Outbreak of Dysentery Associated with Ceftriaxone-Resistant *Shigella sonnei*: First Report of Plasmid-Mediated CMY-2-Type AmpC β -Lactamase Resistance in *S. sonnei*

I-Fei Huang,^{1,3} Cheng-Hsun Chiu,² Mei-Hui Wang,² Chan-Yao Wu,⁴ Kai-Sheng Hsieh,¹ and Christine C. Chiou^{1,3*}

Department of Pediatrics, Veterans General Hospital—Kaohsiung, Kaohsiung, Taiwan¹; Department of Pediatrics, Chang Gung Children's Hospital, Taoyuan, Taiwan²; National Yang-Ming University, Taipei, Taiwan³; and Department of Pediatrics, E-DA Hospital/I-Shou University, Kaohsiung County, Taiwan⁴

Received 21 September 2004/Returned for modification 23 November 2004/Accepted 20 January 2005

We document the first report of plasmid-encoded CMY-2-type AmpC β -lactamase identified among *Shigella sonnei* isolates resistant to ceftriaxone and obtained after an outbreak of bacillary dysentery in Taiwan. One hundred eighty-two children in two elementary schools in Yu-Li, Taiwan, where an outbreak occurred after a typhoon hit this area in 2001, were enrolled in this study. Clinical and epidemiologic data on the infected children were collected. Pulsed-field gel electrophoresis (PFGE) was performed on the isolates to determine the genetic relatedness of outbreak strains. Plasmid analysis and PCR were performed to identify β -lactamase genes responsible for ceftriaxone resistance. Forty-seven children from the two elementary schools were culture positive for *S. sonnei* in this outbreak. Twenty-three children were asymptomatic. Of the total isolates 55.3% were resistant to ampicillin. One hundred percent of the isolates obtained from children in school A were initially susceptible to both ampicillin and ceftriaxone. Of isolates obtained from school B 96.2% were nonsusceptible to ceftriaxone. However, two isolates from school A developed resistance to ampicillin during the course of treatment. All 18 available isolates showed closely related PFGE patterns (4, 4a, 4b, and 4c). CMY-2-type AmpC β -lactamase was responsible for ceftriaxone resistance in ceftriaxone-nonsusceptible isolates; Southern blot hybridization confirmed that such a resistance gene was located on the plasmid. This is the first report of plasmid-mediated CMY-2-type AmpC β -lactamase in *S. sonnei*. Ampicillin-resistant isolates can develop during the course of antibiotic treatment.

Shigellosis is primarily a disease of poor, crowded communities that do not have adequate sanitation or clean water. Among the four *Shigella* species, *S. flexneri* is the most commonly isolated species in the developing world. However, in industrialized countries, the predominant species is *S. sonnei*, which accounts for 90% of the infections caused by *Shigella* spp. (25). Despite the continual improvement of sanitary conditions and the socioeconomic level of the population in Taiwan, outbreaks of shigellosis still occur after natural disasters such as typhoons or earthquakes.

Resistance of *Shigella* spp. to various antibiotics is increasing (26); however, plasmid-encoded resistance of *Shigella* spp. to extended-spectrum cephalosporins is still rare. Genetic testing is required to identify the relationship and mechanism of antimicrobial resistance in outbreak situations. Pulsed-field gel electrophoresis (PFGE) is a useful epidemiological tool but provides limited information about the genetic (plasmid) profile of the microorganisms. The purpose of this study was to investigate the epidemiological, clinical, and microbiological features of an outbreak of *Shigella* dysentery caused by *S. sonnei* in Yu-Li, Hua-Lien, Taiwan. Of note, this is the first report documenting production of CMY-2-type AmpC β -lactamase by *S. sonnei*.

MATERIALS AND METHODS

Patients and population. An outbreak of bacillary dysentery caused by *S. sonnei* occurred in two elementary schools in Yu-Li, Hua-Lien, Taiwan, in September to October 2001, after a typhoon hit Taiwan. The clinical and epidemiologic data for the infected children were collected retrospectively from the medical records. Children who were admitted to Yu-Li Veterans Hospital with shigellosis were enrolled in the study. Stool culture (or rectal swab) was performed for all the students in the two elementary schools after *S. sonnei* was isolated from the first student who had diarrhea.

Methods. (i) Bacterial isolates. Identification of *Shigella* organisms was performed according to accepted bacteriological procedures and completed by agglutination with specific antisera (Wellcome Labs, Poole, Dorset, England) (14).

(ii) Antimicrobial susceptibility. Antimicrobial susceptibility was examined by the disk diffusion method. The antimicrobial agents included ampicillin, chloramphenicol, ciprofloxacin, trimethoprim-sulfamethoxazole, and ceftriaxone. Susceptible and nonsusceptible isolates were defined according to the criteria suggested by the CLSI (formerly NCCLS) (19, 20).

(iii) Chromosomal DNA analysis by PFGE. PFGE was performed following the procedures described previously (32) with modifications. For PFGE, bacterial colonies were suspended in 10 mM Tris-0.1 mM EDTA and cast into a plug, which was treated with ESP buffer (0.5 M EDTA, 1% sodium lauroyl sarcosine, 0.5 mg of proteinase K per ml) for 24 h. The plug was washed and digested at 37°C with 40 U of XbaI in NEB2 (New England Biolabs 2) buffer overnight. The plug was then loaded onto a 1% agarose gel, and PFGE was carried out with a CHEF Mapper XA System (Bio-Rad Laboratories). The gel was run with a linear increase in switching times (from 4 to 70 s) over a period of 26 h, a 120-degree switch angle, and a gradient of 6.0 V per cm. The gel was stained with ethidium bromide and photographed with UV illumination. The DNA fingerprints generated by PFGE were analyzed according to the criteria proposed by Tenover et al. (33).

PCR and Southern blot hybridization. Genes for β -lactamases responsible for ceftriaxone resistance were amplified by PCR using primer sets previously described for detecting *bla*_{SHV-5}, *bla*_{CTX-M}, and *bla*_{CMY-2} genes (4, 10, 15, 18, 24). PCR products were purified by using the Microcon PCR centrifugal filter device

* Corresponding author. Mailing address: Department of Pediatrics, Veterans General Hospital—Kaohsiung, 386, Ta-Chung 1st Road, Kaohsiung, Taiwan. Phone: 886-7-3468203. Fax: 886-7-3468207. E-mail: chenchia@yahoo.com.

TABLE 1. Demographic data and clinical manifestations for the 47 children infected with *S. sonnei* in the two elementary schools

Characteristic	Value for school (n)	
	A (21)	B (26)
Demographic		
% Male (no. male/total)	52.4 (11/21)	57.7 (15/26)
Mean age in mo (range)	97.9 (52–156)	104.6 (26–133)
No. symptomatic/no. asymptomatic	20/1	4/22
% with symptom/sign (no. with symptom/total)		
Fever	83.3 (20/24)	12.5 (3/24)
Diarrhea	70.8 (17/24)	4.2 (1/24)
Abdominal pain	58.3 (14/24)	8.3 (2/24)
Vomiting	41.7 (10/24)	8.3 (2/24)
Bloody stool	12.5 (3/24)	4.2 (1/24)

(Millipore) and were sequenced with an ABI 377 automatic sequencer (Perkin-Elmer Applied Biosystems). The nucleotide sequences obtained were compiled and analyzed using Pgene software (Intelligenetics). The search for homologous sequences was done in the GenBank database using FASTA software through the Internet.

Plasmid profiles of the isolates were determined by the alkaline lysis method (13) and then transferred to a membrane for the following hybridization experiment. To locate the resistance gene, DNA-DNA hybridization with the PCR product as a probe was performed by the method of Southern (31).

RESULTS

Description of the outbreak. Yu-Li is located on the east side of Taiwan, where sanitary conditions are generally poorer than those of the west side. In September 2001, a typhoon hit the area and caused extensive flooding. Two elementary schools, located about 30 kilometers apart in Yu-Li, were affected in this outbreak. There were 98 students at school A and 84 students at school B. Twenty children in school A initially presented with fever, abdominal pain, and bloody stool and were admitted to Yu-Li Veterans Hospital in mid-September. *S. sonnei* was isolated from the stool cultures of all 20 students. These isolates were all susceptible to ampicillin. Surveillance cultures were performed on the other 78 students in school A. One asymptomatic child was also culture positive for *S. sonnei*. Subsequently, four children in school B had the same symptoms and were admitted during early October. Another 22 asymptomatic children from school B were found to be culture positive for *S. sonnei*. All the stool cultures obtained from school B yielded *S. sonnei* strains that were nonsusceptible to ampicillin.

Demographics and clinical features. Altogether 47 children from the two elementary schools were culture positive for *S. sonnei* in this outbreak (21 from school A and 26 from school B). The demographic data and symptoms at presentation are shown in Table 1. Fifty-one percent (24/47) of the culture-positive children exhibited signs and symptoms of shigellosis (20 from school A and 4 from school B). Twenty-three children were asymptomatic.

Antimicrobial susceptibility, course of treatment, and outcome. The antibiotic susceptibility data are shown in Table 2. All the isolates from the children of school A were susceptible to ampicillin initially. The 21 culture-positive children from school A received a 5-day course of ampicillin. Fifty-seven percent (12/21) had negative follow-up cultures, while *S. sonnei* was still isolated after 5 days of treatment in 42.8% (9/21). Twenty-two percent (two/nine) of these isolates were now nonsusceptible to ampicillin. These nine children received another 2-day treatment with ceftriaxone. The follow-up cultures were all negative after ceftriaxone treatment.

In contrast, all the isolates from the children of school B were nonsusceptible to ampicillin. Ampicillin was the initial treatment for the 26 children of school B before the susceptibility data were available. Ampicillin was used for 3 days, and then the antibiotic was switched to ceftriaxone in 20 children. The treatment of the 20 children was switched to a 5-day course of ciprofloxacin due to the continued stool positivity after 2 days of ceftriaxone treatment. The treatment of the other six children was directly shifted from ampicillin to the 5-day course of ciprofloxacin.

No patient developed bacteremia or other complications such as hemolytic-uremic syndrome, toxic megacolon, intestinal perforation, encephalopathy, or death. The follow-up stool cultures performed 24 and 48 h after discontinuance of the last course of antibiotics were all negative.

PFGE. Eighteen isolates were available for PFGE analysis (nine isolates from school A and nine isolates from school B). The results of PFGE are shown in Fig. 1. These 18 isolates appeared to originate from the same source, as their PFGE patterns were closely related according to the Tenover criteria (33). The isolates from school A showed the same PFGE pattern (pattern 4) except for one isolate (pattern 4a). The isolates from school B also showed an identical PFGE pattern (pattern 4b) except for two isolates (pattern 4c and pattern 4). Furthermore, the genotype of the nonsusceptible isolates from school A that were obtained after treatment was iden-

TABLE 2. Initial antimicrobial susceptibilities of 47 *S. sonnei* isolates from the two elementary schools

Drug	% of total isolates (n = 47)					
	School A (n = 21)			School B (n = 26)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin	100 (90.5) ^b	0	0 (9.5) ^b	0	0	100
Ceftriaxone	100	0	0	3.8	88.5	7.7
TMP-SMX ^a	0	0	100	0	0	100
Ciprofloxacin	100	0	0	100	0	0
Chloramphenicol	100	0	0	100	0	0

^a TMP-SMX, trimethoprim-sulfamethoxazole.

^b The number in parentheses is the percentage of antimicrobial susceptibility of isolates obtained from second stool cultures.

FIG. 1. The pulsed-field gel electrophoresis patterns of 18 isolates (nine from school A, nine from school B). Lane M, molecular mass markers.

tical to that of the susceptible isolates from that school (Table 3).

PCR and Southern blot hybridization. Ten isolates (one from school A and nine from school B) were tested by PCR. AmpC β -lactamase was responsible for ceftriaxone resistance

in all ceftriaxone-resistant isolates, and Southern blot hybridization confirmed that the resistance gene was located on the plasmid (Table 3). DNA sequencing of the PCR products confirmed that all ceftriaxone-resistant isolates harbored CMY-2 and SHV-5 β -lactamase genes. Two isolates

TABLE 3. Results of antibiograms, PFGE, and PCR for 18 *S. sonnei* isolates from the two elementary schools

Isolate	Response to drug ^a :					PFGE type	Presence of resistance gene:		
	Ampicillin	Chloramphenicol	Ciprofloxacin	Ceftriaxone	Trimethoprim-sulfamethoxazole		CMY-2	SHV-5	CTX-M
A-1	S	S	S	S	R	4			
A-2	S	S	S	S	R	4			
A-3	S	S	S	S	R	4b			
A-4	S	S	S	S	R	4			
A-5	S	S	S	S	R	4			
A-6	S	S	S	S	R	4			
A-7	S	S	S	S	R	4			
A-8	R	S	S	S	R	4			
A-9	R	S	S	S	R	4	—	+	—
B-1	R	S	S	I	R	4a	+	+	—
B-2	R	S	S	I	R	4a	+	+	—
B-3	R	S	S	I	R	4a	+	+	—
B-4	R	S	S	R	R	4c	+	+	—
B-5	R	S	S	I	R	4a	+	+	—
B-6	R	S	S	I	R	4a	+	+	—
B-7	R	S	S	S	R	4	—	+	—
B-8	R	S	S	R	R	4a	+	+	—
B-9	R	S	S	I	R	4a	+	+	—

^a S, susceptible; R, resistant; I, intermediate.

contained the SHV-5 gene only and remained susceptible to ceftriaxone.

DISCUSSION

Shigella spp. have managed to survive the antibiotic era via an ingenious mechanism of resistance, the production of β -lactamases. High rates of resistance to ampicillin among *Shigella* isolates are due to the production of β -lactamases similar to TEM-1 or OXA-1 (21, 28, 29). In the past 2 decades both the isolation frequencies and the types of extended-spectrum β -lactamase (ESBL) have gradually increased. ESBLs are detected most commonly in *Klebsiella pneumoniae* and *Escherichia coli* but have been noted in other members of the family *Enterobacteriaceae* as well. However, no ESBL-producing *Shigella* strains were reported until 1999, when an SHV-11 ESBL-producing *S. dysenteriae* strain was reported in India (2). Recently, four other ESBL-producing strains have been reported from four different countries: an *S. sonnei* strain with a CTX-M-14-type ESBL from Korea (22), an *S. sonnei* strain with a CTX-M-2-type ESBL from Argentina (23), an *S. flexneri* strain with an SHV-2-type ESBL from France (8), and an *S. sonnei* strain with a CTX-M-3-type ESBL from Turkey (1). To the authors' knowledge, this is the first report that CMY-2-type AmpC β -lactamase was responsible for ceftriaxone resistance in resistant isolates of *S. sonnei*; Southern blot hybridization confirmed that the resistance gene was located on the plasmid.

The most common typing procedures currently used with *Shigella* spp. are plasmid analysis and PFGE (9, 11, 16, 17, 30). *Shigella* species usually harbor a heterogeneous population of plasmids, ranging in numbers from 2 to as many as 10 (12). Plasmid analysis has proven to be a useful typing technique (9, 11); it is inexpensive and quick to perform. However, many plasmids are unstable and may be easily gained and/or lost, thus limiting this method's capacity in delineating genetic relatedness. Conversely, PFGE has a high discriminatory power, yet the procedure is time-consuming and expensive. In this outbreak, PFGE analysis demonstrated that all the isolates expressed similar or identical DNA fingerprints, indicating that these isolates might originate from the same source. Remarkably, the antibiograms were distinctively different among the isolates from the two schools. Shigellosis is relatively infrequent in Taiwan, with the incidence being 0.006% in the year 2001. Although the direct association with a food/water source was not established, the outbreaks happened very close together in terms of timing and geographic distance. Thus, it is biologically plausible that the outbreaks at the two schools were closely related while a parental strain might have acquired the resistant plasmid, leading to the expression of different antibiograms and spread in one of the schools.

In community-based observational studies, *Shigella* species usually are isolated from children with diarrhea and are seldom isolated from control patients who do not have diarrhea (3, 5, 6). Therefore, it has been inferred that infection with *Shigella* is virtually synonymous with disease. However, in this study, nearly half of the patients (23/47) were asymptomatic. The symptoms/signs of infected children comprise a clinical spectrum ranging from asymptomatic infections and secretory diarrhea to bloody diarrhea. Notably, bloody stool was noted in only 16.7% of the symptomatic patients.

Of note, antibiotic resistance appears to develop during the course of treatment, as shown in the two isolates obtained from patients of school A who received 5-day ampicillin treatment. Emergence of the resistance gene because of selective pressure may have contributed to the newly developed resistance. In this study, nine children from school A received a 2-day course of treatment to eradicate the organism from the stool. A treatment course of 5 days has been recommended for shigellosis. However, a shorter treatment course has been proposed as well (7, 27) and has demonstrated clinical and bacteriological cure rates comparable to those of the longer course. This would limit the environmental pressure for selection and dissemination of resistance genes among bacterial populations. In those instances when antibiotics are mandatory, short but effective courses of treatment may be warranted. A double-blind randomized study is mandatory to determine the optimal regimen and duration of treatment for shigellosis.

In summary, this is the first report on the CMY-2-type AmpC β -lactamase in *S. sonnei*. The gene was located on a plasmid and might be responsible for the spread of ceftriaxone resistance among isolates. Emergence of resistance could occur during the course of treatment; thus, close monitoring is mandatory.

ACKNOWLEDGMENTS

We thank Marilyn M. Wagener for her expertise and insight in reviewing the manuscript and for her helpful comments.

This work was supported by the Veterans General Hospital Kaohsiung grant number VGHKS 93-085.

REFERENCES

- Acikgoz, Z. C., Z. Gulay, M. Biemen, S. Gocer, and S. Gamberzade. 2003. CTX-M-3 extended-spectrum β -lactamase in a *Shigella sonnei* clinical isolate: first report from Turkey. *Scand. J. Infect. Dis.* **35**:503–505.
- Ahamed, J., and M. Kundu. 1999. Molecular characterization of the SHV-11 β -lactamase of *Shigella dysenteriae*. *Antimicrob. Agents Chemother.* **43**:2081–2083.
- Baqui, A. H., R. B. Sack, R. E. Black, K. Haider, A. Hossain, A. R. Alim, M. Yunus, H. R. Chowdhury, and A. K. Siddique. 1992. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children less than 5 years of age. *J. Infect. Dis.* **166**:792–796.
- Bauernfeind, A., I. Stemmlinger, R. Jungwirth, and H. Giamarellou. 1996. Characterization of the plasmidic β -lactamase CMY-2, which is responsible for cephamycin resistance. *Antimicrob. Agents Chemother.* **40**:221–224.
- Bennish, M. L., and B. J. Wojtyniak. 1991. Mortality due to shigellosis: community and hospital data. *Rev. Infect. Dis.* **13**:S245–S251.
- Echeverria, P., D. N. Taylor, U. Lexsomboon, M. Bhaibulaya, N. R. Blacklow, K. Tamura, and R. Sakazaki. 1989. Case-control study of endemic diarrheal disease in Thai children. *J. Infect. Dis.* **159**:543–548.
- Eidlitz-Marcus, T., Y. H. Cohen, M. Nussinovitch, I. Elian, and I. Varsano. 1993. Comparative efficacy of two- and five-day courses of ceftriaxone for treatment of severe shigellosis in children. *J. Pediatr.* **123**:822–824.
- Fortineau, N., T. Naas, O. Gaillot, and P. Nordmann. 2001. SHV-type extended-spectrum β -lactamase in a *Shigella flexneri* clinical isolate. *J. Antimicrob. Chemother.* **47**:685–688.
- Gebre-Yohannes, A., and B. S. Drasar. 1991. Molecular epidemiology of plasmid patterns in *Shigella flexneri* types 1–6. *Epidemiol. Infect.* **107**:321–334.
- Gniadkowski, M., I. Schneider, A. Palucha, R. Jungwirth, B. Mikiewicz, and A. Bauernfeind. 1998. Cefotaxime-resistant *Enterobacteriaceae* isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β -lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. *Antimicrob. Agents Chemother.* **42**:827–832.
- Haider, K., M. I. Huq, K. A. Talukder, and Q. S. Ahmad. 1989. Electrophoretotyping of plasmid DNA of different serotypes of *Shigella flexneri* isolated in Bangladesh. *Epidemiol. Infect.* **102**:421–428.
- Jamieson, A. F., D. A. Bremner, P. L. Bergquist, and H. E. D. Lane. 1979. Characterization of plasmids from antibiotic-resistant *Shigella* isolates by agarose gel electrophoresis. *J. Gen. Microbiol.* **113**:73–81.
- Kado, C. I., and S. T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* **145**:1365–1373.

14. Kelly, M. T., D. J. Brenner, and J. J. Farmer III. 1985. *Enterobacteriaceae*, p. 263–277. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
15. Koeck, J. L., G. Arlet, A. Philippon, S. Basmaciogullari, H. V. Thien, Y. Buisson, and J. D. Cavallo. 1997. A plasmid-mediated CMY-2 β -lactamase from an Algerian clinical isolate of *Salmonella* senftenberg. FEMS Microbiol. Lett. 152:255–260.
16. Litwin, C. M., A. L. Storm, S. Chipowsky, and K. J. Ryan. 1991. Molecular epidemiology of *Shigella* infections: plasmid profiles, serotype correlation, and restriction endonuclease analysis. J. Clin. Microbiol. 29:104–108.
17. Liu, P. Y., Y. J. Lau, B. S. Hu, M. J. Shyr, Z. Y. Shi, W. S. Tsai, Y. H. Lin, and C. Y. Tseng. 1995. Analysis of clonal relationships among isolates of *Shigella sonnei* by different molecular typing methods. J. Clin. Microbiol. 33:1779–1783.
18. Mabilat, C., and S. Goussard. 1993. PCR detection and identification of genes for extended-spectrum β -lactamases, p. 553–559. In D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
19. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. National Committee for Clinical Laboratory Standards. 2000. Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, Pa.
21. 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.
22. Pai, H., E. H. Choi, H. J. Lee, J. Y. Hong, and G. A. Jacoby. 2001. Identification of CTX-M-14 extended-spectrum β -lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli*, and *Klebsiella pneumoniae* in Korea. J. Clin. Microbiol. 39:3747–3749.
23. Radice, M., C. González, P. Power, M. C. Vidal, and G. Gutkind. 2001. Third-generation cephalosporin resistance in *Shigella sonnei*, Argentina. Emerg. Infect. Dis. 7:442–443.
24. Rasheed, J. K., C. Jay, B. Metchock, F. Berkowitz, L. Weigel, J. Crellin, C. Steward, B. Hill, A. A. Medeiros, and F. C. Tenover. 1997. Evolution of extended-spectrum β -lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. 41:647–653.
25. Sack, D. A., C. Lyke, C. McLaughlin, and V. Suwanvanichkij. 2001. Antimicrobial resistance in shigellosis, cholera and campylobacteriosis. WHO/CDS/CSR/DRS/2001.8. [Online.] World Health Organization, Geneva, Switzerland. http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_DRS_2001_8/en/.
26. Sack, R. B., M. Rahman, M. Yunus, and E. H. Khan. 1997. Antimicrobial resistance in organisms causing diarrheal disease. Clin. Infect. Dis. 24:S102–S105.
27. Salam, M. A., and M. L. Bennish. 1991. Antimicrobial therapy for shigellosis. Rev. Infect. Dis. 13:S332–S341.
28. Schumacher, H., M. Nir, B. Mansa, and A. Grassy. 1992. β -Lactamases in *Shigella*. APMIS 100:954–956.
29. Siu, L. K., J. Y. Lo, K. Y. Yuen, P. Y. Chau, M. H. Ng, and P. L. Ho. 2000. β -Lactamases in *Shigella flexneri* isolates from Hong Kong and Shanghai and a novel OXA-1-like β -lactamase, OXA-30. Antimicrob. Agents Chemother. 44:2034–2038.
30. Soldati, L., and J. C. Piffaretti. 1991. Molecular typing of *Shigella* strains using pulsed field gel electrophoresis and genome hybridization with insertion sequences. Res. Microbiol. 142:489–498.
31. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.
32. Su, L. H., H. S. Leu, Y. P. Chiu, J. H. Chia, A. J. Kuo, C. F. Sun, T. Y. Lin, and T. L. Wu. 2000. Molecular investigation of two clusters of nosocomial bacteraemia caused by multiresistant *Klebsiella pneumoniae* using pulsed-field gel electrophoresis and infrequent-restriction-site PCR. J. Hosp. Infect. 46:110–117.
33. 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.