

Letters to the Editor

Newly Emerged Multiple-Antibiotic-Resistant *Shigella dysenteriae* Type 1 Strains in and around Kolkata, India, Are Clonal

Several parts of India, including Kolkata, witnessed a devastating epidemic of shigellosis with increased (35.6%) isolation of *Shigella dysenteriae* type 1 strains during 1984 (10). In the postepidemic period, shigellosis became endemic in Kolkata and *S. flexneri* (58%) was the most prevalent species, followed by *S. sonnei* (28%), *S. boydii* (9%), and *S. dysenteriae* (5%) (5, 7). In the year 2002, a sudden resurgence in the isolation of *S. dysenteriae* type 1 strain was noticed, with the occurrence of several dysentery outbreaks, which swept from northern to southern districts of West Bengal, including Kolkata (4, 11; K. Sarkar, S. Ghosh, S. K. Niyogi, and S. K. Bhattacharya, Letter, Lancet **361**:785, 2003). These newly emerged strains of *S. dysenteriae* type 1 were multidrug resistant, with resistance to commonly used antimicrobials such as ampicillin, tetracycline, cotrimoxazole, amoxicillin, nalidixic acid, and fluoroquinolones (such as ciprofloxacin and norfloxacin), which had high MICs (4, 6). Plasmid analysis and pulsed-field gel electrophoresis (PFGE) have long been used as molecular tools for subtyping a group of similar strains and for studying their genetic relatedness (1, 2, 8, 9). This suddenly increased rate of isolation of *S. dysenteriae* type 1 (36%) from dysentery patients in Kolkata after a gap of almost 18 years was noteworthy (4). Hence the present study was undertaken to monitor the antibiotic resistance profile of recently emerged *S. dysenteriae* type 1 strains and to determine the molecular subtyping of the strains by plasmid DNA analysis and PFGE.

A cluster of 36 strains of *S. dysenteriae* type 1 was included in this study, of which 35 strains were isolated from July 2002 to October 2002 as the sole causative agents of dysentery cases admitted to Infectious Disease Hospital and Dr. B. C. Roy Memorial Children's Hospital, two referral hospitals in Kolkata (4, 6). The remaining strain was isolated from a patient in a dysentery outbreak (Sarkar et al., letter) affecting the northern part of West Bengal. The study strains were further characterized for antimicrobial resistance profiles and subjected to plasmid DNA analysis and PFGE by standard techniques (8, 9).

The commonest antimicrobial resistance profile, observed in 97% (35 of 36) strains, was resistance to seven antimicrobials: ampicillin, tetracycline, nalidixic acid, amoxicillin, cotrimoxazole, ciprofloxacin, and norfloxacin. Plasmid profile analysis of 34 strains of *S. dysenteriae* type 1 revealed four different profiles (types I to IV), of which the type I profile was the most predominant and was found in 88% (30 of 34) strains. All strains had a heavy plasmid of 210 kb. Other types (types II to IV) showed gain or loss of two or three smaller plasmids.

PFGE patterns of all isolates were compared, and Dice coefficients were calculated for each pattern (3). *Xba*I restriction enzyme (New England Biolabs) digests of genomic DNA of *S. dysenteriae* type 1 strains produced a PFGE pattern with 14 to 17 DNA bands between 380 and 40 kb (Fig. 1). When analyzed according to the methods of Chiou et al. (2), the PFGE patterns of the isolates were classified into a single pulsotype (X1) with four subtypes (X1₁, X1₂, X1₃, and X1₄). If PFGE patterns of two phenotypically similar strains were different from each other by four or more bands, the strains were

considered to be genetically different and to belong to different pulsotypes. On the other hand, in one pulsotype, if two strains had three or fewer band differences or shifts with a Dice coefficient of ≥ 80 , they were designated subtypes, which were genetically related to the main type (12). The phenomenon could be explained by simple insertion or deletions or gain or loss of restriction sites.

Of 36 strains of *S. dysenteriae* type 1, 32 (88%) belonged to the PFGE main type (X1) (Fig. 1; lanes 1 to 4, 6 to 8, and 10 to 12) and were considered genetically identical (clonal). Each of the remaining four strains belonged to one of four different subtypes (X1₁, X1₂, X1₃, or X1₄) (Fig. 1, lanes 5 [X1₁] and 9 [X1₂]). Subtypes X1₃ and X1₄ are not shown in Fig. 1.

The present study revealed that most (27 of 36; 75%) of the recently emerged multidrug-resistant *S. dysenteriae* type 1 strains were indistinguishable by their antimicrobial resistance patterns, plasmid DNA profiles, and PFGE profiles, indicating their genetic similarity. The emergence of one predominantly circulating multidrug-resistant clone of *S. dysenteriae* type 1 during a specified period was alarming and might be responsible for significant number of the recently reported dysentery outbreaks in eastern India. Therefore, urgent preventive intervention is necessary to contain further spread of this deadly organism.

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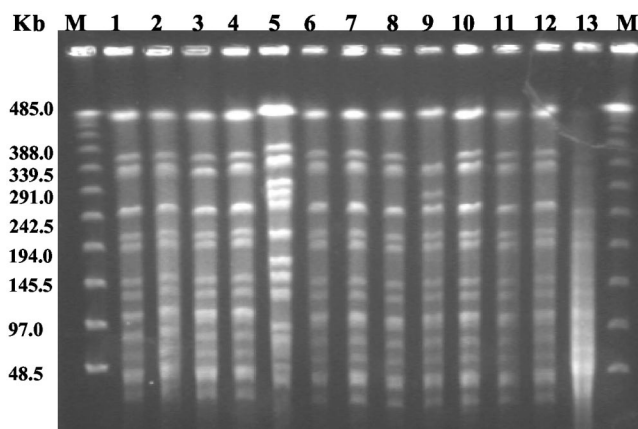


FIG. 1. PFGE patterns of *S. dysenteriae* type 1 strains isolated from Kolkata after digesting genomic DNA with the *Xba*I restriction endonuclease enzyme. Lanes M, 48.5-kb concatemers of bacteriophage λ DNA (Bio-Rad, Hercules, Calif.) used as molecular size standards; lane 1, outbreak strain (X1 pulsotype); lanes 2 to 4, 6 to 8, and 10 to 12, strains from hospitalized cases (X1 pulsotype); lane 5, hospital strain (X1₁ subtype); lane 9, hospital strain (X1₂ subtype); lane 13, smearing of DNA.

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