Increasing Ceftriaxone Resistance in Salmonellae, Taiwan

Lin-Hui Su, Wen-Shin Teng, Chyi-Liang Chen, Hao-Yuan Lee, Hsin-Chieh Li, Tsu-Lan Wu, and Cheng-Hsun Chiu

In Taiwan, despite a substantial decline of *Salmonella enterica* serotype Choleraesuis infections, strains resistant to ciprofloxacin and ceftriaxone persist. A self-transferable bla_{CMY-2}-harboring IncI1 plasmid was identified in *S. enterica* serotypes Choleraesuis, Typhimurium, Agona, and Enteritidis and contributed to the overall increase of ceftriaxone resistance in salmonellae.

Salmonella enterica serotype Choleraesuis usually causes invasive infection (*1*). When resistant *Salmonella* infection is encountered, fluoroquinolones or extended-spectrum cephalosporins are frequently used (*2*). Fluoroquinolone resistance has been common in this invasive serotype (*3*). Isolation of SC-B67, a strain of *S. enterica* ser. Choleraesuis that was resistant to ciprofloxacin and ceftriaxone (CIP^r/CRO^r), has exacerbated the problem (*4*). Ceftriaxone resistance in SC-B67 was attributed to a plasmid-mediated bla_{CMY-2}, located on a specific IS*Ecp1*-bla_{CMY-2}-*blc*-*sugE* structure (*4*). This conserved DNA fragment, subsequently named Tn*6092* (*5*), has been reported from different geographic areas and is widely distributed among various *Salmonella* serotypes and other Enterobacteriaceae (*6*).

The Study

Since 1999, computerized records of bacterial culture results have been stored at Chang Gung Memorial Hospital, a 3,500-bed medical center in northern Taiwan. Periodic review of these records indicated a reverse trend in the prevalence of serogroups D (increase) and B (decrease) isolates during the past decade (Figure 1, panel A). A significant decrease in the prevalence of *S. enterica* ser. Choleraesuis was also evident (Figure 1, panel A). Nevertheless, in recent years, ceftriaxone resistance has increased from <5% to >10% in *S. enterica* ser. Choleraesuis and in serogroup B salmonellae (Figure 1, panel B).

Since isolation of SC-B67 in 2002 (*4*), 10 CIP^r/CRO^r *S. enterica* ser. Choleraesuis isolates have been recovered. All CIP^r/CRO^r isolates were resistant to nalidixic acid and ciprofloxacin, but SC-B134 remained susceptible to ciprofloxacin (Table 1). PCR and sequencing with specific primers (online Technical Appendix Table 1, www.cdc.gov/EID/content/17/6/1086-Techapp.pdf) revealed 3 identical amino acid changes in GyrA and ParC among all CIP^r/CRO^r isolates except SC-B134 (Table 1). Reduced fluoroquinolone susceptibility of SC-B134 could be explained by the single amino acid change at codon 87 of GyrA. Amino acid changes were not found in GyrB or ParE.

In terms of clinical features (online Technical Appendix Table 2), most patients with these infections were adults who had a wide spectrum of underlying diseases. Antimicrobial agents were prescribed for all patients, and extended-spectrum cephalosporins were used most frequently. Two patients died. Seven blood isolates from the 10 patients with CIP^r/CRO^r *S. enterica* ser. Choleraesuis infections, together with the ceftriaxone-resistant isolates noted below, were investigated further. SC-B67 was used as a reference.

During the first 6 months of 2010, a total of 6 cases of ceftriaxone-resistant *Salmonella* infection were found: serogroup B in 5 patients (*S. enterica* ser. Agona, n = 1; *S.*

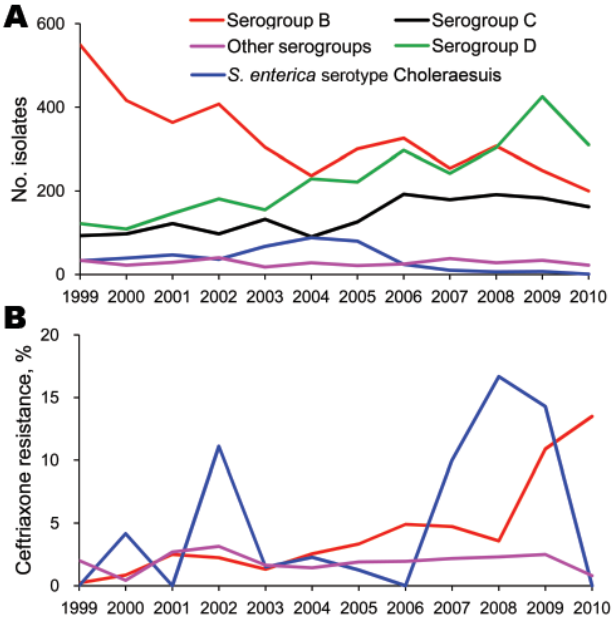

Figure 1. Secular trends in annual numbers (A) and rates (B) of ceftriaxone resistance among various serogroups or serotype of nontyphoidal *Salmonella enterica* isolates in Chang Gung Memorial Hospital, 1999–2010.

Author affiliations: Chang Gung Memorial Hospital, Taoyuan, Taiwan (L.-H. Su, H.-C. Li, T.-L. Wu); Chang Gung University College of Medicine, Taoyuan (L.-H. Su, W.-S. Teng, H.-Y. Lee, T.-L. Wu, C.-H. Chiu); and Chang Gung Children's Hospital, Taoyuan (W.-S. Teng, C.-L. Chen, H.-Y. Lee, C.-H. Chiu)

DOI: 10.3201/eid1706.101949

Table 1. Characteristics of the resistant *Salmonella enterica* isolates from 17 patients at Chang Gung Memorial Hospital, Taiwan*

Serotype and isolate (no. patients)	Year	Type of specimen	Susceptibility profile†	Puls‡	Plasmid profile, kb§	DNA–DNA hybridization¶			PCR sequencing#		
							rep		*gyrA*		*parC*
						spvC	FIIA/FIB	Rep_3	Ser(83)	D(87)	Ser(80)
Choleraesuis											
SC-B67	2002	Blood	R/R/R	C-1-a	50, **138**	50	50	138	F	N	I
SC-B104 (1)	2003	Blood	R/R/R	C-1-b	50, **150**	50	50	150	F	N	I
SC-B93 (2)	2003	Blood	R/R/R	C-1-b	50, **115**	50	50	50,115	F	N	I
SC-B98 (3)	2004	Blood	R/R/R	C-1-c	50, **138**	50	50	138	F	N	I
NA (4)	2004	Pus	R/R/R	NA	NA	NA	NA	NA	NA	NA	NA
SC-B131 (5)	2004	Blood	R/R/R	C-1-d	50, **138**	50	50	138	F	N	I
SC-B132 (6)	2004	Blood	R/R/R	C-1-b	50, **150**	50	50	150	F	N	I
NA (7)	2005	Pus	R/S/R	NA	NA	NA	NA	NA	NA	NA	NA
SC-B134 (8)	2007	Blood	R/S/R†	C-1-e	40, 65, **105**	65	65	40	Ser	N	Ser
SC-B136 (9)	2008	Blood	R/R/R	C-2	115, **138**	115	115	115	F	N	I
NA (10)	2009	Pus	R/S/R	NA	NA	NA	NA	NA	NA	NA	NA
Typhimurium var. Copenhagen SB-5	2010	Feces	R/R/R	B-1-a	7, **125**, 180, 260	Neg	Neg	Neg	Ser	D	Ser
Typhimurium											
SB-28	2010	Urine	R/R/S	B-2	**115**, 210	Neg	Neg	Neg	Ser	D	Ser
SB-151	2010	Feces	S/S/S	B-1-b	**85**	Neg	Neg	Neg	Ser	D	Ser
SB-193	2010	Feces	R/R/S	B-2	**105**, 210	Neg	Neg	Neg	Ser	D	Ser
Agona SB-105	2010	Feces	R/S/S	B-3	**95**	Neg	Neg	Neg	Ser	D	Ser
Enteritidis SD-166	2010	Feces	R/S/S	D-1	45, 60, **95**	60	Neg	Neg	Ser	D	Ser

*Puls, pulsotype; R, resistant; F, phenylalanine; N, asparagine; I, isoleucine; NA, not available; S, susceptible; ser, serine; neg, negative reaction; var., variant; D, aspartic acid.
†Antimicrobial drug susceptibility to chloramphenicol, trimethoprim/sulfamethoxazole, and quinolones. Results were the same for the 2 quinolones (nalidixic acid and ciprofloxacin) tested, except that SC-B134 was resistant to nalidixic acid and susceptible to ciprofloxacin. All isolates were resistant to ampicillin and ceftriaxone.
‡Pulsed-field gel electrophoresis; pulsotypes are expressed as serogroup-major type-subtype.
§Plasmids harboring the bla_{CMY-2}-carrying Tn*6092* element are shown in **boldface**. IncI1 plasmids are underlined. Both are evidenced by DNA–DNA hybridization.
¶The size (kb) of the plasmid showing positive results in the respective DNA–DNA hybridization experiments is indicated. Rep_3, replicon of pSC138, the Tn*6092*-containing resistant plasmid of strain SC-B67.
#Amino acid changes compared with the quinolone resistance–determining regions of *gyrA* (codons 83 and 87) and *parC* (codon 80) in *S. enterica* serotype Typhimurium LT2. No mutation was found in *gyrB* and *parE*.

enterica ser. Typhimurium, n = 4, including 1 Copenhagen variant) and serogroup D (*S. enterica* ser. Enteritidis) in 1 patient (Table 1). All isolates were derived from fecal specimens of patients <3 years of age, except *S. enterica* ser. Typhimurium SB-28, which was isolated from the urine of a 77-year-old patient. In contrast to *S. enterica* ser. Choleraesuis, these ceftriaxone-resistant isolates generally remained susceptible to fluoroquinolones (Table 1).

Pulsed-field gel electrophoresis performed as described showed close association among all *S. enterica* ser. Choleraesuis isolates, including SC-B67 (Table 1; Figure 2, panel A) (*7*). Only strain SC-B136, recovered in 2008, demonstrated a relatively different pattern. Two pulsotypes, with minor differences, were found among the 4 *S. enterica* ser. Typhimurium isolates (Table 1).

Ceftriaxone resistance was investigated by using PCR and sequencing as described (*6*). The specific bla_{CMY-2}-carrying Tn*6092* was present in all isolates tested (Table 1). Tn*6092* was located within a *finQ* gene at a position identical to that in SC-B67. The only difference was in SC-B134; a 1,338-bp insertion sequence, IS*10*, was inserted 262 bp upstream of the bla_{CMY-2}. No CTX-M and SHV genes were found in these isolates.

Using an alkaline lysis method (*8*), we found various numbers of plasmids among the isolates studied (Table 1). DNA–DNA hybridization indicated that Tn*6092* was located on large (85-kb to 150-kb) plasmids (Table 1) (*9*). An identical Rep_3 replicon was found in all CIP^r/CRO^r *S. enterica* ser. Choleraesuis isolates studied (*5*). Similar to SC-B67, the Rep_3 replicon was located on the Tn*6092*-harboring resistance plasmid in the 5 resistant isolates recovered before 2004 (Table 1). However, in SC-B134, the Rep_3 replicon was found on the smaller 40-kb plasmid, and in SC-B136, the Rep_3 replicon was on the 115-kb large virulence plasmid that contained the *spvC* gene (Table 1; Figure 2, panels B, C). Replicons FIIA and FIB were simultaneously present in all the *spvC*-containing virulence plasmids among the *S. enterica* ser. Choleraesuis isolates studied (Table 1). Virulence plasmids in the 2 recent isolates, SC-B134 and SC-B136, appeared larger than those in earlier isolates, including SC-B67 (Table 1; Figure 2, panels B, C). The 2 bands

demonstrated by the *spvC* probe in SC-B134 were from the same single virulence plasmid, as proven by hybridization experiments on *Bam*HI-digested plasmid DNA of SC-B134 (data not shown).

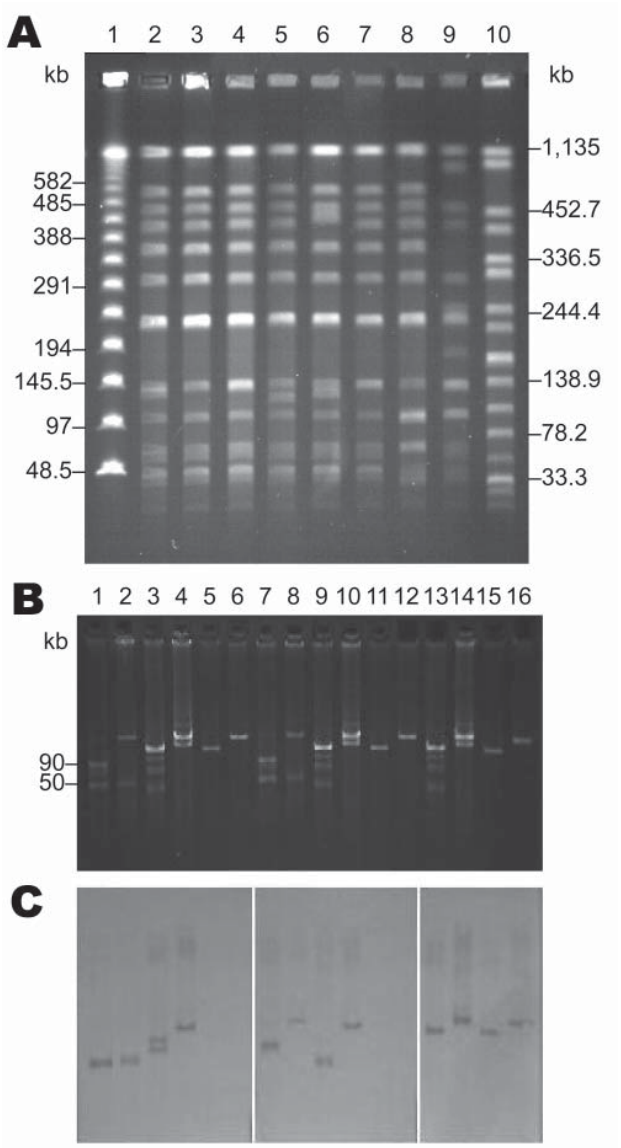

Figure 2. Analyses of *Salmonella enterica* serotype Choleraesuis isolates from Chang Gung Memorial Hospital, 1999–2010. A) Pulsed-field gel electrophoresis patterns. Lanes 1 and 10, DNA size markers demonstrated by a λ DNA concatemer standard and *S. enterica* ser. Braenderup H9812, respectively; lanes 2 to 9, *S. enterica* ser. Choleraesuis SC-B67, SC-B104, SC-B93, SC-B98, SC-B131, SC-B132, SC-B134, and SC-B136. B) Plasmid analysis and C) DNA–DNA hybridization. Probes for DNA–DNA hybridization of lanes 1–6, 7–12, and 13–16 were prepared from amplicons of *spvC*, Rep_3 replicon of pSC138 in SC-B67, and repI1, respectively; lanes 1 and 7, *S. enterica* ser. Choleraesuis OU7529 containing 2 plasmids of known sizes, 50 kb and 90 kb, was used as the size marker; lanes 2 and 8, SC-B67; lanes 3, 9, and 13, SC-B134; lanes 4, 10, and 14, SC-B136; lanes 5, 11, and 15, *Escherichia coli* J53/pSC-B134; lanes 6, 12, and 16, *E. coli* J53/pSC-B136.

Replicon typing through a published multiplex PCR system revealed a replicon I1 from the Tn*6092*-harboring resistance plasmids in SC-B134 and SC-B136 (Table 1; Figure 2, panel C) (*10*). Similarly, Tn*6092*-carrying plasmids among the other ceftriaxone-resistant salmonellae isolates all belonged to the IncI1 group (Table 1). Conjugation experiments using a filter mating method showed that all IncI1 resistant plasmids were self-transferrable (*11*). With azide-resistant *Escherichia coli* J53 and *S. enterica* ser. Typhimurium LBNP4417 as the recipients, the IncI1-resistant plasmids were confirmed to be self-transferrable.

Subtyping of the 8 conjugated IncI1 plasmids was achieved by using a recently described plasmid multilocus sequence typing (pMLST) method specifically set up for IncI1 plasmids (*12*). Six combinations of allele variants were obtained (Table 2). Because these pMLST patterns differed from those reported elsewhere, 6 new sequence types (STs) were designated (Table 2). Two major groups were further derived: ST54 (pSB28, pSB193) and ST52 (pSC-B136) that differed only in *trbA*, and ST56 (pSD166, PSB105) and ST53 (pSB5) that only differed in *pilL* (Table 2). pMLST patterns of representative bla_{CMY-2}-carrying IncI1 plasmids published in recent years (Table 2) were derived from *E. coli* or various *Salmonella* serotypes in Europe or North America (*12–14*). Nine STs and 2 major clonal complexes, CC-2 and CC-12, were observed. pMLST patterns found in the present study differed from these STs by at least 3 alleles (Table 2).

Conclusions

Resistance to ciprofloxacin and ceftriaxone remains high, indicating persistence of antimicrobial drug–resistant traits in *S. enterica* ser. Choleraesuis. The conserved genotypes found in the clinical isolates suggest a mode of clonal dissemination. However, plasmid analysis indicates that the location of the Tn*6092*-containing resistance element had shifted from the nonconjugative Rep_3 plasmids in early isolates to the self-transferable IncI1 plasmids in recent isolates. The emergence of such self-transferable resistance plasmids seems to provide an efficient way for *S. enterica* ser. Choleraesuis to spread its ceftriaxone resistance trait.

Because infections with nontyphoid salmonellae are rampant in Asia, emergence of a conjugative IncI1 resistance plasmid in ceftriaxone-resistant salmonellae from an Asian country is of public health concern. Presence of bla_{CMY-2}-carrying IncI1 plasmids in a variety of *Salmonella* serotypes has been reported, but to our knowledge, not in *S. enterica* serotypes Enteritidis or Choleraesuis (*12–14*). IncI1 plasmids of the same or similar STs have been found in isolates of different bacterial species; with different resistance genes; or from different countries or sources, including human,

Table 2. Characteristics of conjugative *bla*$_{CMY-2}$-harboring IncI1 plasmids derived in this study and comparison of pMLST patterns with similar plasmids published previously*

IncI1 plasmid	*Salmonella enterica* serotype or *Escherichia coli*	Susceptibility profile†	Country	Year of isolation	pMLST‡ repI1	*ardA*	*trbA*	*sogS*	*pilL*	ST§	Clonal complex	Reference
pSC-B134	Choleraesuis	R/S/S	Taiwan	2007	1	1	15	9	3	51	NA	This study
pSC-B136	Choleraesuis	R/R/S	Taiwan	2008	1	4	15	11	2	52	NA	This study
pSB-5	Typhimurium variant Copenhagen	R/R/R	Taiwan	2010	2	1	15	11	2	53	NA	This study
pSB28	Typhimurium	S/S/S	Taiwan	2010	1	4	5	11	2	54	NA	This study
pSB151	Typhimurium	S/S/S	Taiwan	2010	4	5	15	11	3	55	NA	This study
pSB193	Typhimurium	S/S/S	Taiwan	2010	1	4	5	11	2	54	NA	This study
pSB105	Agona	R/S/S	Taiwan	2010	2	1	15	11	3	56	NA	This study
pSD166	Enteritidis	R/S/S	Taiwan	2010	2	1	15	11	3	56	NA	This study
398T	*E. coli*	NA	Italy	2006	1	2	3	2	1	2	CC-2	(*12*)
05–1909	Heidelberg	NA	Canada	2005	1	2	3	2	1	2	CC-2	(*13*)
1358T	Thompson	NA	USA	1996	1	3	3	4	1	4	CC-12	(*12*)
DH-20406	Heidelberg	NA	USA	2004	1	4	3	4	1	12	CC-12	(*14*)
06–3048	4,5,12:i:–	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
06–3539	Agona	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
N07–0084	*E. coli*	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
05–2835	Heidelberg	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
06–3985	Litchfield	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
05–5567	Typhimurium	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
N06–523	*E. coli*	NA	Canada	2006	1	4	3	2	1	18	CC-2/ CC-12	(*13*)
N06–0537	*E. coli*	NA	Canada	2006	1	3	3	4	3	19	CC-12	(*13*)
05–6117	4,5,12:1:–	NA	Canada	2006	1	1	3	9	1	20	NA	(*13*)
N07–0093	*E. coli*	NA	Canada	2005	1	1	3	9	1	20	NA	(*13*)
06–0753	Heidelberg	NA	Canada	2006	1	2	11	3	3	21	CC-5	(*13*)
N07–0079	*E. coli*	NA	Canada	2005	1	6	3	4	1	22	CC-12	(*13*)
N07–0081	*E. coli*	NA	Canada	2005	1	2	3	1	1	23	CC-2	(*13*)

*pMLST, plasmid multilocus sequence typing; ST, sequence type; R, resistant; S, susceptible; NA, not applicable.
†Antimicrobial drug susceptibility to chloramphenicol, trimethoprim/sulfamethoxazole, and quinolones (nalidixic acid and ciprofloxacin). The transconjugants were also resistant to ampicillin and ceftriaxone.
‡pMLST results were compared to those published in the plasmid MLST website (http://pubmlst.org/plasmid).
§STs were designated according to the different combinations of allele variants observed among the IncI1 plasmids.

animals, and the environment (*12–15*). Emergence of the IncI1 plasmid in Taiwan represents a need for continuous efforts to monitor and control its further spread.

This work was supported by the grants CMRPG350023, CMRPG381591, and CMRPG460102 from Chang Gung Memorial Hospital, Taoyuan, Taiwan.

Ms Su is an infection control practitioner and clinical microbiologist at the Chang Gung Memorial Hospital and an associate professor at the Chang Gung University College of Medicine. Her research interests include antimicrobial drug resistance mechanisms, molecular epidemiology of infectious diseases, and microbial pathogenesis.

References

1. Vugia DJ, Samuel M, Farley MM, Marcus R, Shiferaw B, Shallow S, et al. Invasive *Salmonella* infections in the United States, FoodNet, 1996–1999: incidence, serotype distribution, and outcome. Clin Infect Dis. 2004;38(Suppl 3):S149–56. doi:10.1086/381581
2. Su LH, Chiu CH, Chu C, Ou JT. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin Infect Dis. 2004;39:546–51. doi:10.1086/422726
3. Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, et al. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype choleraesuis. N Engl J Med. 2002;346:413–9. doi:10.1056/NEJMoa012261
4. Chiu CH, Su LH, Chu C, Chia JH, Wu TL, Lin TY, et al. Isolation of *Salmonella enterica* serotype choleraesuis resistant to ceftriaxone and ciprofloxacin. Lancet. 2004;363:1285–6. doi:10.1016/S0140-6736(04)16003-0
5. Ye J, Su LH, Chen CL, Hu S, Wang J, Yu J, et al. Complete nucleotide sequence of pSC138, the multidrug resistance plasmid of *Salmonella enterica* serotype Choleraesuis SC-B67. Plasmid. 2011;65:132–40. doi:10.1016/j.plasmid.2010.11.007
6. Su LH, Chen HL, Chia JH, Liu SY, Chu C, Wu TL, et al. Distribution of a transposon-like element carrying *bla*$_{CMY-2}$ among *Salmonella* and other Enterobacteriaceae. J Antimicrob Chemother. 2006;57:424–9. doi:10.1093/jac/dki478
7. Su LH, Leu HS, Chiu YP, Chia JH, Kuo AJ, Sun CF, et al. 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. 2000;46:110–7. doi:10.1053/jhin.2000.0815
8. Kado CI, Liu ST. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol. 1981;145:1365–73.
9. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 1975;98:503–17. doi:10.1016/S0022-2836(75)80083-0

10. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63:219–28. doi:10.1016/j.mimet.2005.03.018
11. Jacoby GA, Han P. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli.* J Clin Microbiol. 1996;34:908–11.
12. García-Fernández A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, et al. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J Antimicrob Chemother. 2008;61:1229–33. doi:10.1093/jac/dkn131
13. Mataseje LF, Baudry PJ, Zhanel GG, Morck DW, Read RR, Louie M, et al. Comparison of CMY-2 plasmids isolated from human, animal, and environmental *Escherichia coli* and *Salmonella* spp. from Canada. Diagn Microbiol Infect Dis. 2010;67:387–91. doi:10.1016/j.diagmicrobio.2010.02.027
14. Folster JP, Pecic G, Bolcen S, Theobald L, Hise K, Carattoli A, et al. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from humans in the United States. Foodborne Pathog Dis. 2010;7:181–7. doi:10.1089/fpd.2009.0376
15. Cloeckaert A, Praud K, Lefevre M, Doublet B, Pardos M, Granier SA, et al. IncI1 plasmid carrying extended-spectrum-β-lactamase gene $bla_{CTX-M-1}$ in *Salmonella enterica* isolates from poultry and humans in France, 2003 to 2008. Antimicrob Agents Chemother. 2010;54:4484–6. doi:10.1128/AAC.00460-10

Address for correspondence: Cheng-Hsun Chiu, Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, 5 Fu-Hsin St, Kweishan, Taoyuan 333, Taiwan; email: chchiu@adm.cgmh.org.tw