

Electropherotyping of plasmid DNA of different serotypes of *Shigella flexneri* isolated in Bangladesh

K. HAIDER, M. I. HUQ, K. A. TALUKDER AND Q. S. AHMAD

*International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B),
GPO Box 128, Dhaka 1000, Bangladesh*

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SUMMARY

One hundred and twenty-five *Shigella flexneri* strains, isolated during January–December 1984, at the Dhaka treatment centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, were serotyped using absorbed rabbit antisera specific for all type- and group-factor antigens, as well as a group of ten mouse and rat monoclonal antibodies. Electropherotypes of the plasmid deoxyribonucleic acid (DNA) were also determined. *S. flexneri* 2a was the predominant serotype followed by 3b, 1a, and 2b. The recently described E1037 antigen was also found in three strains of *S. flexneri* serotype 6. Electropherotyping of the plasmid DNA showed that three plasmids of approximately 140, 2·7, and 2 megadalton (MDa) were present, respectively, in 97, 97 and 94% of the 125 strains. Additional plasmids of various other sizes were also present in different serotypes except in serotype 2a. The additional plasmids again appeared to be specific for that particular serotype. For example, all 12 strains of *S. flexneri* 2b harboured an additional plasmid of approximately 1 MDa. Thus, electropherotyping of plasmid DNA of different serotypes of *S. flexneri* might be useful to differentiate *S. flexneri* from other species of *Shigella* and in identifying different serotypes of *S. flexneri*. Therefore, the common plasmids, plus the additional plasmids, could be used to identify epidemic, as well as sporadic, subclones of *S. flexneri* strains.

INTRODUCTION

Shigellosis is one of the major causes of diarrhoeal diseases, especially among children in Bangladesh, as well as in other developing countries. Among the four species of *Shigella*, *Shigella flexneri* is the most prevalent species in Bangladesh. Serotyping and sub-serotyping of *S. flexneri* depends upon determination of the O-antigen and O-antigen factors (Ewing & Lindberg, 1984). Little information is, however, available on the epidemiology of the serotypes and sub-serotypes of *S. flexneri* in Bangladesh.

Surveys carried out in 1975 and 1979 in Bangladesh showed that serotypes 1 and 2 of *S. flexneri* were predominant (Mutanda, Kibriya & Mansur, 1981). This is in contrast to earlier studies in which serotypes 1–4 and 6 were present with more or less similar frequency (Mutanda *et al.* 1980). A study carried out in East Africa (Mutanda, Kaviti & Wamola, 1979) revealed a change in the predominance

of serotypes. In Kenya, *S. flexneri* 2 was the most predominant in the 1960s but changed to type 6 in about 1977. Type 2 was also the most frequently isolated in Uganda around 1957, but had changed to type 3 by 1966.

Plasmids are considered to be potential strain-markers and have been shown to be a useful tool in epidemiological studies of various microorganisms (Farrer, 1983; O'Brien *et al.* 1982). No reports have so far been published on the plasmid profile of *S. flexneri* and its association with different serotypes. Recently *Shigella sonnei* strains have been shown to harbour a 3.4 megadalton (MDa) plasmid (Prado *et al.* 1987) along with the previously described 120 MDa plasmid (Sansone, Kopecko & Formal, 1982) irrespective of their place of isolation. Similarly, we have observed that three plasmids of molecular size 160, 6 and 2 MDa are consistently associated with the strains of *S. dysenteriae* type 1 isolated from different geographical locations of the world (Haider *et al.* 1988). Thus, it has been suggested that caution should be used in identifying new strains of *S. sonnei* and/or *S. dysenteriae* type 1 by plasmid profile marker. Plasmids, other than the serotype specific, should be sought and used to identify a possible pandemic strain, as well as individual epidemic strains. No such report on the presence of common plasmids, with the possible exception of the 140 MDa plasmid, is available for the *S. flexneri* strain. This study was, therefore, undertaken to determine the prevalence of different serotypes and sub-serotypes of *S. flexneri* in Bangladesh and to compare their association with the plasmid DNA of these strains.

MATERIALS AND METHODS

Bacterial strains

S. flexneri strains were isolated from either stool specimens or rectal swabs from 2915 diarrhoeal patients in the 4%-systematic surveillance system at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) treatment centre from January to December 1984. Details of the surveillance system have been described elsewhere (Stoll *et al.* 1982). Three hundred and eighty-two *Shigella* strains were isolated, 166 (44%) were further identified as *S. flexneri*. One hundred and twenty-five strains of *S. flexneri* were studied. Pure cultures of all strains were identified biochemically and serologically by standard methods (Edwards & Ewing, 1972). A single colony of a confirmed *S. flexneri* strain was grown in trypticase soy broth with 0.3% yeast extract, containing 15% glycerol and was stored at -70°C for further studies. Reference *S. flexneri* strains of the National Shigella Centre, Warsaw, Poland, were used for producing antisera. Absorption and evaluation of *S. flexneri* typing antisera were done in collaboration with Professor Hanna of the National Shigella Centre. The E1037 4X strain was obtained from the Central Public Health Laboratory, Colindale, England. The Y-300/81 4a, without the E1037 antigen, and the ATCC 12023 4a strain with the E1037 antigen were kindly provided by Dr Nils Curlin, Department of Bacteriology Laboratory, Stockholm, Sweden. *Escherichia coli* strains used as standards for molecular mass estimations of plasmids were obtained from the Center for Disease Control (Atlanta, GA).

Serotyping of S. flexneri

The type-specific antisera and group-factor antisera of *S. flexneri* used in this study were raised by immunization of adult albino rabbits (2.5 kg). The rabbits were injected with formalin-killed bacteria; subsequent injections were of whole live cells. The antisera were absorbed with cross-reacting antigens and stored at -40°C after determination of titre by dilution. Strains were cultured for 18 h on trypticase soy agar plates and serological reactions were performed, using the above antisera, by the slide agglutination test. All strains were also serotyped with monoclonal antibody reagents specific for all *S. flexneri* type- and group-factor antigens (Carlin & Lindberg, 1983, 1986*a, b*) to compare the results obtained with the rabbit monovalent-absorbed antisera.

Analysis of plasmid DNA

Plasmid DNA from *S. flexneri* strains was extracted by the method of Birnboim & Doly (1979) and was separated by vertical gel electrophoresis in 0.7% agarose (Meyers *et al.* 1976). The molecular size of the unknown plasmid DNAs was determined on the basis of its mobility through agarose gel and was compared with the mobility of known molecular size plasmids. Plasmids present in strains PDK 9 (140 and 105 MDa), R₁ (62 MDa), RP₄ (36 MDa), and Sa (23 Ma) were used as molecular size controls.

RESULTS

The predominant serotypes of the 125 *S. flexneri* strains, isolated in the 4%-systematic surveillance system were 2a (33%), 3b (19%), 1a (10%), and 2b (8%). All known serotypes and sub-serotypes, except 4b and 3C, were isolated during this period (Table 1). Serotyping by the absorbed rabbit monovalent antisera correlated well with the monoclonal antibody of *S. flexneri* (MASF). Three strains of *S. flexneri* serotype 6 were shown to have the provisional E1037 antigen as detected by monoclonal antibody of the MASF *S. flexneri* IV-1 antibody (Curlin & Lindberg, 1986*b*).

Analysis of plasmid DNA by agarose gel electrophoresis showed that the serotypes 1-3 of *S. flexneri* strains isolated in Dhaka contained three plasmids in common. A 140, 2.7, and 2.0 MDa plasmid was present respectively, in 97, 97 and 94% of the 125 strains (Table 1). However, only serotype 2a contained the three common plasmids in all 63 strains studied. Serotypes 1a, 1b, 2b, 3a and 3b of *S. flexneri* contained additional plasmids, in the range of 140-1.0 MDa, which seemed to form a unique banding pattern in many of these serotypes (Table 1; Fig. 1).

The three strains of *S. flexneri* serotype 6 with the E1037 antigen were found to have a 4 MDa plasmid (Fig. 2: Lanes A, C and E) which was not seen in serotype 6 strains without the provisional antigen (Table 1; Fig. 2: Lane B). Interestingly, the former strains all lacked a 65 MDa plasmid which was consistently seen in the latter group of *S. flexneri* strains. Plasmid profiles of the *S. flexneri* 4 strains are shown in Table 2. The E1037 4X strain (Fig. 2: Lane D) containing the E1037 antigen had the 4 MDa plasmid whereas the ATCC 12023 4a strain (Fig. 2: Lane F) although containing E1037 antigen, did not contain the plasmid. Other 4a strains

Table 1. *Plasmid profile of different serotypes of S. flexneri isolated in Bangladesh*

Serotype	Number strains studied	Molecular size (in MDa)													
		140	100-20	6.0	4.4	4.0	3.4	2.7	2.0	1.8	1.4	1.0	0.5	< 0.5	
1a	20	17	10	—	—	—	—	18	20	20	—	—	—	—	—
1b	9	9	—	—	—	—	—	9	9	9	7	—	—	—	—
2a	62	62	3	—	—	—	—	62	62	—	—	—	—	—	—
2b	12	12	—	—	—	—	—	12	12	—	—	—	—	—	1
3a	5	5	—	—	5	5	—	5	5	—	—	—	—	—	—
3b	9	8	—	—	5	4	—	9	9	—	—	—	—	—	—
4a	1	1	—	—	1	1	—	1	—	—	—	—	—	—	—
5X ⁺	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
5X ⁻	1	1	—	—	—	—	—	—	—	—	—	—	—	—	1
6	2	2	—	—	—	—	—	2	—	—	—	—	—	—	—
6 with E1037	3	3	—	—	3	—	—	3	—	—	—	—	—	—	—
125	121 (97)	16 (13)	7 (6)	1 (1)	14 (11)	12 (10)	121 (97)	118 (94)	29 (23)	7 (6)	43 (34)	1 (1)	2 (2)		

Numbers in parentheses indicate percentage of strains that harboured the plasmid.

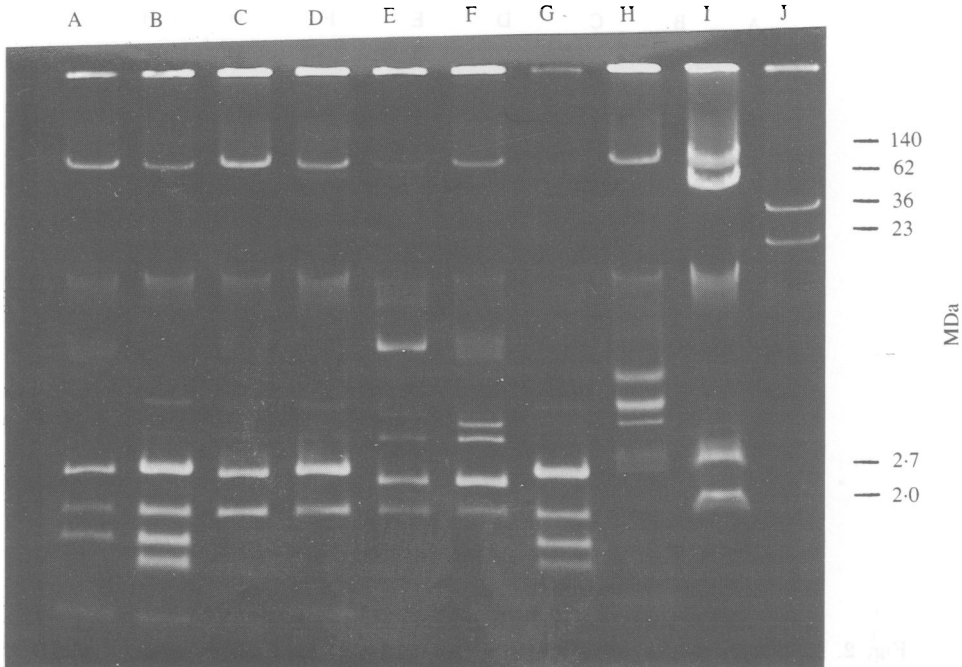


Fig. 1. Plasmid profiles of seven representative strains of *S. flexneri* are shown. *S. flexneri* serotype 1a, 1b, 2a, 2b, 3a, 3b, 1b, and 4a are shown, respectively, in lanes A through H. The control was a composite of plasmids obtained from PDK-9 and R₁ (lane I), and RP₄ and Sa (lane J).

Table 2. Plasmid profiles in strains of *S. flexneri* 4 with or without the provisional antigen E1037

Strain no.	Source	Presence of		
		E1037	Plasmid profiles (MDa)	
E1037 4X	Dr Nils Curlin	+	105, 4.4, 4	2.7, 2.0
Y300/81 4a	Dr Nils Curlin	-	80, 4.4	2.7, 2.2, 1 < 1
ATCC 12023 4a	Dr Nils Curlin	+	4.4	2.7, 2.0 < 1
Z-2118 4a	ICDDR,B	-	4.4, 4, 3.4	2.7

isolated either in Bangladesh (Fig. 1: Lane H) or in Sweden (Fig. 2: Lane G) did not show any association between the presence of the E1037 antigen and the 4 MDa plasmid.

DISCUSSION

In our study, the most common serotype of *S. flexneri* was 2a. Changes in the serotypes of *Shigella* with time in a particular community have been reported by various workers (Mutanda, Kibriya & Mansur, 1981; Mutanda, Kaviti & Wamola, 1979; Arya *et al.* 1977). In 1967, *S. flexneri* serotypes 1-4 and 6 occurred almost with the same frequency in Bangladesh, while during 1969-70, serotypes 2, 3, and 6 were commonly encountered. In 1975, serotypes 3 and 6 had been surpassed in predominance by serotype 1 (Mutanda, Kibriya & Mansur, 1981). These changes in serotype prevalence have also been described in other countries (Arya *et al.* 1977).

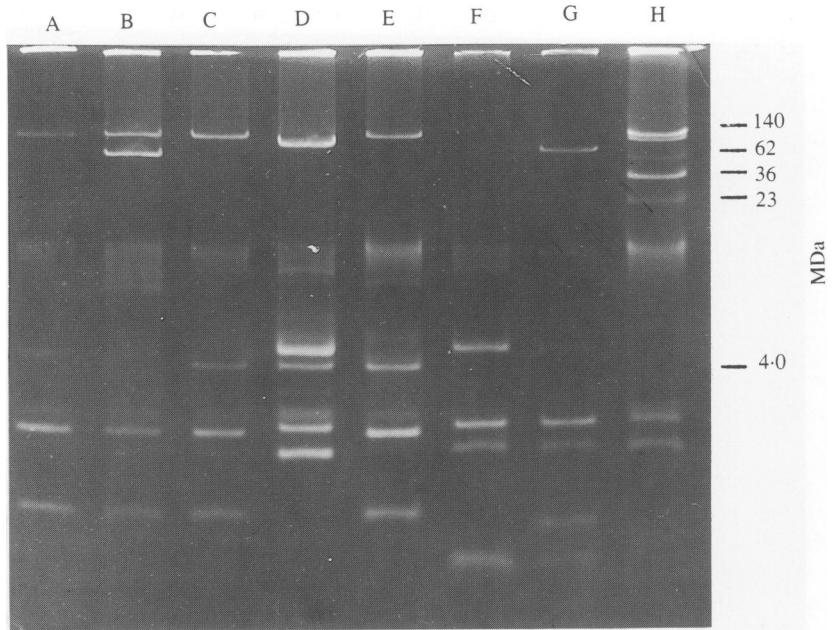


Fig. 2. Electropherotypes of representative strains of *S. flexneri* with and without provisional antigen E1037 are shown. Plasmid profiles are shown as follows: *S. flexneri* 6 with E1037 (lanes A, C and E), without E1037 (lane B); *S. flexneri* E1037 4X and ATCC 12023 4a with E1037 antigen (lanes D and F, respectively); and *S. flexneri* Y300/81 4a without E1037 (lane G) with reference marker plasmids (lane H).

Although there is little published data available on the association of the plasmid profile of *S. flexneri* and their serotypes, previously published reports have revealed a heterogeneous plasmid populations in strains of *S. flexneri*, most are smaller than 6 MDa (Tacket *et al.* 1984; Jamieson *et al.* 1979). This study confirms that most plasmids in *S. flexneri* strains are smaller than 6 MDa. However, we describe an association between serotypes and plasmid profiles. This may be explained by the occurrence of a single strain in the community for the year of the study.

The presence of the 140, 2.7, and 2.0 MDa plasmids in serotypes 1a, 1b, 2a, 2b, 3a, and 3b suggests that a significant association may exist between the presence of these small plasmids and the ecology and/or pathogenicity of the isolates. The presence of the additional plasmids in patterns related to particular serotypes suggests that plasmid profiles may be useful in distinguishing between serotypes of *S. flexneri*. It may also be possible to document the appearance of any new strains in a community by these patterns.

Sasakawa *et al.* (1986) reported that multiple plasmids of sizes 140, 7.2, and 2.0 MDa were present in virulent strains of *S. flexneri* 2a isolated in Japan, which agrees with our observations on strains from Bangladesh. Analysis of plasmid profiles of different serotypes of *S. flexneri*, isolated from different geographical locations at different time intervals is necessary, since it may help us in studying the epidemiology of *S. flexneri* strains and in understanding their epidemiological patterns.

The presence of the recently described E1037 antigen in *S. flexneri* serotype 6 might be due to form variation and have an important epidemiological value. The presence of 4.0 MDa plasmid in the strains of serotype with its provisional antigen E1037 and its absence in serotype 6 without this antigen indicate that the 4 MDa plasmid may have some role in the expression of E1037 antigen in serotype 6 which deserves further investigation. However, this association between the additional E1037 antigen and the 4 MDa plasmid has not been observed in strains of serotype 4a, although a 4 MDa plasmid is present in E1037 4X strain.

In our study no association was found between the presence of plasmids in the range of 30–100 MDa and serotypes of *S. flexneri* strains. However, these plasmids may code for drug resistance as was observed previously (Haider *et al.* 1985). Conjugational transfer of these plasmids to an *E. coli* K-12 strain, followed by curing of the plasmid, is necessary to demonstrate the role of these plasmids in the antibiotic resistance or any unknown functions.

The presence of 140, 2.7, and 2.0 MDa plasmids in different serotypes of *S. flexneri* and the unique association of the additional plasmids in different serotypes suggest that caution should be exercised when using plasmid profiles as an epidemiological marker for interpretation of common-source outbreak of *S. flexneri*. The presence of the common plasmids in different serotypes of *S. flexneri* within a wide geographical distribution needs to be studied in order to understand more carefully the epidemiological patterns of plasmids.

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