Transferable or mobilisable antibiotic resistance in *Shigella dysenteriae* types 1, 2, 3, 4, 6 and 7 isolated in Ethiopia during 1974–85

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Summary. A total of 199 *Shigella dysenteriae* isolates resistant to one or more antibiotics and belonging to types 1, 2, 3, 4, 6 and 7 was examined by one-step broth mating with *Escherichia coli* K12 and, if non-conjugative, additionally by triparental crosses with the conjugative plasmids X and Δ . Of the *S. dysenteriae* type 1 (Shiga's bacillus) strains, 96% harboured conjugative plasmids. During 1974–79, isolates of Shiga's bacillus carried conjugative plasmids coding for ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamide, tetracycline) resistance that transferred at low frequencies ($<10^{-4}$). After 1980, however, about 50% of isolates of Shiga's bacillus with this resistance (R)-type carried conjugative plasmids that transferred at high frequencies (10^0 – 10^{-2}) and that expressed the ACT determinant only. The introduction of a new clone of Shiga's bacillus into Ethiopia in 1980 is suspected. Conjugative plasmids coding for SSuT resistance were detected in *S. dysenteriae* types 2, 3, and 4. Non-conjugative SSu determinants in *S. dysenteriae* type 3 were mobilised by conjugative plasmids X and Δ . R-type CSSuT in strains of types 2 and 7, and R-type ACST in type-3 strains were neither transferable nor mobilisable and are probably determined chromosomally.

Introduction

The phenomenon of R-plasmid mediated multiple antibiotic resistance was first noted in Japan during the 1950s in bacterial strains that were resistant simultaneously to chloramphenicol, streptomycin, sulphonamides and tetracycline (Suzuki *et al.*, 1956). Akiba *et al.* (1960) proposed the theory that the factor governing such multiresistance could be transmitted by cellular contact. At about the same time, Ochiai *et al.* (1959) transmitted resistance *in vitro* among strains of the genus *Shigella* and *Escherichia coli*.

Within the genus *Shigella*, R-plasmid mediated antibiotic multiresistance has been especially common in *S. dysenteriae* type 1 (Shiga's bacillus). The most dramatic incident so far reported has been the Central American epidemic in 1969–1971 (Mata *et al.*, 1970). The outbreak in Mexico City (Olarie *et al.*, 1976), and the recent Central African epidemic (Frost *et al.*, 1981) are other examples.

In Ethiopia, the prevalence of various *Shigella*

serotypes has been studied (Gebre-Yohannes and Habte-Gabr, 1984a), and their resistance patterns determined (Gedebou and Tassew, 1982; Gebre-Yohannes and Habte-Gabr, 1984b). However, reports on R-plasmids are few (Gedebou and Tassew, 1979). The present study was undertaken to determine the prevalence of R-plasmids in specific serotypes of *S. dysenteriae*, by use of standardised methods which included determination of transfer frequency and mobilisation of non-conjugative plasmids.

Materials and methods

Shigella strains

S. dysenteriae strains isolated from cases of endemic shigellosis and referred to the National Research Institute of Health, Addis Ababa were used. These strains were collected between 1974 and 1985 and were stored at -70°C in trypticase soy yeast broth with glycerol 25% v/v.

Serotype identification. Serotypes were identified by slide agglutination with commercial serotype-specific antisera (Difco).

Antibiotic susceptibility

Sensitivity tests for each isolate were performed by the method of Bauer *et al.* (1966). *E. coli* (ATCC 25922) was used to control medium and sensitivity disks. The agents tested were: ampicillin (A), chloramphenicol (C), cephalothin (Ce), kanamycin (K), gentamicin (G), polymyxin B (Px), streptomycin (S), sulphadiazine (Su), tetracycline (T) and trimethoprim (Tp).

A total of 199 strains resistant to one or more drugs was selected for the genetic transfer studies.

Genetic transfer studies

Direct transfer of plasmids was examined by the methods of Anderson and Threlfall (1974). Broth cultures of donor *Shigella* strains and the recipient strain (*E. 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 exconjugant colonies were counted. From each selective plate, 5–10 colonies were

resistance typed by the agar dilution method with Steer's replicating apparatus (Steers *et al.*, 1959). Transfer frequency was expressed as the proportion of resistant progeny per recipient cell.

Non-conjugative plasmids were mobilised by triparental crosses (Anderson and Threlfall, 1974) with the Fi⁺, group F_{II} 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).

Results

Table I shows the spectrum of R-types encountered, the number with conjugative plasmids, the R-phenotype transferred to *E. coli* K12 and the estimated transfer frequencies (for *S. dysenteriae* type I). Of *S. dysenteriae* type-1 isolates, 96% harboured conjugative plasmids, and most of these transferred at high frequencies.

Table II shows comparative transfer frequencies of conjugative plasmids in *S. dysenteriae* type 1 with R-type ACSSuT isolated during the years 1974–79 and 1980–85. Conjugative plasmids coding

Table I. Transferable antibiotic resistance in 132 strains of *S. dysenteriae* type 1 isolated in Addis Ababa, Ethiopia (1974–1985)

Donor R-type*	Number tested	Number with R-plasmid	Recipient R-type†	Transfer frequency‡		
				High	Medium	Low
Su	1	1	Su	+		
ACT	5	5	ACT	+		
CSSu	1	1	CSSu	+		
SSuT	3	3	T		+	
CSSuT	9	8	CSSuT	+		
ACSSuT	112	107	ACT	+§	+¶	
ACKSSuT	1	1	AC	+		
			AK	+		
			ACKS,SuT			+
			ACS,SuT			+
ACCSuTTp	1	1	AC	+		
			ACS,SuTTp	+		
			ACSuTTp	+		
			TTp	+		
Total	132	127				

* Resistance type.

† *Escherichia coli* K12, F⁻, Lac⁺, Nal^r, prototrophic; Enteric Reference Laboratory no. 14R525.

‡ High: 10⁰–10⁻²; Medium: 10⁻³–10⁻⁵; Low: 10⁻⁶–10⁻⁸.

§ 57.4%.

¶ 42.6%.

A = ampicillin, C = chloramphenicol, K = kanamycin, S = streptomycin, Su = sulphadiazine, T = tetracycline, Tp = trimethoprim, Nal = nalidixic acid.

Subscript i shows intermediate resistance.

Table II. Transfer kinetics of conjugative plasmids in *S. dysenteriae* type 1 with R-type ACSSuT isolated during the years 1974–79 and 1980–1985.

Transfer* frequency	Number (%) of transfer plasmids in isolates from		
	1974–1979	1980–1985	
		Plasmid	R-type ACT
10 ⁰			1 (1.9)
10 ⁻¹		5 (9.3)	13 (24.1)
10 ⁻²			12 (22.2)
10 ⁻³			
10 ⁻⁴	2 (3.8)		
10 ⁻⁵	10 (18.9)	4 (7.4)	
10 ⁻⁶	24 (45.3)	10 (18.5)	
10 ⁻⁷	16 (30.2)	9 (16.7)	
10 ⁻⁸	1 (1.9)		
Total	53 (100.1)	28 (51.9)	26 (48.1)

* Overnight crosses in broth mating at 37°C with selection on media containing chloramphenicol, ampicillin or tetracycline.

for ACSSuT resistance transferred at low frequencies from strains isolated during 1974–79. After 1980, *c.* 50% of the conjugative plasmids transferred at high frequencies and expressed only the ACT determinant in recipients.

Transferable or mobilisable antibiotic resistance detected in *S. dysenteriae* type 2, 3, 4, 6, and 7 is shown in table III. Conjugative plasmids coding for SSuT resistance were detected in *S. dysenteriae* types 2, 3, and 4. Non-conjugative SSu determinants in *S. dysenteriae* type 3 were mobilised by transfer factors X and Δ . The CSSuT phenotype in types 2 and 7 and the ACST phenotype in type 3 were neither transferable nor mobilisable.

Discussion

Howe (1982) concluded that the appearance of conjugally transferable resistance among bacteria has been a recognised consequence of the use of antibiotics for some 25 years. Among antibiotic-resistant bacteria, *S. dysenteriae* type 1 (Shiga's bacillus) has been associated with troublesome antibiotic multiresistance mediated by R plasmids. During 1969–71, Shiga's bacillus with plasmid-mediated antibiotic multiresistance to CSSuT appeared in populous central America with devastating effects (Mata *et al.*, 1969). This was closely followed by a severe but limited outbreak in Mexico City (Olarte *et al.*, 1976). The strain contained a transmissible plasmid (R-type CSSuT) and a mobilisable plasmid encoding ampicillin resistance. R-plasmid mediated drug resistance types CSSuT and

ACSSuT occurred in India (Macaden *et al.*, 1980) and Bangladesh (Crosa *et al.*, 1977).

The present study clearly demonstrated that > 96% of strains of Shiga's bacillus isolated in Ethiopia harboured conjugative plasmids, most of which transferred at high frequencies. About 84% of these isolates were characteristically associated with R-type ACSSuT. It is interesting to note that there were two distinct patterns of transfer frequency associated with this R-type. The ACSSuT determinant in strains isolated before 1980 transferred at low frequencies, whereas > 50% of conjugative plasmids encountered after this time transferred at high frequencies and expressed the ACT determinant only. The introduction of a new clone of Shiga's bacillus into Ethiopia after 1980 is suspected. Genetic and molecular studies are in progress to substantiate this hypothesis.

According to Frost *et al.* (1981), isolates of Shiga's bacillus with R-type ACSSuT from Somalia carried conjugative group-X plasmids coding for ACT, which mobilised the SSu-determinant. On the other hand, isolates of Shiga's bacillus with the above R-type from Central Africa had conjugative ACT plasmids that failed to mobilise the SSu-determinant (Ebright *et al.*, 1984; Frost *et al.*, 1985). It is possible that some of our Shiga's bacillus isolates are related to these African strains. Genetic and molecular studies are, however, needed to establish whether the determinants of drug resistance are carried on related plasmids.

Preliminary plasmid finger-print analysis of *S. dysenteriae* type-1 isolates and their R-plasmid

Table III. Transferable or mobilisable antibiotic resistance in *S. dysenteriae* types 2, 3, 4, 6 and 7 isolated in Addis Ababa, Ethiopia (1974–1985)

Sero-type	Donor R-type	Direct transfer*		Mobilisation†	
		Number tested	Number conjugative (R-type)	Number tested	Number mobilised (R-type)
2	S	12	0	4	0
	SSuT	2	2 (SSuT) (T)		
3	CSSuT	17	0	3	0
	SSu	2	0	2	2 (Δ , SSu)‡ (X, SSu)
	SSuT	2	2 (SSuT) (T)		
	ACST	2	0	2	0
	ACSSuT	17	0	3	3 (Δ , SSu)‡ (X, SSu)
4	SSu	5	0	3	0
	SSuT	1	1 (SSuT)		
6	SSu	3	0	2	0
7	SSu	2	0	2	0
	CSSuT	1	0	1	0

* Overnight broth culture at 37°C and 28°C with *E. coli* K12, F⁻, Lac⁺, NaI^r, prototrophic; Enteric Reference Laboratory (ERL no. 14R525).

† Triparental crosses with plasmids X (ERL no. 48R626) and Δ (ERL no. RT641).

‡ Higher transfer in Δ -mediated transfer (10^{-3} – 10^{-4}).

bearing *E. coli* counterparts has shown that the strain, which first appeared in Ethiopia in 1980, was identical with the Zairian strain (Frost *et al.*, 1985), whereas the second strain was endemic to Ethiopia and may have an independent R-plasmid evolution.

A few of our *S. dysenteriae* type-1 isolates showed the CSSuT R-type characteristic of strains of Shiga's bacillus from Central America (Mata *et al.*, 1970). Previous studies in Ethiopia demonstrated that antibiotic multiresistance in *S. dysenteriae*, other than type 1, is not common (Gebre-Yohannes and Limenih, 1980; Gebre-Yohannes and Habte-Gabr, 1984b). The present study also demonstrated the relative paucity of conjugative plasmids in these serotypes. It was interesting to note that CSSuT determinants in *S. dysenteriae* types 2 and 7, and ACST determinants in type 3, were neither transferable nor mobilisable. However, the SSu-determinant in *S. dysenteriae* type 3 with R-type ACSSuT was efficiently mobilised by plasmids X and Δ . It is possible that some of the above R-types are chromosomally mediated.

According to Frost and Rowe (1983), conjugative plasmids coding for tetracycline resistance have

been detected in the genus *Shigella*. In this study, *S. dysenteriae* types 2, 3 and 4 with R-type SSuT carried conjugative plasmids coding for tetracycline resistance and, in some cases, the SSu-determinant was mobilised by the plasmid coding for tetracycline resistance.

The present study has demonstrated that R-type ACSSuT is especially prevalent in Shiga's bacillus isolates from Ethiopia. A similar situation also exists in *S. flexneri* type 2, which is the most commonly encountered serotype in Ethiopia (Gebre-Yohannes and Drasar, 1987). The WHO Scientific Working Group (1983) on antimicrobial resistance reports that there is a higher prevalence of resistance to ACSSuT in developing countries. These drugs are often freely available in the open market and are taken without medical advice. A critical reappraisal of the use of antimicrobials in developing countries is, therefore, urgently required.

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