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Nosocomial Outbreak of Pediatric Gastroenteritis Caused by CTX-M-14-Type Extended-Spectrum β-Lactamase-Producing Strains of *Salmonella enterica* Serovar London

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CTX-M-14-type extended-spectrum β-lactamase was first detected in *Salmonella enterica* serovar London strains which were isolated from three hospitalized pediatric patients with gastroenteritis. The isolates had pulsed-field gel electrophoresis patterns identical to those of the previously isolated antimicrobial-susceptible strains from community-acquired gastroenteritis, suggesting the susceptible clone acquired the resistance.

Nontyphoidal salmonella (NTS) is one of the most important enteric pathogens even in developed countries, and it may also cause bloodstream and other extraintestinal infections for which antimicrobial therapy is required. *Salmonella* spp. are usually susceptible to many antimicrobial agents, but a recent increase of resistance has become a concern (18). *Salmonella enterica* serovar London is not a common serovar; the infection was rarely reported, but an epidemic occurred in Hungary in 1980 (9). Only 4 of 1,306 (0.3%) *Salmonella* isolates were of this serovar in 1998 in Korea (7). However, the number increased to 74 in the period from 2000 to 2001 (8), indicating a wide dissemination. In 2000, an infant formula-associated community-acquired gastroenteritis outbreak affecting 31 infants was caused by *Salmonella* serovar London (16), and even a rare endophthalmitis infection in a previously healthy 3-month-old infant was similarly caused (20). There were no reports of outbreaks of *Salmonella* serovar London infection in 2002 or 2003, but the National Institute of Health, Korea, reported that 23 of 632 (3.6%) *Salmonella* isolates were of this serovar in 2003 (http://dis.cdc.go.kr/cdmr/eng_cdmr.asp).

A small outbreak of gastroenteritis occurred, affecting three hospitalized pediatric patients during an 11-day period in June 2004. Serogroup E *Salmonella* strains with cefotaxime resistance were isolated from watery stool specimens, and this prompted us to determine a possible relationship between these strains and the recently reported *Salmonella* serovar London strains (8, 16) and to determine the mechanism of β-lactam resistance.

The serovar of the isolates was determined at the National Institute of Health, Korea. Antimicrobial susceptibility was tested by the disk diffusion method (14), and extended-spectrum β-lactamase (ESBL) was screened by the double-disk synergy test with amoxicillin-clavulanic acid versus cefotaxime and ceftazidime disks with distances of 15 mm from edge to edge. Etest strips and Etest ESBL strips (AB BIODISK, Solna, Sweden) were used to determine MICs of cefotaxime and ceftazidime and to confirm ESBL production, respectively. Isoelectric points (pIs) of the β-lactamases were determined as described previously (11). The bla_{TEM} and bla_{SHV} alleles were detected by PCR as described previously (2, 10). The primers shown in Table 1 were used to detect or sequence the bla_{CTX-M} gene with the following conditions: 30 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 45 s. *Proteus mirabilis* strain YMC 03/01/U226, which harbors the $bla_{CTX-M-14}$ gene, was used as a positive control. The sequencing was performed as reported previously (11). Resistance transfer was tested by both broth- and plate-mating methods using an azide-resistant recipient, *Escherichia coli* strain J53. Pulsed-field gel electrophoresis (PFGE) of XbaI-restricted genomic DNA was performed according to the manufacturer's instructions (Bio-Rad, Hercules, Calif.), and the patterns were visually compared with those of recently reported *Salmonella* serovar London strains (8, 16).

The three patients developed diarrhea 4, 17, and 22 days after hospitalization, respectively (Table 2). All three *Salmonella* isolates from the patients were identified as *Salmonella* serovar London. However, contrary to the previously reported strains (8), the isolates were resistant to ampicillin, cefotaxime, aminoglycosides, and trimethoprim-sulfamethoxazole. ESBL production was suspected by the double-disk synergy test, and the MICs by Etest, i.e., ≥256 μg/ml for cefotaxime, 0.25 μg/ml for cefotaxime-clavulanic acid, 3 to 6 μg/ml for ceftazidime, and 0.38 μg/ml for ceftazidime-clavulanic acid, suggested the enzyme being of the CTX-M type. All three isolates had β-

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TABLE 1. Primers used to detect and sequence CTX-M-type ESBL genes

PCR target	Utility	Primer	Nucleotide sequence (5′ to 3′)	Position of primer	Expected size (bp)	GenBank accession no.	Source or reference
CTX-M	Detection	CTX-Uni-F	CVA TGT GCA GYA CCA GTA A	209–227	585	AJ416341	A. Bauernfeind[a]
		CTX-Uni-R	ARG TSA CCA GAA YMA GCG G	775–793			
CTX-M-1 group	Detection	CTX-M-1 Gr-F	TCA ATG GGA CGA TGT CAC TG	350–366	500	X92506	This study
		CTX-M-1 Gr-R	CGC CGA CGC TAA TAC ATC G	831–849			
CTX-M-2 group	Detection	CTX-M-2 Gr-F	AAA GTG ACG GCG TTT GCT C	448–466	360	X92507	This study
		CTX-M-2 Gr-R	CGG TTG GGT AAA GTA GGT CAC	787–807			
CTX-M-8 group	Detection	CTX-M-8 Gr-F	AGA CGC TTC GCA ATC TGA C	572–590	236	AF189721	This study
		CTX-M-8 Gr-R	TGG CTG GGT GAA GTA AGT G	789–807			
CTX-M-9 group	Detection	CTX-M-9 Gr-F	GAT GAA CGC TTT CCA ATG T	196–214	463	AJ416345	This study
		CTX-M-9 Gr-R	CGG TCG TAT TGC CTT TGA G	640–658			
CTX-M-14	Sequencing	CTX-M-14-F1	GAG TGT TGC TCT GTG GAT AAC	−227 to −207	1,347	AF252622	11
		CTX-M-14-R2	GGC AAG GTC AGA ATA GCG CTG	224–244			
		CTX 14S-F	AAA AAT GAT TGA AAG GTG GTT GT	−162 to −131			This study
		CTX 14S-R	TTA CAG CCC TTC GGC GAT GA	857–876			
		CTX 14S-Seq-F	TGG CTC AAA GGC AAT ACG A	637–655			

[a] Personal communication.

lactamase bands of pIs 5.4 and >8.0. The bla_{TEM} and bla_{CTX-M} alleles, but not the bla_{SHV} allele, were detected by PCR, and the sequences were identical to those of bla_{TEM-1} and $bla_{CTX-M-14}$.

A majority of the ESBL-producing *Salmonella* isolates have been *Salmonella enterica* serovar Typhimurium (4) and *Salmonella enterica* serovar Enteritidis (17). Nosocomial infections caused by ESBL-producing NTS are not often reported (4). In Korea, a few isolates of TEM-52-type ESBL-producing NTS were reported, but they were not *Salmonella* serovar London (10).

Among the six groups of CTX-M enzymes (1), the CTX-M-1, -2, and -9 groups have been reported in serovars Typhimurium, Virchow, Enteritidis, Kentucky, Infantis, and Oranienburg (1, 3, 5, 12, 19). However, to the best of our knowledge this is the first report of a CTX-M-14 enzyme-producing *Salmonella* serovar London, which suggests a gradual spread of this resistance to various serovars of *Salmonella*. ESBL genes have the potential to spread to other organisms because they reside on plasmids or in class 1 integrons (1, 3). Repeated attempts failed to transfer the cefotaxime resistance by conjugation, as was reported with other CTX-M-type ESBL-producing isolates (4).

The PFGE patterns of XbaI-digested genomic DNA of all three $bla_{CTX-M-14}$-positive isolates in this study were identical, suggesting an outbreak by a single clone (Fig. 1). We could not determine the origin of the outbreak. The pattern was also identical to that from community-acquired pediatric patients (8, 16), suggesting an identical clone had been spreading. However, it is interesting that our isolates were multidrug resistant, while the other strains reported were susceptible to multiple antimicrobial agents, including ampicillin. Acquisition of bla_{TEM-52} by NTS during hospitalization was documented in a previous study (9). The *Salmonella* serovar London strains in this study also probably acquired the bla_{CTX-M} gene from other gram-negative bacilli. CTX-M-14 enzyme-producing *Klebsiella pneumoniae* and *E. coli* isolates were detected from blood at the same hospital (15).

Antimicrobial treatment for NTS gastroenteritis is generally not required, but treatment with ceftriaxone or trimethoprim-sulfamethoxazole is recommended if a patient is under six months or over 50 years of age or has underlying diseases (6). Two of our patients did not receive antimicrobial therapy for the gastroenteritis until the persistence of the organisms for 9 and 24 days was known, which provided ample opportunity to disperse the strains. Fluoroquinolone is not a recommended drug for pediatric patients, but a short treatment with oral ciprofloxacin is considered safe and allows a rapid recovery (13). The isolates were susceptible to nalidixic acid, and ciprofloxacin therapy eliminated the *Salmonella* from two patients. The one remaining patient received cefazolin for the treatment of other conditions but did not receive any antimicrobial agent to treat gastroenteritis because she was discharged before the *Salmonella* isolation report was available.

In conclusion, the CTX-M-14-type extended-spectrum β-lactamase was first detected in *Salmonella* serovar London strains which were isolated from three hospitalized pediatric patients with gastroenteritis. The isolates had PFGE patterns identical to those of the previously isolated antimicrobial-susceptible strains from community-acquired gastroenteritis, suggesting the susceptible clone acquired the resistance.

TABLE 2. Clinical features of the gastroenteritis patients infected with ESBL-producing *Salmonella* serovar London isolates

Case no.	Age/sex	Underlying disease	Department	Admission date	Date of *Salmonella* culture/persistence	Treatment/outcome
1	18 mo/male	Acute lymphocytic leukemia	Hematooncology	1 June 2004	18 June 2004/9 days	Ciprofloxacin/cured
2	4 yr/male	Ganglioneuroblastoma	Hematooncology	6 June 2004	28 June 2004/24 days	Ciprofloxacin/cured
3	3 mo/female	Meningocele	Neurosurgery	19 June 2004	23 June 2004/unknown	Cefazolin/unknown

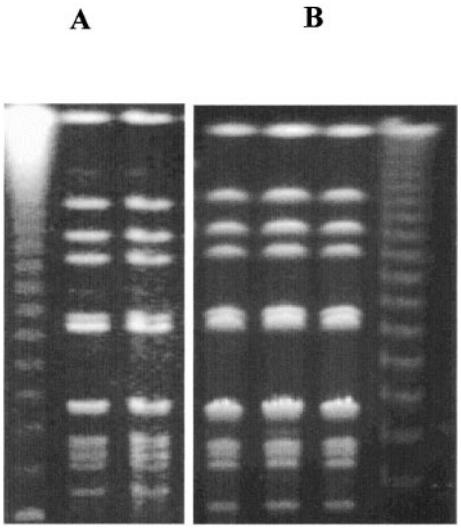

FIG. 1. Comparison of PFGE patterns of XbaI-digested genomic DNA of *Salmonella* serovar London isolates. (A) Two antimicrobial-susceptible strains isolated from infants (8). (B) Three CTX-M-14-producing strains in this study. The patterns are apparently identical, although the PFGE conditions were different: an initial 2.2 s and a final 63.8 s for 16 h (for panel A) and an initial 0.5 s and a final 60 s for 20 h (for panel B).

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REFERENCES

1. **Bonnet, R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
2. **Chong, Y., K. Lee, R. Okamoto, and M. Inoue.** 1997. Characteristics of extended-spectrum β-lactam hydrolyzing activity of *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from clinical specimens. Korean J. Infect. Dis. **29:**477–485.
3. **Di Conza, J., J. A. Ayala, P. Power, M. Mollerach, and G. Gutkind.** 2000. Novel class 1 integron (InS21) carrying $bla_{CTX-M-2}$ in *Salmonella enterica* serovar Infantis. Antimicrob. Agents Chemother. **46:**2257–2261.
4. **Edelstein, M., M. Pimkin, T. Dmitrachenko, V. Semenov, N. Kozlova, D. Gladin, A. Baraniak, and L. Stratchounski.** 2004. Multiple outbreaks of nosocomial salmonellosis in Russia and Belarus caused by a single clone of *Salmonella enterica* serovar Typhimurium producing an extended-spectrum β-lactamase. Antimicrob. Agents Chemother. **48:**2808–2815.
5. **Gierczynski, R., J. Szych, A. Cieslik, W. Rastawicki, and M. Jagielski.** 2003. The occurrence of the first two CTX-M-3 and TEM-1 producing isolates of *Salmonella enterica* serovar Oranienburg in Poland. Int. J. Antimicrob. Agents **21:**497–499.
6. **Guerrant, R. L., T. Van Gilder, T. S. Steiner, N. M. Thielman, L. Slutsker, R. V. Tauxe, T. Hennessy, P. M. Griffin, H. DuPont, R. B. Sack, P. Tarr, M. Neill, I. Nachamkin, L. B. Reller, M. T. Osterholm, M. L. Bennish, and L. K. Pickering.** 2001. Practice guidelines for the management of infectious diarrhea. Clin. Infect. Dis. **32:**331–351.
7. **Kim, H. H., M. S. Park, Y. H. Kang, J. Y. Yu, S. H. Kim, Y. H. Shin, M. J. Kim, and B. K. Lee.** 1999. Epidemiological characteristics of *Salmonella* strains isolated recently in Korea. J. Korean Soc. Microbiol. **34:**595–598.
8. **Kim, S., Y. H. Kang, H. J. Nam, O. Y. Lim, W. S. Seok, J. K. Park, and B. K. Lee.** 2003. A virulent strain of *Salmonella enterica* serovar London isolated in infants with enteritis traced by active surveillance and molecular epidemiological study. J. Korean Med. Sci. **18:**325–330.
9. **Lantos, J., and E. Marjai.** 1980. In vitro transfer of multiple resistance observed in vivo during a *Salmonella* London epidemic. Acta Microbiol. Acad. Sci. Hung. **27:**47–53.
10. **Lee, K., D. Yong, J. H. Yum, H. H. Kim, and Y. Chong.** 2003. Diversity of TEM-52 extended-spectrum β-lactamase-producing non-typhoidal *Salmonella* isolates in Korea. J. Antimicrob. Chemother. **52:**493–496.
11. **Lee, K., D. Yong, J. H. Yum, Y. S. Lim, H. S. Kim, B. K. Lee, and Y. Chong.** 2004. Emergence of multidrug-resistant *Salmonella enterica* serovar Typhi in Korea. Antimicrob. Agents Chemother. **48:**4130–4135.
12. **Liebana, E., M. Batchelor, C. Torres, L. Brinas, L. A. Lagos, B. Abdalhamid, N. D. Hanson, and J. Martinez-Urtaza.** 2004. Pediatric infection due to multiresistant *Salmonella enterica* serotype Infantis in Honduras. J. Clin. Microbiol. **42:**4885–4888.
13. **Moulin, F., H. Sauve-Martin, E. Marc, M. M. Lorrot, M. Soulier, S. Ravilly, J. Raymond, and D. Gendrel.** 2003. Ciprofloxacin after clinical failure of β-lactam antibiotics in children with salmonellosis. Arch. Pediatr. **10:**608–614.
14. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement. M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.
15. **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.
16. **Park, J. K., W. S. Seok, B. J. Choi, H. M. Kim, B. K. Lim, S. S. Yoon, S. Kim, Y. S. Kim, and J. Y. Park.** 2004. *Salmonella enterica* serovar London infections associated with consumption of infant formula. Yonsei Med. J. **29:**43–48.
17. **Romero, L., L. Lopez, L. Martinez-Martinez, B. Guerra, J. R. Hernandez, and A. Pascual.** 2004. Characterization of the first CTX-M-14-producing *Salmonella enterica* serotype Enteritidis isolate. J. Antimicrob. Chemother. **53:**1113–1114.
18. **Stephen, J. M., M. A. Toleman, T. R. Walsh, and R. N. Jones.** 2003. *Salmonella* bloodstream infections: report from the SENTRY antimicrobial surveillance program (1997–2001). Int. J. Antimicrob. Agents **22:**395–405.
19. **Weill, F. X., J. D. Perrier-Gros-Claude, M. Demartin, S. Coignard, and P. A. Grimont.** 2004. Characterization of extended-spectrum-β-lactamase (CTX-M-15)-producing strains of *Salmonella enterica* isolated in France and Senegal. FEMS Microbiol. Lett. **238:**353–358.
20. **Yu, Y. S., S. W. Hwang, and H. J. Lee.** 2002. *Salmonella* London endogenous endophthalmitis in a healthy infant. Pediatr. Infect. Dis. J. **21:**578.