

Clonal Multidrug-Resistant *Shigella dysenteriae* Type 1 Strains Associated with Epidemic and Sporadic Dysenteries in Eastern India

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Multidrug-resistant strains of *Shigella dysenteriae* type 1 were implicated in three outbreaks and sporadic cases of dysentery in eastern India in 2002 and 2003. After a hiatus of 14 years, this pathogen reemerged with an altered antibiotic resistance pattern. In addition to ampicillin, co-trimoxazole, tetracycline, chloramphenicol, and nalidixic acid, all the recent strains were resistant to norfloxacin, lomefloxacin, pefloxacin, and ofloxacin and showed reduced susceptibility to ciprofloxacin. Pulsed-field gel electrophoresis identified a new clone of *S. dysenteriae* type 1 that was associated with the recent outbreaks and sporadic cases. Based on the spatial and temporal spread of multidrug-resistant *S. dysenteriae* type 1, we predict that this clonal type may spread further in this region.

Shigellosis caused by multiply antibiotic-resistant *Shigella* strains constitutes an increasing problem in developing countries. The most virulent serotype of *Shigella dysenteriae*, *S. dysenteriae* type 1, has been responsible for large dysentery epidemics in India (S. C. Pal, Letter, Lancet **i**:1462, 1984), Guatemala and other parts of Central America (9), Zaire (2), Kenya (Y. Iijima, J. O. Oundo, K. Taga, S. M. Saidi, and T. Honda, Letter, Lancet **345**:69-70, 1995), Bangladesh (M. Benish, A. Eusof, B. Kay, and T. Weirzba, Letter, Lancet **ii**:441, 1985), and recently West Africa (3). To our knowledge, the last cases of dysentery caused by *S. dysenteriae* type 1 were reported from eastern India in 1988 (D. Sen, P. Dutta, B. C. Deb, and S. C. Pal, Letter, Lancet **ii**:911, 1988), and except for one strain in 1995, this serotype was not found again till 2001 (14, 15). We report here a comparative study of *S. dysenteriae* type 1 strains isolated from an early dysentery outbreak in 1988 and in recent outbreaks and sporadic cases in eastern India.

From March to June 2002, a total of 1,124 patients reported with acute bacillary dysentery to the subdivisional hospital, Diamond Harbor, West Bengal. During almost the same time (April to May 2002), a similar outbreak occurred in a northern part of West Bengal (Alipurduar, Siliguri) among tea garden laborers, affecting 1,728 persons with an attack rate and death rate of 25.6 and 6%, respectively. The details of these two outbreaks have been published elsewhere (K. Sarkar, S. Ghosh, S. K. Niyogi, and S. K. Bhattacharya, Letter, Lancet **361**:785, 2003; D. Sur, S. K. Niyogi, S. Sur, K. K. Datta, Y. Takeda, G. B. Nair, and S. K. Bhattacharya, Letter, Emerg. Infect. Dis. **9**:404-405, 2003). A year later (2003), another outbreak of dysentery from the first week of April until the first week of June was reported from a remote village of Aizal in

Mizoram State. The total number of reported cases was 169 with an attack rate of 17%. For bacteriological examination, 51 stool specimens were collected from dysentery patients from these three affected areas.

Strains of *S. dysenteriae* type 1 isolated from 904 and 950 acute diarrheal patients with sporadic cases who sought treatment at the Infectious Diseases Hospital (IDH) and B. C. Roy Children's Hospital (BCRCH), Calcutta, respectively, between January and December 2002 were also analyzed in this study. Stool specimens were collected from patients with dysentery, by the use of sterile catheters, into MacCartney bottles or with rectal swabs and tested for common enteric pathogens by standard microbiological techniques (18). The identity of these isolates was confirmed by biochemical tests (18). *Shigella* strains were serotyped with an antiserum kit (Denka Seiken Co., Ltd., Tokyo, Japan).

The antimicrobial susceptibility test was done by the standard disk diffusion method, according to the National Committee for Clinical Laboratory Standards guidelines (12). The *Escherichia coli* strain ATCC 25922 was used for quality control. Strains of *S. dysenteriae* type 1 were tested for susceptibility by using commercially available disks (HiMedia, Mumbai, India, and Difco) of ampicillin (10 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), lomefloxacin (10 µg), pefloxacin (5 µg), ofloxacin (5 µg), and tetracycline (30 µg). MICs of nalidixic acid, norfloxacin, ciprofloxacin, and ofloxacin were determined by the E-test (AB Biodisk, Solna, Sweden).

DNA fingerprinting was done by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Xba*I (Takara Shuzo, Otsu, Japan) according to a standard procedure (4). New and old strains of *S. dysenteriae* type 1, representing outbreak and sporadic cases, were included for comparison. Run conditions were generated by the autoalgorithm mode of the CHEF Mapper PFGE system with a size range of 30 to 600 kb (Bio-Rad Laboratories, Hercules, Calif.). After electrophore-

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TABLE 1. Antimicrobial resistance and PFGE profiles of *S. dysenteriae* type 1 strains isolated from patients with sporadic and outbreak cases of dysentery

Strain	Place (location)/yr of isolation	Case type	MIC ($\mu\text{g/ml}$)				Resistance profile ^a	Pulsotype
			Nalidixic acid	Ciprofloxacin	Norfloxacin	Ofloxacin		
HU8	Tripura (northeastern state)/1988	Outbreak	1	0.08	0.027	0.047	ACoTCNa	A
HU10	Tripura (northeastern state)/1988	Outbreak	1.25	0.012	0.047	0.047	ACoTCNa	A
HU29	Tripura (northeastern state)/1988	Outbreak	1	0.006	0.023	0.047	ACoTCNa	A
BCH518	Calcutta (West Bengal)/1995	Sporadic	>256	0.094	0.19	0.038	ACoTCNa	B ₁
D1	Diamond Harbor (West Bengal)/2002	Outbreak	>256	6	16	8	ACoTCNaNxLoOfPe	B ₂
D2	Diamond Harbor (West Bengal)/2002	Outbreak	>256	4	12	8	ACoTCNaNxLoOfPe	B ₃
D44	Diamond Harbor (West Bengal)/2002	Outbreak	>256	4	16	12	ACoTCNaNxLoOfPe	B
18	Siliguri (North Bengal)/2002	Outbreak	>256	2	12	12	ACoTCNaNxLoOfPe	B
21	Siliguri (North Bengal)/2002	Outbreak	>256	6	16	12	ACoTCNaNxLoOfPe	B
25	Siliguri (North Bengal)/2002	Outbreak	>256	2	12	12	ACoTCNaNxLoOfPe	B ₄
NK2217	Calcutta (West Bengal)/2002	Sporadic	>256	6	24	12	ACoTCNaNxLoOfPe	B ₅
NK2490	Calcutta (West Bengal)/2002	Sporadic	>256	4	12	12	ACoTCNaNxLoOfPe	B ₆
NK2678	Calcutta (West Bengal)/2002	Sporadic	>256	4	12	12	ACoTCNaNxLoOfPe	B ₇
NT4359	Calcutta (West Bengal)/2002	Sporadic	>256	4	12	12	ACoTCNaNxLoOfPe	C
H14174	Calcutta (West Bengal)/2002	Sporadic	>256	6	16	10	ACoTCNaNxLoOfPe	B ₈
H16576	Calcutta (West Bengal)/2002	Sporadic	>256	6	16	8	ACoTCNaNxLoOfPe	B ₉
Az11	Aizal (Mizoram State)/2003	Outbreak	>256	3	6.0	16	ACoTCNaNxLoOfPe	B

^a Abbreviations: A, ampicillin; Co, co-trimoxazole; T, tetracycline; C, chloramphenicol; Na, nalidixic acid; Nx, norfloxacin; Lo, lomefloxacin; Of, ofloxacin; Pe, pefloxacin.

sis, the ethidium bromide (Sigma, St. Louis, Mo.)-stained agarose gel was visualized and the gel images were digitized for computer-aided analysis (Gel Doc 2000; Bio-Rad) and stored in a personal computer. The images were retrieved and analyzed with the Diversity Database fingerprinting software (Bio-

Rad). The differences in the banding patterns of the PFGE profiles were compared to ascertain the clonal relationship between the strains. An unrooted dendrogram was made with the unweighted pair group with arithmetic mean (UPGMA) method.

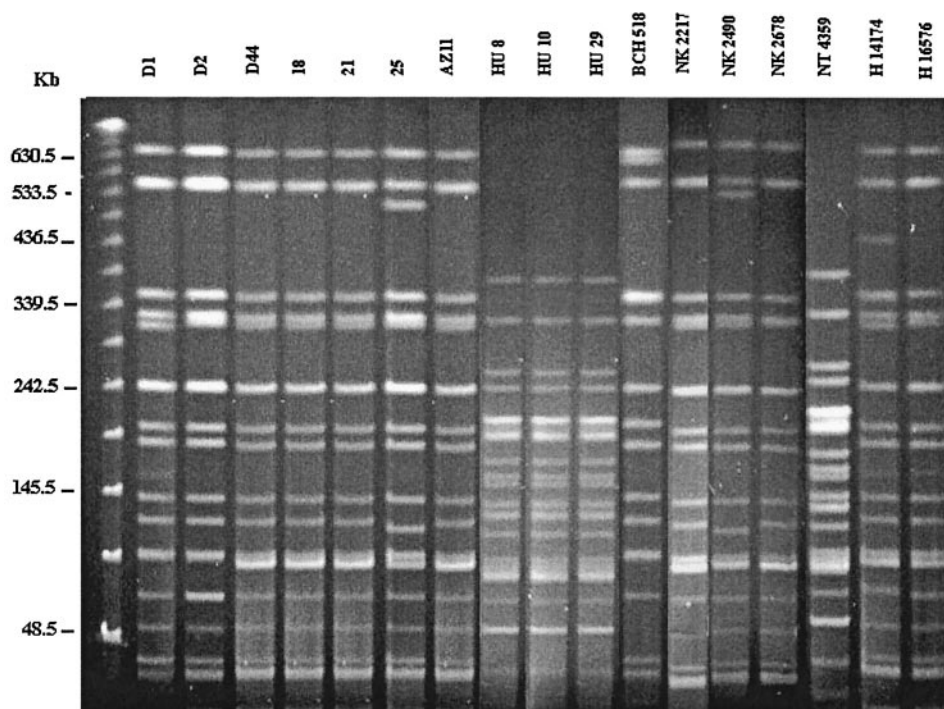


FIG. 1. PFGE profiles of *S. dysenteriae* type 1 strains after digestion with *Xba*I. Strains D1, D2, D44, 18, 21, and 25 represent the 2002 dysentery epidemic. Strain AZ11 was isolated from Aizal in 2003. Strains HU8, HU10, and HU29 were isolated during a 1988 outbreak in Tripura. BCH518 was isolated from a patient with a sporadic case in 1995 in Calcutta. Strains NK2217, NK2490, and NK2678 and strains NT4359, H14174, and H16576 were isolated from patients with sporadic cases of dysentery at BCRCH and IDH, respectively, in 2002. The molecular size marker (leftmost lane) used here is the PFG lambda ladder.

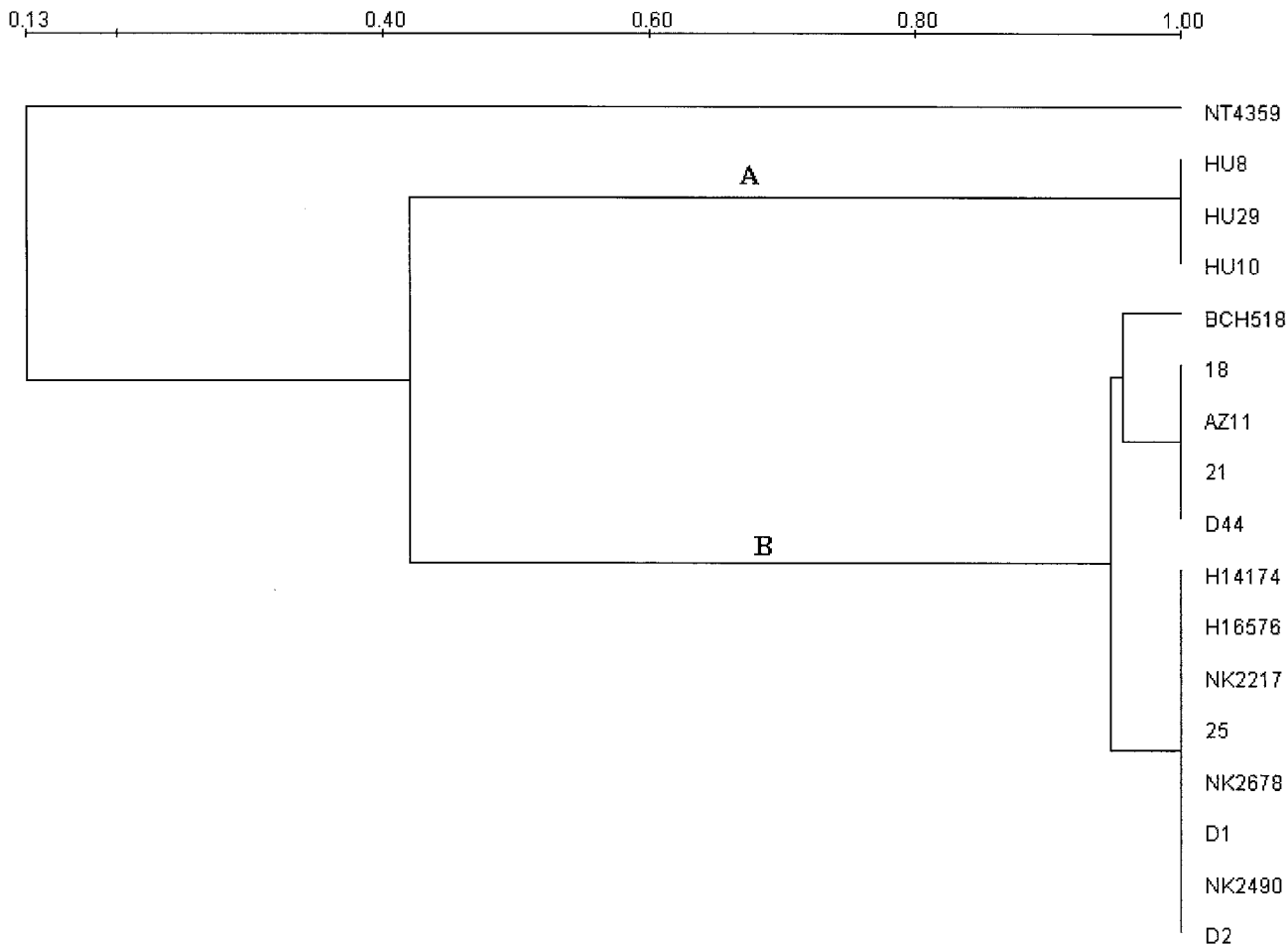


FIG. 2. Dendrogram analysis of the *S. dysenteriae* type 1 strains based on the PFGE profiles with Diversity Database software (Bio-Rad) employing the UPGMA method, which grouped clusters A and B.

Seventeen strains of *Shigella* spp. were isolated from the outbreak-affected regions, and sporadic case strains were serologically confirmed as *S. dysenteriae* type 1. The incidence rate of *S. dysenteriae* type 1 among sporadic cases was 0.8 and 0.95% in IDH and BCRCH, respectively. No other enteric pathogens were detected from the *S. dysenteriae* type 1-positive stool specimens. Cyclic epidemic patterns with major shifts in serogroups, with *S. dysenteriae* type 1 strains initially predominating and subsequently being replaced by *S. flexneri*, which is then replaced by *S. sonnei*, have been observed since the turn of the 20th century (7). This trend seems to be true in Calcutta (1, 14). Even though other *Shigella* serotypes prevailed (1, 14), *S. dysenteriae* type 1 appeared in this region after a hiatus of 14 years. The incidence of *S. dysenteriae* type 1 appears to be a recurrent phenomenon, associated with large-scale morbidity and mortality in eastern India (Pal, letter; Sarkar et al., letter; Sen et al., letter; Sur et al., letter).

Antibiotic susceptibility testing showed that *S. dysenteriae* type 1 strains were resistant to ampicillin, co-trimoxazole, nalidixic acid, chloramphenicol, and tetracycline (Table 1). In addition, the strains isolated in epidemic (2002 to 2003) and sporadic (2002) cases were resistant to norfloxacin, lomefloxacin, ofloxacin, and pefloxacin and showed reduced susceptibil-

ity to ciprofloxacin. The MIC ranges of ciprofloxacin, norfloxacin, and ofloxacin for recent *S. dysenteriae* type 1 strains were found to be 2 to 6, 6 to 24, and 8 to 16 $\mu\text{g/ml}$, respectively (Table 1). Antibiotic therapy is essential in the treatment of shigellosis, especially when the patients are infected with *S. dysenteriae* type 1. In this study, we recorded the isolation of *S. dysenteriae* strains with unique resistance profiles compared to those isolated previously in this region. The emergence of multidrug-resistant strains of *Shigella* was previously reported in Bangladesh (16), Indonesia (8), Tanzania (13), and India (15). An interesting observation in this study was the emergence of *S. dysenteriae* type 1 strains with altered antibiotic resistance profiles during each epidemic in eastern India. Nalidixic acid-resistant strains caused an epidemic in 1988 (Sen et al., letter), followed by the 2002 epidemic in which all the *S. dysenteriae* type 1 strains were resistant to fluoroquinolones (Sarkar et al., letter; Sur et al., letter).

S. dysenteriae type 1 strains resistant to nalidixic acid, a narrow-spectrum quinolone, were reported as early as 1981 from Rwanda (Iijima et al., letter). The subsequent appearance of nalidixic acid-resistant strains was reported in the Indian subcontinent (11; Sen et al., letter), and since then the proportion of resistance is increasing in other species of *Shi-*

gella (14). Treatment of patients with nalidixic acid was successful in 1984 when an *S. dysenteriae* type 1 epidemic occurred in northeastern India (R. Bose, J. N. Nashipuri, P. K. Sen, P. Dutta, S. K. Bhattacharya, D. Datta, D. Sen, and M. K. Bhattacharya, Letter, *Lancet* **ii**:1160, 1984). In many areas nalidixic acid still remains the drug of choice for shigellosis (5; A. Mahmood, Letter, *J. Pak. Med. Assoc.* **51**:101, 2001) including West Africa (3).

Norfloxacin and ciprofloxacin, expanded-spectrum quinolone derivatives, are effective in the treatment of shigellosis (6). These drugs have helped to control and manage shigellosis in areas of endemicity (10). The current epidemic is particularly disturbing due to the emergence of *S. dysenteriae* type 1 strains resistant to norfloxacin and ciprofloxacin, which are the drugs of choice for dysentery. Ampicillin and co-trimoxazole were formerly the drugs of choice for treating shigellosis in developing countries due to their low cost and broad spectrum of activity (13). These drugs are no longer useful because of the emergence of widespread resistance (16).

The PFGE patterns of the *S. dysenteriae* type 1 strains isolated in the 1988 epidemic and a strain isolated in 1995 in a sporadic case in Calcutta differed from those isolated during 2002 and 2003. All the recent epidemic strains were found to be closely related (Fig. 1), and the strains isolated from patients with sporadic cases admitted to the BCRCH during almost the same time frame exhibited strong similarity to the epidemic strains. However, discrete clones were also detected among patients with sporadic cases from Calcutta (strains BCH518 and NT4359) (Fig. 1). Dendrogram analysis indicated that the 1988 epidemic strains are identical (Fig. 2, cluster A) but different from seven recent epidemic strains (Fig. 2, cluster B).

Considering the PFGE clonal criteria (17), we observed that many strains from patients with sporadic cases were clonally related to epidemic strains isolated during the same period (Table 1). Chronologically, we first detected a new clone of *S. dysenteriae* type 1 among 2002 epidemic case patients, followed by sporadic diarrheal case patients admitted to the BCRCH, Calcutta, and in outbreak cases in Mizoram in 2003. This result indicates that the recent multiply drug-resistant strains of *S. dysenteriae* type 1 are spreading from the outbreak-affected areas to other regions. The reemergence of *S. dysenteriae* type 1 with epidemic potential is cause for concern. This trend should be carefully monitored in India and neighboring countries.

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REFERENCES

1. Dutta, P., D. Dutta, S. K. Bhattacharya, D. Sen, U. Mitra, A. R. Ghose, M. Lahiri, and B. C. Deb. 1989. Clinical and bacteriological profiles of shigellosis in Calcutta before and after an epidemic (1984–87). *Indian J. Med. Res.* **89**:132–137.
2. Goma Epidemiology Group. 1995. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire, in July 1994? *Lancet* **345**:339–344.
3. Guerin, P. J., C. Brasher, E. Baron, D. Mic, F. Grimont, M. Rayan, P. Aavitsland, and D. Legros. 2003. *Shigella dysenteriae* serotype 1 in West Africa: intervention strategy for an outbreak in Sierra Leone. *Lancet* **362**:705–706.
4. Gunderson, K., and G. Chu. 1991. Pulsed-field electrophoresis of megabase-sized DNA. *Mol. Cell. Biol.* **11**:3348–3354.
5. Iwalokun, B. A., G. O. Gbenle, S. I. Smith, A. Ogunledun, K. A. Akinsinde, and E. A. Omonigbehin. 2001. Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. *J. Health Popul. Nutr.* **19**:183–190.
6. Khan, W. A., C. Seas, U. Dhar, M. A. Salam, and M. L. Bennis. 1997. Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. A double-blind randomized, control trial. *Ann. Intern. Med.* **126**:697–703.
7. Kostrzewski, J., and H. S. Misiurewicz. 1968. Changes in the epidemiology of dysentery in Poland and the situation in Europe. *Arch. Immunol. Ther. Exp.* **16**:429–451.
8. Malakooti, M. A., J. Alaii, G. D. Shanks, and P. A. Phillips-Howard. 1997. Epidemic dysentery in western Kenya. *Trans. R. Soc. Trop. Med. Hyg.* **91**:541–543.
9. Mendizabal-Morris, C. A., I. Malta, E. J. Gangarisa, and G. Guzman. 1971. Epidemic Shiga-bacillus dysentery in Central America. Derivation of the epidemic and its progression in Guatemala, 1968–69. *Am. J. Trop. Med. Hyg.* **20**:927–933.
10. Moolasart, M. A., B. Eampokalap, and M. Ratanarithong. 1999. Comparison of the efficacy of cefitibuten and norfloxacin in the treatment of acute gastrointestinal infection in children. *Southeast Asian J. Trop. Med. Public Health* **30**:764–769.
11. Munshi, M. H., K. Haider, M. M. Rahaman, D. A. Sack, Z. U. Ahmed, and M. G. Morshed. 1987. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. *Lancet* **ii**:419–421.
12. National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial disk susceptibility tests. 12th informational supplement. Document M100–512. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. Navia, M. M., L. Capitano, J. Ruiz, M. Vargas, H. Urassa, D. Schelleberg, J. Gascon, and J. Vila. 1999. Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. *J. Clin. Microbiol.* **37**:3113–3117.
14. Niyogi, S. K., U. Mitra, and P. Dutta. 2001. Changing patterns of serotypes and antimicrobial susceptibilities of *Shigella* species isolated from children in Calcutta, India. *Jpn. J. Infect. Dis.* **54**:121–122.
15. Niyogi, S. K., and G. P. Pazhani. 2003. Multiresistant *Shigella* species isolated from childhood diarrhoea cases in Kolkata, India. *Jpn. J. Infect. Dis.* **56**:33–34.
16. Sack, R. B., M. Rahamn, M. Yunus, and E. H. Khan. 1997. Antimicrobial resistance in organisms causing diarrhoeal disease. *Clin. Infect. Dis.* **24**:S102–S105.
17. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
18. World Health Organization. 1987. Manual for laboratory investigation of acute enteric infections. CDD/83.3. World Health Organization, Geneva, Switzerland.