Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in *Escherichia coli*: 20 years of surveillance in resource-limited settings from Latin America

A. Bartoloni[1,2], L. Pallecchi[3], E. Riccobono[3], A. Mantella[1], D. Magnelli[1], T. Di Maggio[3], A. L. Villagran[4], Y. Lara[5], C. Saavedra[6], M. Strohmeyer[1], F. Bartalesi[2], C. Trigoso[7] and G. M. Rossolini[3,8]

1) *Dipartimento Area Critica Medico Chirurgica, Clinica Malattie Infettive, Università Di Firenze, Florence, Italy,* 2) *Malattie Infettive e Tropicali, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy,* 3) *Dipartimento di Biotecnologie, Sezione di Microbiologia, Università di Siena, Siena, Italy,* 4) *Hospital Basico Villa Montes, Villa Montes, Bolivia,* 5) *Hospital San Antonio de los Sauces, Monteagudo, Bolivia,* 6) *Hospital Dermatologico, Monteagudo, Bolivia,* 7) *Facultad de Medicina, Enfermería, Nutrición y Tecnología Médica, Universidad Mayor de San Andrés, La Paz, Bolivia and* 8) *Dipartimento di Emergenza, Urgenza e dei Servizi Diagnostici, UO Microbiologia e Virologia, Azienda Ospedaliera-Universitaria Senese, Siena, Italy*

Abstract

Previous studies on commensal *Escherichia coli* from healthy children in the Bolivian Chaco have shown remarkable resistance rates to the old antibiotics since the early 1990s, and the emergence of resistance to newer drugs (fluoroquinolones and expanded-spectrum cephalosporins) in the 2000s. Here we report the results of a new survey conducted in 2011 in the same setting. Rectal swabs were obtained from 482 healthy children (aged 6–72 months) from three urban areas of the Bolivian Chaco. Screening for antibiotic-resistant *E. coli* was performed by a direct plating method, as in the previous studies. The $bla_{CTX\text{-}M}$ genes were investigated by PCR/sequencing, and CTX-M-producing isolates were subjected to genotyping and detection of several plasmid-mediated quinolone resistance mechanisms. Results showed high rates of resistance to nalidixic acid (76%), ciprofloxacin (44%) and expanded-spectrum cephalosporins (12.4%), demonstrating a relentless increase of resistance to those drugs over the past two decades. CTX-M-type extended-spectrum beta-lactamases were found to be widespread (12%, 97% of extended-spectrum beta-lactamase producers). Compared with the previous studies, CTX-M-producing *E. coli* underwent a dramatic dissemination (120-fold increase since early 2000s) and a radical change of dominant CTX-M groups (CTX-M-1 and CTX-M-9 groups versus CTX-M-2 group). Most CTX-M producers were not susceptible to quinolones (91%), and 55% carried plasmid-mediated quinolone resistance genes (different combinations of *aac(6′)-Ib-cr*, *qnrB* and *qepA*). This study shows the rapid and remarkable increasing trend for resistance to fluoroquinolones and expanded-spectrum cephalosporins in one of the poorest regions of Latin America, and underscores the need for urgent control strategies aimed at preserving the efficacy of those drugs in similar settings.

Keywords: Bolivia, commensals, CTX-M, enterobacteria, healthy children
Original Submission: 7 November 2011; **Revised Submission:** 22 January 2012; **Accepted:** 5 February 2012
Editor: R. Cantón
Article published online: 9 February 2012
Clin Microbiol Infect 2013; **19:** 356–361
10.1111/j.1469-0691.2012.03807.x

Corresponding author: L. Pallecchi, Dipartimento di Biotecnologie, Sezione di Microbiologia, Università di Siena, Policlinico Santa Maria alle Scotte, 53100 Siena, Italy
E-mail: lucia.pallecchi@unisi.it

Introduction

Increasing antibiotic resistance is a global public health concern, with particularly serious consequences in countries of limited resources, where resistance has dramatic effects on morbidity and mortality rates and threatens the viability of local healthcare systems [1,2].

Surveillance of antibiotic resistance is a crucial element for the implementation of intervention strategies aimed at preserving the efficacy of antibiotics [1]. Beside clinical isolates, there is increasing agreement about the importance of monitoring commensal bacteria, which constitute a reservoir not only of resistant strains that can cause infections but also of resistance genes that are potentially transferable to pathogens [1,3].

From this perspective, several studies have monitored resistance in commensal *Escherichia coli*, which is the predominant aerobic species of the gut, in addition to being one of the most common pathogens both in hospital and in community settings [3]. Reliable, simple and low-cost methods for investigating resistance in commensal *E. coli* have been implemented and were shown to represent a valid tool for performing large-scale surveillance studies in resource-limited settings (where microbiological diagnosis is usually not performed, and data on antibiotic resistance are scarce or totally absent) [4–6].

In previous studies aimed at monitoring antibiotic resistance in commensal *E. coli* from healthy children in the Bolivian Chaco, we observed very high rates of resistance to the old antibiotics (i.e. ampicillin, tetracycline, trimethoprim-sulphamethoxazole, chloramphenicol) since the early 1990s, and the emergence of resistance to newer drugs (namely fluoroquinolones and expanded-spectrum cephalosporins) in the 2000s [5–7]. Further studies demonstrated that resistance to expanded-spectrum cephalosporins in commensal *E. coli* from that setting was mainly related to the emergence of CTX-M-type extended-spectrum beta-lactamase (ESBL) determinants [8,9].

Here we report on the dramatic increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins shown by a new survey conducted in 2011 in the same area, and describe the molecular epidemiology of the widespread dissemination of CTX-M-type ESBLs that occurred in that setting.

Materials and Methods

Study design and population

The study was conducted in March 2011 in three urban areas of the Bolivian Chaco: Camiri (Santa Cruz Department, *c.* 34 000 inhabitants), Villa Montes (Tarija Department, *c.* 25 000 inhabitants) and Monteagudo (Chuquisaca Department, *c.* 11 500 inhabitants). Although located in different Departments, the three urban areas are connected by roads (distances of about 100 km from Camiri to Monteagudo, and about 160 km from Camiri to Villa Montes) which favour exchanges of goods and people. As in previous surveys [5–7], the study population was represented by children (160, 156 and 166 from Camiri, Villa Montes and Monteagudo, respectively), aged 6–72 months, who have not had diarrhoea in the previous 24 h. Only one child in the target age cohort per household was included. The studied households were selected by a modified cluster sampling, as described previously [5]. A rectal swab was obtained from each enrolled child, after informed consent was obtained from parents or legal guardians, who were also interviewed about antibiotics possibly administered to the child during the previous 2 weeks. In case of antibiotics consumption, parents/legal guardians were also interviewed about antibiotic prescription. Full ethical clearance was obtained from the qualified local authorities (Convenio de Salud, Ministerio de Salud—Vicariato de Camiri) who revised and approved the study design.

Screening for faecal carriage of antibiotic-resistant *E. coli*

Screening for the presence of antibiotic-resistant *E. coli* in the faecal samples was performed by a direct plating method (essentially based on direct application of antibiotic disks onto a MacConkey No. 3 agar plate inoculated with the faecal swab), as described previously [4,5]. Antibiotics tested are listed in Table 1.

Characterization of ESBL-producing *E. coli*

All samples showing the presence of isolates with reduced susceptibility to expanded-spectrum cephalosporins (n = 60) were further plated onto MacConkey no. 3 agar plates containing ceftriaxone 2 μg/mL, and one isolated coliform colony per sample was collected. Identification was confirmed by

TABLE 1. Antibiotic resistance rates in commensal *Escherichia coli* from healthy children in the Bolivian Chaco over the past two decades

Year	No. of studied children	Urban areas (no. of children)[a]	Antibiotic resistance rates (%)[b]							Reference
			AMP	TET	SXT	CHL	NAL	CIP	ESC	
1992	296	C (296)	97	92	94	69	4	0	0	[7]
2002	1594	C (794), VM (790)	97	94	96	70	36	16	0.1	[5]
2005	1600	C (800), VM (800)	97	92	94	67	51	26	1.9	[6]
2011	482	C (160), VM (156), M (166)	98	95	94	78	76	44[c]	12[d]	This study

[a]C, Camiri; VM, Villa Montes; M, Monteagudo.
[b]AMP, ampicillin; TET, tetracycline; SXT, trimethoprim-sulphamethoxazole; CHL, chloramphenicol; NAL, nalidixic acid; CIP, ciprofloxacin; ESC, expanded-spectrum cephalosporins (ceftriaxone and/or ceftazidime).
[c]Camiri 39%, Villa Montes 40%, Monteagudo 51%.
[d]Camiri 9%, Villa Montes 17%, Monteagudo 11%.

the API20E identification system (bioMérieux, Marcy l'Étoile, France). ESBL confirmatory tests were performed according to CLSI [10]. Identification of CTX-M-type determinants and characterization of CTX-M groups was carried out by PCR [9,11], followed by complete sequencing of $bla_{CTX\text{-}M}$ genes. Primers designed in this study were used for amplification and sequencing of variants belonging to the CTX-M-9 group (5′-GATGTAACACGGATTGACC and 5′- GAACTTTTG CTGAGTTGAAGG) and CTX-M-8 group (5′-CACGG ATTCAATTTTCAGGAG and 5′-GAGCGCTCCACATT TTTTAG), whereas other groups were sequenced as described previously [9]. Genotyping of CTX-M producers was performed by determination of the main *E. coli* phylogenetic groups (A, B1, B2, D) according to the Clermont method [9], random amplification of polymorphic DNA (RAPD) with the 1290 and 1254 decamers [9], and multilocus sequence typing using protocols and conditions described on the *E. coli* multilocus sequence typing website [http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primersColi_html].

All CTX-M producers were also investigated for quinolone susceptibility by the disk diffusion method [10,12], and for the presence of plasmid-mediated quinolone resistance genes by PCR, as described previously (*qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, *qepA* [13]; *qnrC* [14]; *qnrD* [15]).

Statistical analysis

Data entry and analysis were performed with the Epi Info software package version 2008 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Statistical differences were determined by the chi-squared test.

Results and Discussion

Evolution of antibiotic resistance rates in commensal *E. coli* from healthy children in the Bolivian Chaco over the past two decades

The 2011 survey confirmed the very high resistance rates to the old antibiotics (i.e. ampicillin, tetracycline, trimethoprim-sulphamethoxazole, chloramphenicol) recorded in the previous studies [5–7], and showed an alarmingly relentless increase of resistance to quinolones (including fluoroquinolones) and expanded-spectrum cephalosporins (Table 1). In particular, *E. coli* isolates with acquired resistance to nalidixic acid, ciprofloxacin and expanded-spectrum cephalosporins were found in 76%, 44% and 12.4% of enrolled children, respectively ($p < 0.0001$ compared with the 2005 survey). Resistance rates were overall similar in the three urban areas studied, with the exception of higher resistance rates observed for ciprofloxacin in Monteagudo (statistically significant, $p < 0.05$) and for expanded-spectrum cephalosporins in Villa Montes (statistically significant compared with Camiri, p 0.03) (Table 1).

Data from interviews showed that 33% of children had been administered antibiotics during the 2 weeks preceding the study: 17% had received beta-lactams (14% ampicillin, 3% penicillin, 1% expanded-spectrum cephalosporins), 11% trimethoprim-sulphamethoxazole, 2% erythromycin, 2% tetracyclines and 2% other antibiotics (including nalidixic acid, chloramphenicol, metronidazole and aminoglycosides). Data on antibiotic prescription were available for 89% of children who received antibiotics, and showed that they were mostly prescribed by doctors (83%), although in some cases they were recommended by other healthcare providers (nurse 3%, 'promotor de salud' 5%), pharmacy staff (2%), or were taken without prescription (7%).

Household antibiotic use in the three urban areas was overall comparable, except for a significantly higher use of ampicillin in Villa Montes (p 0.005 and p 0.002 compared with Camiri and Monteagudo, respectively), which could possibly have contributed to the higher level of resistance to expanded-spectrum cephalosporins observed in this urban area. However, no significant difference in carriage of isolates resistant to expanded-spectrum cephalosporins was observed between children who had been administered ampicillin in the 2 weeks preceding the survey and those who had not (p 0.21), excluding a direct role of ampicillin in promoting colonization by CTX-M producers. The reasons for the higher resistance rates to fluoroquinolones observed in Monteagudo remain unclear and will be the subject of further studies.

Increasing resistance to fluoroquinolones and expanded-spectrum cephalosporins in clinical isolates of *Enterobacteriaceae* is an emerging worldwide phenomenon affecting the management of both hospital-acquired and community-acquired infections, and intestinal colonization has been found to represent a risk factor for subsequent infections caused by those resistant microorganisms [3]. The results from this study underscore the magnitude of such a relevant phenomenon in one of the poorest regions of Latin America, calling for urgent control strategies aimed at preserving the efficacy of fluoroquinolones and expanded-spectrum cephalosporins in similar settings, where alternative therapeutic options are often unavailable or too expensive.

Dramatic increase and changed epidemiology of CTX-M-type ESBLs

Of the 60 children carrying isolates with reduced susceptibility to expanded-spectrum cephalosporins, 58 (97%) were found to be colonized by CTX-M-producing *E. coli*.

The remaining two children carried *E. coli* isolates producing other types of ESBLs, which were not further investigated.

Characterization of $bla_{CTX\text{-}M}$ genes identified variants belonging to CTX-M-1 (*n* = 25, 43%), CTX-M-9 (*n* = 25, 43%), CTX-M-8 (*n* = 7, 12%) and CTX-M-2 (*n* = 2, 3%) groups, with one isolate carrying $bla_{CTX\text{-}M}$ genes of different groups (CTX-M-1 and CTX-M-9 groups) (Tables 2 and 3). No significant difference in the distribution of CTX-M groups among the three study areas was observed, although CTX-M-2 and CTX-M-8 groups were not detected in Monteagudo and Camiri, respectively.

TABLE 2. Dissemination and changed epidemiology of CTX-M-type extended spectrum beta-lactamases in commensal *Escherichia coli* from healthy children in the Bolivian Chaco

Year	No. of studied children	% of carriers of ESC-resistant *E. coli* (*n*)[a]	% of carriers of CTX-M-producing *E. coli* (*n*)	Distribution of different CTX-M-groups (%)[b]				Reference
				CTX-M-1	CTX-M-2	CTX-M-8	CTX-M-9	
1992	296	0	0	0	0	0	0	[7]
2002	1594	0.1 (2)	0.1 (2)	0	100	0	0	[8]
2005	1600	1.9 (30)	1.6 (26)	38	62	0	0	[9]
2011	482	12.4 (60)	12.0 (58)	43	3	12	43	This study

[a]ESC, expanded-spectrum cephalosporins (ceftriaxone and/or ceftazidime).
[b]Prevalence of each CTX-M group over the total number of children carrying CTX-M-producing *E. coli*. In the 2011 study, one *E. coli* isolate harboured $bla_{CTX\text{-}M}$ genes of different groups (CTX-M-1 and CTX-M-9 groups).

TABLE 3. Features of CTX-M-producing *Escherichia coli* from the 2011 survey

Group	CTX-M type	RAPD type (no. of isolates)	Phylogenetic group	Origin (no. of isolates)[a]	Resistance to quinolones[b]	PMQR genes[c]
CTX-M-1	CTX-M-15	1 (6)	A	C (3), VM (1), M (2)	NAL/CIP	*aac(6′)-Ib-cr*
		2 (2)	A	VM (2)	NAL/CIP	*aac(6′)-Ib-cr*
		3 (1)	A	VM (1)	NAL/CIP	*aac(6′)-Ib-cr*
		4 (2)	A	VM (1), M (1)	NAL/CIP	*aac(6′)-Ib-cr*
		5 (3)	A	VM (1), M (2)	NAL/CIP	*aac(6′)-Ib-cr*
		6 (1)	A	C (1)	NAL/CIP	*aac(6′)-Ib-cr*
		7 (1)	A	VM (1)	NAL/CIP	*aac(6′)-Ib-cr*
		8 (1)	A	VM (1)	NAL/CIP	*aac(6′)-Ib-cr*
		9 (1)	A	M (1)	NAL/CIP	*qnrB*, *aac(6′)-Ib-cr*
		10 (2)	A	M (2)	NAL/CIP	*qepA*
		11 (1)	A	C (1)	NAL/CIP	*aac(6′)-Ib-cr*
		12 (1)	B1	M (1)	nal	*qnrB*, *aac(6′)-Ib-cr*
		13 (1)	D	VM (1)	NAL/CIP	*qepA*
	CTX-M-3	14 (1)	A	M (1)	–	–
CTX-M-9	CTX-M-65	15 (1)	A	C (1)	NAL/CIP	*qnrB*
		16 (1)	A	C (1)	nal	*qnrB*
		17 (1)	A	VM (1)	–	–
		18 (1)	A	VM (1)	NAL/CIP	–
		19 (1)	A	VM (1)	NAL/cip	*qnrB*
		20 (1)	A	M (1)	NAL/CIP	–
		21 (1)	A	M (1)	-	–
		22 (4)	B1	VM (3), M (1)	NAL/CIP	–
		23 (2)	B1	C (1), M (1)	NAL	–
		24 (2)	B1	C (2)	NAL/CIP	–
		25 (1)	B1	VM (1)	NAL/CIP	–
		26 (1)	B1	M (1)	NAL/CIP	–
		27 (2)	D	C (2)	NAL/CIP	–
		28 (1)	D	C (1)	NAL/CIP	–
	CTX-M-14	29 (1)	A	VM (1)	NAL/CIP	–
		30 (1)	B1	VM (1)	NAL/CIP	–
		31 (1)	B1	VM (1)	NAL	–
		32 (1)	D	M (1)	NAL/CIP	*qepA*
CTX-M-8	CTX-M-8	33 (1)	A	VM (1)	NAL/CIP	*qnrB*
		34 (1)	A	VM (1)	NAL/cip	*qnrB*
		35 (1)	A	VM (1)	NAL/CIP	*qnrB*
		36 (1)	A	M (1)	–	–
		37 (1)	B1	VM (1)	NAL/CIP	–
		38 (1)	D	VM (1)	NAL/CIP	–
		39 (1)	D	M (1)	–	–
CTX-M-2	CTX-M-2	40 (1)	D	C (1)	nal/cip	*qnrB*
		41 (1)	D	VM (1)	NAL/CIP	-
CTX-M-1+CTX-M-9	CTX-M-15+CTX-M-14	42 (1)	D	VM (1)	NAL/CIP	*qepA*, *aac(6′)-Ib-cr*

[a]C, Camiri; VM, Villa Montes; M, Monteagudo.
[b]NAL, nalidixic acid; CIP, ciprofloxacin. Resistant and intermediate phenotypes are indicated in upper-case and lower-case letters, respectively.
[c]PMQR, plasmid-mediated quinolone resistance.

Sequence analysis of $bla_{CTX\text{-}M}$ genes identified $bla_{CTX\text{-}M\text{-}15}$ (n = 24), $bla_{CTX\text{-}M\text{-}65}$ (n = 20), $bla_{CTX\text{-}M\text{-}8}$ (n = 7), $bla_{CTX\text{-}M\text{-}14}$ (n = 5), $bla_{CTX\text{-}M\text{-}2}$ (n = 2) and $bla_{CTX\text{-}M\text{-}3}$ (n = 1) (Table 3). Phylogenetic grouping and RAPD typing revealed an overall genetic heterogeneity among CTX-M producers, although some events of clonal expansion were observed (mainly among CTX-M-15 producers) (Table 3). Multilocus sequence typing analysis of two CTX-M-15-producing isolates of phylogenetic group A (the most prevalent phylogenetic group among CTX-M-15 producers) identified ST10 (RAPD type 1) and ST617 (RAPD type 6). ST617 was also assigned to a CTX-M-15-producing isolate of phylogenetic group A from the 2005 survey (representative of an RAPD type circulating both in Camiri and Villa Montes [9]), although the RAPD pattern apparently differed from that of the ST617 isolate collected in 2011 (data not shown). ST10 and ST617 belong to the ST10 complex, which has recently been found to represent the predominant sequence type among *E. coli* isolates assigned to phylogenetic group A [16]. Two CTX-M-65-producing isolates of phylogenetic group B1 (the most prevalent phylogenetic group among CTX-M-65 producers) were assigned to ST602 (RAPD type 22) and ST58 (RAPD type 23).

Most CTX-M producers were non-susceptible to quinolones (91%), and more than half (55%) were shown to carry one of the plasmid-mediated quinolone resistance genes investigated: *aac(6′)-Ib-cr* (n = 21), *qnrB* (n = 9) and *qepA* (n = 5) (Table 3). In particular, *aac(6′)-Ib-cr* was identified in 21 of the 24 CTX-M-15-producing isolates (suggesting a possible genetic linkage between the two resistance determinants), whereas *qnrB* and *qepA* were found in isolates producing different CTX-M variants.

Results from this study demonstrate that, after their first appearance in 2002 [8], CTX-M-type ESBLs underwent a dramatic dissemination in the Bolivian Chaco during the last decade (from 0.1 to 12%, 120-fold, $p < 0.0001$). Furthermore, the present findings underscore the complex dynamics of CTX-M-type ESBL dissemination in the study setting, with the remarkably increased prevalence being associated with a radical change of circulating CTX-M groups. CTX-M-1 and CTX-M-9 groups are emerging worldwide as the dominant CTX-M variants [17]. Here, we demonstrated that those CTX-M groups have successfully spread also in Bolivia, where they have almost completely replaced the CTX-M-2 group, which has been endemic and historically dominant in clinical isolates of *Enterobacteriaceae* in Latin America since the first identification of the CTX-M-2 variant in Argentina in the late 1980s [18]. The reasons accounting for the remarkable dissemination of CTX-M-type ESBLs in the study area are not easy to investigate because of the difficulties in collecting reliable data on drug consumption. Further studies on the genetic supports of $bla_{CTX\text{-}M}$ genes will be performed to investigate the possible causes for the changed molecular epidemiology of CTX-M enzymes in that setting. To the best of our knowledge, this is also the first report of $bla_{CTX\text{-}M\text{-}65}$ and *qepA* genes in South America.

Acknowledgements

We wish to thank Patricia Rops, Claudia Quispe, Claudia Padilla and Mariela Antezana for their valuable support in the laboratory activities, and the students of the Escuela de Salud del Chaco Tekove Katu for their professionalism and enthusiasm in performing the field work.

These results were presented in part at the 51th ICAAC, 17–20 September 2011, Chicago, IL, USA.

Transparency Declaration

All authors declare that there are no conflicts of interest.

The study was partially supported by grants from the Italian Ministry for Foreign Affairs ('Fortalecimiento de la red de salud del Chaco Boliviano: una perspectiva comunitaria'), the Ente Cassa di Risparmio di Firenze (Florence, Italy), the Regione Toscana (Italy) ('Toscana e Chaco, 25 anni di cooperazione sanitaria: un passo decisivo verso il contenimento della diffusione delle resistenze batteriche agli antibiotici'), and the European Community's Seventh Framework Programme (EvoTAR, HEALTH-F3-2011-282004).

References

1. World Health Organization. *Global strategy for containment of antimicrobial resistance*. Geneva: WHO, 2001. Available from http://www.who.int/drugresistance/en. (last accessed 22 August 2011).
2. DiazGranados CA, Cardo DM, McGowan JE Jr. Antimicrobial resistance: international control strategies, with a focus on limited-resource settings. *Int J Antimicrob Agents* 2008; 32: 1–9.
3. Alekshun MN, Levy SB. Commensals upon us. *Biochem Pharmacol* 2006; 71: 893–900.
4. Bartoloni A, Benedetti M, Pallecchi L *et al.* Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut. *Trans R Soc Trop Med Hyg* 2006; 100: 119–125.
5. Bartoloni A, Pallecchi L, Benedetti M *et al.* Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerg Infect Dis* 2006; 12: 907–913.
6. Bartoloni A, Pallecchi L, Fiorelli C *et al.* Increasing resistance in commensal *Escherichia coli*, Bolivia and Peru. *Emerg Infect Dis* 2008; 14: 338–340.

7. Bartoloni A, Cutts F, Leoni S *et al.* Patterns of antimicrobial use and antimicrobial resistance among healthy children in Bolivia. *Trop Med Int Health* 1998; 3: 116–123.
8. Pallecchi L, Malossi M, Mantella A *et al.* Detection of CTX-M-type beta-lactamase genes in fecal *Escherichia coli* isolates from healthy children in Bolivia and Peru. *Antimicrob Agents Chemother* 2004; 48: 4556–4561.
9. Pallecchi L, Bartoloni A, Fiorelli C *et al.* Rapid dissemination and diversity of CTX-M extended-spectrum beta-lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* 2007; 51: 2720–2725.
10. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Supplement M100-S21. 2011: Wayne, PA, USA: CLSI.
11. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum beta-lactamases. *J Antimicrob Chemother* 2006; 57: 154–155.
12. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial disk susceptibility tests*. Supplement M02-A10. 2009: Wayne, PA, USA: CLSI.
13. Riccobono E, Pallecchi L, Mantella A *et al.* Carriage of antibiotic-resistant *Escherichia coli* among healthy children and home-raised chickens: a household study in a resource-limited setting. *Microb Drug Resist* 2011; 18: 83–87.
14. Kim HB, Park CH, Kim CJ *et al.* Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother* 2009; 53: 639–645.
15. Cavaco LM, Hasman H, Xia S *et al. qnrD* a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and *Bovis morbificans* strains of human origin. *Antimicrob Agents Chemother* 2009; 53: 603–608.
16. Gordon DM, Clermont O, Tolley H *et al.* Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. *Environ Microbiol* 2008; 10: 2484–2496.
17. Oteo J, Pérez-Vázquez M, Campos J. Extended-spectrum beta-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. *Curr Opin Infect Dis* 2010; 23: 320–326.
18. Radice M, Power P, Di Conza J *et al.* Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother* 2002; 46: 602–604.