 | B6-831/84 | Sensitive | 217 | | | | | 4·5 | 3·3 | | 2·8 | |

* Transferable plasmids are italicized.
† iS, partial resistance to streptomycin.
Drug abbreviations as in Table 1.

However, a plasmid of about 6·5 to 6·7 Kb was consistently found in all of these isolates. *Shigella flexneri* type 2 strains with R-types ACST or AiCST were always non-conjugative and their plasmid profile lacked middle-size plasmids likely to code for drug resistance.

Table 3 shows characterization of R plasmids in *S. flexneri* types 1 and 2. It is interesting to note the presence of R plasmids with diverse molecular weights and incompatibility groups in both serotypes.

Plasmids profile analysis of *S. flexneri* type 3 (Table 4) showed a diversity of small plasmids, and the number of conjugative plasmids was limited. Besides, this serotype has not acquired the 6·7 Kb plasmid commonly found in other serotypes of *S. flexneri*.

Table 5 shows plasmid profiles of *S. flexneri* types 4 and 5. The usual ubiquity of small plasmids is clearly shown. Several strains that were resistant to SSu or had an SSu-resistance component in their R-types showed the 6·7 Kb plasmid, described earlier. The single isolate of *S. flexneri* type 5 showed only two cryptic plasmids.

Plasmid profiles of *S. flexneri* type 6 (Table 6) demonstrated a uniform pattern of small plasmids. Most strains contained cryptic plasmids of about 4·3, 3·4 and 2·8 Kb. Like other serotypes, *S. flexneri* type 6 had a 6·7 Kb plasmid which correlated with SSu resistance. Table 7 shows plasmids transferred from *S. flexneri* types 4 and 6 to *Escherichia coli* K12. Relevant resistance markers, molecular weights and incompatibility groups are indicated.

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Table 7. Shigella flexneri *types 4 and 6: resistance plasmids transferred to* Escherichia coli *K12 hosts**

| No. | Strain/year | Resistance type | R-type(s)† | *Escherichia coli* K12 hosts Plasmid(s)† size in kilobases | Plasmid name (Incompatibility) |
|---|---|---|---|---|---|
| 1 | B4-774/83 | ACKSSuT | ACKT | 113 | pYH62 (NT)§ |
| | | | CT | 109 | pYH63 (B) |
| 2 | B4-583/82 | AiCSSuTTM ‖ | ACSSuTTm | 71 | pYH64 (N) |
| | | | CSSuTTm | 60 | pYH65 (N) |
| 3 | B4-118/78 | ACSSuT | ACiSSuT | 90 | pYH66 (N) |
| 4 | B4-569/81 | ACSSuT | ACT | 65 | pYH67 (X) |
| 5 | B4-042/78 | ACiST | ACT | 54 | pYH68 (X) |
| 6 | B4-771/83 | ACT | ACT | 60 | pYH69 (X) |
| 7 | B4-189/79 | SSuT | SSuT | 90, 7·3, 4·6 | |
| | | | | 3·7, 2·8, 2·5 | — |
| 8 | B-T14/74 | iST | T | 53 | pYH70 (N) |
| 9 | B6-788/83 | ACSSuT | Su | 91, 6·5, 4·6, 3·4 | — |
| 10 | B6-057/78 | SSuT | SSuT | 87 | pYH72 (UNC)¶ |
| 11 | B6-761/83 | iST | T | 81 | pYH73 (N) |
| 12 | B6-562/81 | SuT | T | 87 | pYH74 (N) |

* *Escherichia coli* K12, $F^-$, $Lac^+$, $Nx^r$, prototrophic; Enteric Reference Laboratory number 14R525.
† Resistance type.
‡ Plasmids were correlated with parent plasmid profile.
§ Lack of a suitable marker (not tested).
‖ iC and iS, partial resistance to chloramphenicol and streptomycin, respectively.
¶ Compatible with all reference plasmids (unclassified).
Drug abbreviations as in Table 1.

## DISCUSSION

There was a remarkably uniformity of small plasmids within the plasmid profile of *S. flexneri* type 1 (Table 1). Plasmids of molecular weight 4·6, 4·2, 3·7, 3·4, 3·1 and 2·5 Kb were found during the years 1976–85, indicating a probable clonal origin. In spite of the small plasmid uniformity, however, the strains contained R-plasmids with diverse resistance phenotype and molecular weights. An interesting observation in this serotype was the lack of the 6·5 Kb SSu-resistance plasmid commonly found in other serotypes of *S. flexneri*. Strains carrying SuT and Su resistance did not have middle-size plasmids likely to code for drug resistance, and were earlier found to be non-conjugative and non-mobilizable [8]. These resistance patterns may be chromosomally mediated.

An earlier study has shown that *S. flexneri* type 2 became dominant in Ethiopia after 1980; and that this was directly linked to an increase in multi-drug resistance [20]. Plasmid profile analysis showed that the significant increase in isolates with R-type ACSSuT correlated with two independent R-determinants: the SSu-determinant which correlated with a 6·5 Kb plasmid in the parent plasmid profile (Table 2), and an ACT determinant which was neither transferable nor mobilizable. The evolution of drug resistance in *S. flexneri* type 2 parallels that of *S. dysenteriae* type 3 with R-type ACSSuT [7], the only difference being that the SSu-determinant in the latter was efficiently mobilized by transfer factors while the SSu-determinant in the former serotype was non-mobilizable. This lack of transferable and mobilizable antibiotic resistance in *S. flexneri* type 2 confirms the earlier finding by Frost and coworkers [9], who concluded that over 60% of *S. flexneri* (serotypes unspecified) with R-type ACSSuT could not transfer their resistance directly or by mobilization. Small plasmids of about 4·6, 4·2 and 3·6 Kb seem to characterize the serotype (Table 2). Haider and colleagues [21] have carried out plasmid profiles of *S. flexneri* isolates with R-types AKTTm, AKST and S. According to these authors, small plasmids varying in number from two to four were found, and plasmids of about 4·0 and 2·9 Kb were common. These *S. flexneri* serotypes were not identified at serotype level and it was not possible for us to match our results. Our strains of *S. flexneri* contained R-plasmids with miscellaneous resistance phenotypes and molecular weight.

Plasmid profiles of *S. flexneri* type 3 showed the usual ubiquity of small plasmids, which were comparatively more diverse than those of types 1 and 2. However, most strains contained plasmids of about 4·3 and 3·7 Kb (Table 4). It was interesting to note that type 3, like type 1, did not show the 6·5 Kb plasmid commonly observed in other serotypes of *S. flexneri*.

In conformity with *S. flexneri* type 2, *S. flexneri* type 4 and 6 with SSu-component in their resistance phenotypes showed plasmids of about 6·3 to 6·7 Kb (Tables 5 and 6). The ubiquity and uniformity of small plasmids was also shown in *S. flexneri* types 4 and 6. *S. flexneri* type 4 was characterized by plasmids of about 7·4, 4·6 and 4·0 Kb in size, while type 6 was characterized by plasmids of about 4·6 and 3·4 Kb. Resistance plasmids were less common in these serotypes.

The overall distribution of small plasmids in *S. flexneri* showed that a cryptic plasmid of about 4·6 Kb was found in all serotypes and a plasmid of about 4·0 to 4·2 Kb was found in types 1–4; *Shigella flexneri* type 4 was characterized by a 7·4 Kb plasmid. Reports of plasmid profile analysis in individual *S. flexneri*

serotypes are scanty. There is, however, a single report of plasmid profile analysis of *S. flexneri* from Asia [22]. Small plasmids of molecular weight 4·0 and 3·1 Kb were found in all *S. flexneri* serotypes investigated, and the authors concluded that there was enough plasmid pattern diversity to help trace epidemic strains of shigella infection. Our results indicate that the 3·1 Kb was common in *S. flexneri* type 1 and was rarely found in other serotypes. However, it was interesting to note that a plasmid of about 4·0 to 4·2 Kb was found in serotypes 1–4, as was reported from Asia [21].

Genetic analysis has established that virulence in *S. flexneri* is associated with chromosomal loci [23]. In addition, a large plasmid of about 217 Kb has been observed [24, 25]. Strains lacking this plasmid failed to invade HeLa cell monolayers and to evoke kerato-conjunctivitis in guinea pigs. These large *S. flexneri* plasmids do not seem to encode functions for 'O' antigen production and hence specify one or more functions for invasiveness. The results reported in our study showed that all *S. flexneri* serotypes harboured a large plasmid of about 217 Kb, even though this plasmid was not detected in every strain examined. The loss of shigella invasiveness in long storage with loss of the large plasmid has been well described [16, 24].

Shigellosis in Bangladesh was reported to be caused by a large number of clones [26]. Our results of the dominant *S. flexneri* subgroup did not bear out this conclusion. There is a remarkable uniformity of plasmid profile within individual serotypes collected over a decade. There is, however, a limited variability which at times could be useful for epidemiological investigation of these strains.

According to Frost and Rowe [9], the most commonly encountered incompatibility (Inc) groups in 111 strains of *S. flexneri* R-plasmids, originating from Asia, Africa and the UK were: 44 Inc B, 24 Inc $I_1$, 10 Inc FII and 9 Inc $H_1$. Five of the nine strains from Africa harboured Inc $I_1$, two Inc FII and one each Inc B and Inc M. This study is important in that it elucidated the distribution of R-plasmid Inc groups in *S. flexneri* the most commonly encountered serogroup in developing countries.

In this study, Inc N and Inc X plasmids were commonly found in *S. flexneri* isolates (Tables 3 and 7). It was interesting to note that, except for a single Inc X plasmid which was found in a strain of *S. flexneri* type 4 isolate of 1978, all Inc X plasmids were detected after 1981. The prevalence of Inc X plasmids in *S. flexneri* coincided with the introduction of the 'Zairian strain' of Shiga bacillus into Ethiopia (unpublished data). It seems that Inc X plasmids are a recent introduction into *S. flexneri* isolates of Ethiopia.

Inc N R-plasmids were common in *S. flexneri* isolates in this study. These plasmids usually coded for ACSSuTTm, SSuT and T resistance. Inc N plasmids coding for SSuT and T resistance ranged between 93 and 108 Kb in size, while those coding for ACSSuTTm resistance had a range of 62 to 77 Kb. Incompatibility group N plasmids were detected during the years 1974–82, and are considered endemic in Ethiopia. The previous study of Frost and colleagues [9] did not show Inc N plasmids in *S. flexneri* isolates from Africa. However, Inc N plasmids are commonly reported from enterobacterial isolates from many countries. An Inc N plasmid with a molecular weight of 57 Kb and coding for ASSuTTm resistance was found in *Salmonella typhi* isolates from Southeast Asia [27]. Inc N plasmids of 48·5

to 65 Kb, were commonly isolated in members of Enterobacteriaceae in France [28]. These plasmids were also isolated from faecal coliforms in Indonesia [29]. A plasmid of about 65 Kb coding for SSuTm was found in Chilean isolates of *Escherichia coli* associated with infant diarrhoea [30]. Similarly, a 64 Kb plasmid coding for ACSSuTTm resistance was found in *E. coli* strains causing urinary tract infection in India [31]. It is known that a fairly good correlation exists between Inc groups and molecular weight [32]. The diversity of molecular weight of Inc N plasmids in Ethiopia seems strange and will require further investigation.

In this study, Inc FIme plasmids were detected in *S. flexneri* types 1 and 2. Unlike the atypical Inc FIme plasmids in Shiga bacillus isolates of Ethiopia [33], these were typical Inc FIme plasmids greater than 124 Kb. These plasmids were temporally clustered between 1980 and 1982, and were probably a recent acquisition from other members of Enterobacteriaceae. Plasmids of Inc FIme were found in strains *S. flexneri* originating from India, the Middle East and the Mediterranean [9]. It seems that these plasmids have a wide geographical distribution.

The results of plasmid profile analysis and R-plasmid characterization of *S. flexneri* types 1 to 6 have been informative. To our knowledge, this is the first comprehensive report of *S. flexneri* plasmids in Africa. More studies are required to see if these plasmids have a wider geographical distribution.

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