An unusual cluster of dysentery due to *Shigella dysenteriae* type 4 in Dhaka, Bangladesh

*Shigella* infections occur in both epidemic and sporadic forms in south Asia, including Bangladesh. The genus *Shigella* is composed of four species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. In *S. dysenteriae*, there are 15 recognized serotypes, of which serotype 1 attracts special attention for its epidemic-causing potential, the Shiga toxin it produces and its association with most serious complications (Bennish et al., 1990). Epidemics usually occur in areas with crowding and poor sanitary conditions where transmission from person to person is common, or when food or water is contaminated by the organism (Chiou et al., 2001).

In recent years, *S. dysenteriae* 1 has been responsible for large dysentery epidemics in south Asia, Central America and Africa (Talukder et al., 2003; Mendizabal-Morris et al., 1971; Guerin et al., 2003). In contrast, to our knowledge there are no reports of outbreaks caused by serotypes of *S. dysenteriae* other than type 1 except for an outbreak of *S. dysenteriae* 2 among laboratory workers due to intentional food contamination (Kolavic et al., 1997). We report here an outbreak associated with *S. dysenteriae* type 4 that occurred between June and December 2000 in Dhaka, Bangladesh. This is the first report of an outbreak caused by *S. dysenteriae* type 4.

From January 1999 to December 2002, a total of 358 *S. dysenteriae* strains isolated from patients attending the Dhaka treatment centre operated by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Centre for Health and Population Research, were initially identified in the Clinical Microbiology Laboratory by standard microbiological and biochemical methods (Talukder et al., 2003). Serotyping of these strains was confirmed by using the commercially available antisera kit (Denka Seiken) and slide agglutination test as described previously (Talukder et al., 2003).

Susceptibility patterns of the strains to antimicrobial agents were determined by the disc diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000), using commercial antimicrobial discs (Oxoid). The antibiotic discs used in this study were ampicillin (10 μg), sulfamethoxazole-trimethoprim (25 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and mecillinam (25 μg). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains for susceptibility studies. Plasmid DNA was prepared by the alkaline lysis method of Kado and Liu, with some modifications (Talukder et al., 2002).

Restriction patterns of chromosomal DNA were analysed by PFGE to determine the clonal distribution of the strains isolated at different time intervals according to the procedures described earlier (Talukder et al., 2002). Briefly, a portion of agarose plugs was digested with XbaI restriction enzyme (Gibco-BRL) and the restriction fragments were resolved by using CHEF-DRII system (Bio-Rad) with following pulse times: 1–10 s for 10 h, 3–28 s for 10 h, 3–35 s for 5 h and 5–70 s for 15 h. The gel was photographed by a gel documentation system (UV) and banding patterns were established by using the criteria described elsewhere (Tenover et al., 1995).

Restriction patterns of chromosomal DNA were determined for 358 *S. dysenteriae* isolates, serotypes 1, 2 and 4 were dominant, with the isolation of 122, 67 and 102 strains, respectively (Fig. 1). From 8 June 2000, the number of *S. dysenteriae* type 4 increased dramatically and a total of 71 strains were isolated by December 2000, as compared to 1, 24 and 6 isolates in 1999 (pre-outbreak), 2001 and 2002 (post-outbreak), respectively (Fig. 1). Thus the number of cases of shigellosis caused by *S. dysenteriae* type 4 peaked during the period from June to December 2000, suggesting the clustering of a single serotype of *S. dysenteriae* strains. During this period, 59% of cases of infection occurred in children less than 5 years old and the infection rate was higher in males (61%) as compared to females (39%). This age and sex distribution is similar to that of the patients treated at the ICDDR, B hospital. All of the 102 strains of *S. dysenteriae* 4 were resistant to sulfamethoxazole-trimethoprim, and 12.3% (n = 13) and 4.2% (n = 4) of strains were resistant to ampicillin and nalidixic acid, respectively, while all the strains were sensitive to ciprofloxacin and mecillinam.

Plasmid profiling and PFGE have long been used as molecular tools for investigating outbreaks as well as epidemiological studies (Talukder et al., 1999; Matsumoto et al., 1998; Terajima et al., 2004). Analysis of plasmid DNA showed that 140, 70–60, 4 and 1–6 Mda plasmids were commonly present in all of the 102 strains and no significant changes in plasmid profiles were observed between the outbreak and non-outbreak strains. PFGE identified three different types among the strains, which were designated A, B and C according to published interpretation criteria (Tenover et al., 1995). All of the 71 strains isolated in 2000 were indistinguishable and clustered in a single PFGE type, A, which was designated the outbreak clone. PFGE type B was observed only in the strains isolated in 1999, whereas type C and the outbreak clone A were found to be disseminated throughout the post-outbreak period of 2001 and 2002. PFGE analysis revealed that all of the strains isolated during the outbreak period from June to December 2000 were clonal, suggesting their dissemination from a single origin. This finding underscores the need to monitor the emergence and prevalence of *S. dysenteriae* type 4 in Bangladesh as well as in other parts of the world, as it appears to have the potential to cause outbreaks.

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Fig. 1. Number of S. dysenteriae type 1, 2 and 4 strains isolated each month from January 1999 to December 2002 in Dhaka, Bangladesh. Bars: white, type 1; grey, type 2; black, type 4.

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