

## Usefulness of Plasmid Profiles for Differentiation of *Shigella* Isolates in Bangladesh

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**We studied the plasmid profiles of 136 *Shigella* isolates in Bangladesh to determine whether plasmid profiles could be used for differentiation of strains for epidemiological studies. Many different plasmid patterns were observed within each species, indicating that many genetically different strains of *Shigella* are responsible for illness in Bangladesh.**

In the United States, plasmid profile analysis has been useful as an epidemiological tool in investigating outbreaks of enteric disease. When used as a fingerprint for a strain, the plasmid profile may aid in differentiation of strains, identifying a source of infection, or evaluating the efficacy of control measures (6, 10, 11). The usefulness of plasmid analysis for typing strains in a community in which shigellosis is hyperendemic has not been evaluated. In Bangladesh, most *Shigella* strains have similar antimicrobial susceptibility patterns, suggesting that a few circulating strains produce most cases of dysentery (9). To determine whether plasmid analysis could more precisely discriminate strains of *Shigella*, we studied a sample of *Shigella* isolates from patients treated at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

We examined two groups of *Shigella* isolates. Since isolates recovered within a 1-month period might be more likely to contain similar plasmids, we studied a group of 24 *Shigella* strains of all species isolated from a 4% random surveillance sample of patients treated at ICDDR,B in December 1982. To examine profiles over a longer period, we also studied 112 stocked isolates from patients admitted to the medical ward from January to October 1982, including every third *Shigella flexneri* isolate and all isolates of other species.

Plasmid DNA was extracted by the alkaline precipitation technique described by Birnboim and Doly (1) and electrophoresed on a 0.8% agarose gel in Tris-borate buffer (5). Sereny tests for invasiveness in the guinea pig conjunctiva were performed by standard techniques (8). Susceptibility to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole were determined by disk diffusion.

The isolates examined included 81 (60%) *S. flexneri*, 23 (17%) *Shigella dysenteriae* type I, 13 (10%) *Shigella boydii*, 10 (7%) *Shigella sonnei*, and 9 (7%) *S. dysenteriae* type II. All but one isolate contained one or more plasmids, and three isolates had as many as eight plasmids. Table 1 shows, for each species, the mean number of plasmids per isolate, the number of different plasmid profiles, and the number of different antimicrobial susceptibility patterns. The most common antimicrobial susceptibility pattern was resistance to streptomycin and tetracycline. Within each species, plasmid profiles distinguished more strains than did the anti-

microbial susceptibility pattern; for example, 24 *S. flexneri* strains from surveillance patients had five different antimicrobial susceptibility patterns, but 19 different plasmid profiles.

Most profiles were characterized by the presence of a large-molecular-weight plasmid; 124 (91%) of the 136 isolates of all species contained a plasmid between about 110 and 200 megadaltons. Twelve isolates (7 *S. flexneri*, 3 *S. sonnei*, and 2 *S. dysenteriae* type I) lacked a large plasmid. Sereny tests were performed on all 12 isolates lacking a large plasmid and five isolates (2 *S. flexneri*, 2 *S. dysenteriae* type I, and 1 *S. boydii*) containing a large plasmid. All 12 isolates without large plasmids were Sereny test negative, and all 5 isolates with large plasmids were Sereny test positive.

This study indicates that shigellosis in patients seen at the ICDDR,B is caused by a large number of clones which are not differentiated by antimicrobial susceptibility pattern. This is in contrast to the experience in the developed world, where one or a few clones account for shigellosis in a community. This finding is not surprising since in Bangladesh, even family members of patients frequently excrete *Shigella* species of a different serotype than the index case (2).

A large plasmid has been associated with the invasiveness of *S. flexneri* and *S. sonnei* (3, 7). The consistent presence of a large-molecular-weight plasmid in all four *Shigella* species isolated from patients with diarrhea in this study suggests that the plasmid is related to the invasive ability of other *Shigella* species. Although the large plasmids were not the same size in all strains, these plasmids may nevertheless contain common gene sequences. In the few strains which lacked this plasmid, the Sereny test was negative, indicating that the isolate was no longer capable of causing dysentery (4). The loss of *Shigella* invasiveness in storage associated with loss of a large plasmid has been well described (3, 7); this probably explains the absence of this plasmid in some of these isolates.

The more plasmids an organism contains, the more specific is the plasmid profile as a marker for a single strain. Plasmid profile analysis differentiated very specifically among the strains infecting patients seen at the ICDDR,B. This may be because there are a large number of different circulating strains, because plasmids are easily transferred among the strains circulating in the community, or both. Among strains isolated from the large population of patients served by ICDDR,B, we could not trace the spread of strains through the community or identify clusters of cases that

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TABLE 1. Number of different plasmid profile and antimicrobial susceptibility patterns among *Shigella* isolates in Bangladesh

Species	Mean no. of plasmids per isolate (no. of isolates)	No. of different plasmid profiles (no. of isolates)	No. of different antimicrobial susceptibility patterns (no. of isolates)	No. of strains resistant to TeS <sup>a</sup> only (no. of isolates)
<i>S. flexneri</i> (surveillance patients)	4.2 (24)	19 (24)	5 (24)	16 (24) (67%)
<i>S. flexneri</i> (medical ward patients)	4.5 (57)	38 (57)	7 (42)	32 (42) (76%)
<i>S. dysenteriae</i> type I	3.4 (23)	14 (23)	5 (12)	6 (12) (20%)
<i>S. dysenteriae</i> type II	3.1 (9)	7 (9)	3 (5)	1 (5) (20%)
<i>S. boydii</i>	4.5 (13)	11 (13)	3 (9)	5 (9) (56%)
<i>S. sonnei</i>	3.7 (10)	10 (10)	2 (8)	5 (8) (63%)

<sup>a</sup> TeS, Tetracycline and streptomycin.

might be related by a common source or close contact. Plasmid analysis may be useful in epidemiological studies of less diverse populations such as those in families or villages.

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