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Extended-spectrum and CMY-type β-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain

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Abstract

Infections due to *Escherichia coli* producing extended-spectrum β-lactamase (ESBL) or CMY-type β-lactamase (CMY) are increasingly observed in non-hospitalized patients. The origin of these organisms is uncertain, but retail meat contaminated with *E. coli* may be a source. In the present study, clinical information and strains collected from patients infected or colonized with ESBL-producing and CMY-producing *E. coli* at hospitals in Pittsburgh, USA and Seville, Spain were investigated. Retail meat purchased in these cities was also studied for the presence of these organisms. Twenty-five and 79 clinical cases with ESBL-producing *E. coli* and 22 cases and one case with CMY-producing *E. coli* were identified in Pittsburgh and Seville, respectively. Among them all, community-acquired and healthcare-associated cases together constituted 60% of the cases in Pittsburgh and 73% in Seville. Community-acquired cases were more common in Seville than in Pittsburgh (49% vs. 13%; p <0.001). ESBL-producing and CMY-producing *E. coli* isolates were commonly recovered from the local retail meat. In particular, 67% (8/12) of retail chickens in Seville and 85% (17/20) of those in Pittsburgh contained ESBL-producing and CMY-producing *E. coli* isolates, respectively. Among the ESBL-producing isolates, CTX-M and SHV were the most common ESBL types in both clinical and meat isolates. Approximately half of the ESBL-producing and CMY-producing *E. coli* isolates from meat belonged to phylogenetic groups associated with virulent extra-intestinal infections in humans. Community and healthcare environments are now significant reservoirs of ESBL-producing and CMY-producing *E. coli*. Retail meat is a potential source of these organisms.

Keywords: Antimicrobial resistance, clinical epidemiology, CMY-type β-lactamases, *Escherichia coli*, extended-spectrum β-lactamases, food, molecular epidemiology, β-lactamases
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Introduction

Escherichia coli is a leading cause of community-acquired infections, especially urinary tract infections. In *E. coli*, resistance to oxyimino-cephalosporins, such as ceftriaxone and ceftazidime, occurs by production of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC-type β-lactamases [1]. ESBLs and plasmid-mediated AmpC-type β-lactamases in *E. coli* were first noted in the 1980s [2,3], and have been associated with hospital-acquired infections. In *E. coli*, CMY-type β-lactamase (CMY) is the most commonly encountered plasmid-mediated AmpC-type β-lactamase worldwide [4].

Although it is conventionally perceived as a nosocomial pathogen, ESBL-producing *E. coli* has emerged as a community pathogen in many parts of the world [5]. Community-acquired cases are largely caused by *E. coli* that produces CTX-M-type ESBLs [5]. Recent reports suggest that CMY-producing *E. coli* may cause community-acquired infections as well [6–8]. The spread of ESBL-producing and CMY-producing *E. coli* outside of the hospital environment may compromise the usefulness of penicillins and cephalosporins for infections such as complicated urinary tract infections and bacteraemia [7–12]. In addition, these *E. coli* isolates often have co-resistance to other classes of antimicrobials, medi-

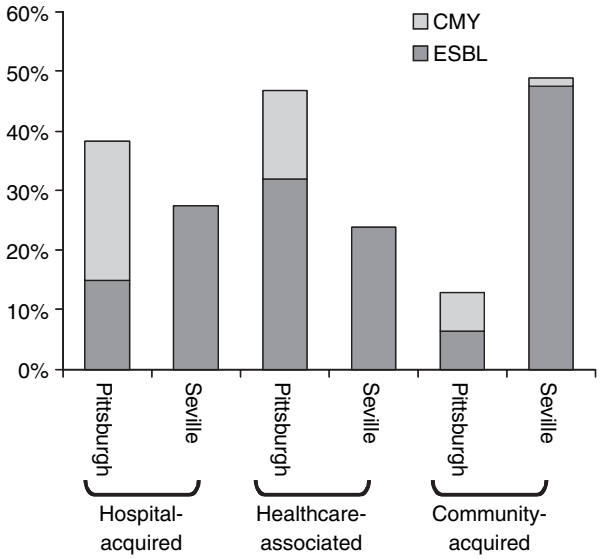

FIG. 1. Distribution of extended-spectrum β-lactamase (ESBL)-producing or CMY-type β-lactamase (CMY)-producing *Escherichia coli* cases according to the place of acquisition at both study sites.

ated by other resistance mechanisms (e.g. aminoglycosides, trimethoprim–sulphamethoxazole and fluoroquinolones) [1].

One potential source of resistant *E. coli* isolates causing infection in the community is food that we eat. Some food animals, including chickens, turkeys, pigs and cattle, may be colonized by ESBL-producing and CMY-producing *E. coli* and have been considered as potential sources of these organisms that cause community-acquired diseases [13].

In the present study, we sought to systematically evaluate clinical infection with these organisms, using rigorous definitions of the place of acquisition, and to identify these organisms from retail meat purchased locally during the same time period.

Patients and Methods

Laboratory surveillance of ESBL-producing and CMY-producing *E. coli*

Prospective laboratory-based surveillance was conducted from September 2006 to March 2007 to identify all cases of ESBL-producing and CMY-producing *E. coli* infection or colonization at the University of Pittsburgh Medical Center (Presbyterian-Shadyside Campus), Pittsburgh, USA and Hospital Universitario Virgen Macarena, Seville, Spain. The University of Pittsburgh Medical Center (Presbyterian-Shadyside Campus) is a 1300-bed tertiary teaching hospital with affiliated outpatient clinics. Hospital Universitario Virgen Macarena is a 950-bed tertiary teaching hospital with affiliated outpatient clinics and primary-care centres. ESBL production was screened for and confirmed by the disk diffusion method defined by the CLSI [14]. For isolates that were non-susceptible (intermediate or resistant) to ceftriaxone and had negative confirmatory test findings for ESBL production, PCR analysis was conducted to identify plasmid-mediated AmpC-type β-lactamases, including CMY [15].

Study sample and data collection

The study was approved by the Institutional Review Boards of the University of Pittsburgh and Hospital Universitario Virgen Macarena, which waived the need to obtain written informed consent, owing to the observational nature of the study. Computerized medical and laboratory records were reviewed to document the place of acquisition of infection [16]. The same patient could be re-enrolled only when a positive culture was identified ≥30 days from the initial enrolment. A hospital-acquired case was defined as a positive culture obtained from a patient who had been hospitalized for ≥48 h. A healthcare-associated case was defined as a positive culture obtained from a patient at the time of hospital admission or <48 h after admission if the patient fulfilled any of the following criteria: (i) received intravenous therapy at home, received wound care or specialized nursing care through a healthcare agency, family, or friends, or had self-administered intravenous medical therapy ≤30 days before enrolment; (ii) attended a hospital or haemodialysis clinic or received intravenous chemotherapy ≤30 days before enrolment; (iii) was hospitalized in an acute-care hospital for ≥2 days in the 90 days before enrolment; or (iv) resided in a nursing home or long-term-care facility [17]. A community-acquired case was defined as one in which a positive culture was obtained from a patient at the time of hospital admission or <48 h after admission and that did not meet the criteria for a healthcare-associated case. Complementary clinical data for patients from Pittsburgh have been published elsewhere [7].

Statistical analysis

Categorical variables were compared using the chi-squared test. SPSS software (version 13.0; SPSS, Chicago, IL, USA) was used for the analyses.

Culturing of retail meat

Chicken, turkey, pork and ground beef were purchased from local supermarkets at least every 2 weeks in metropolitan Pittsburgh and Seville during the study period. After immediate transport of the samples to the research laboratory on ice, approximately 25 g of each sample was suspended in buffered peptone broth with the use of a sterile technique, and homogenized manually or with the use of a stomacher. After overnight incubation at 37°C, an aliquot of the broth was plated on MacConkey agar plates containing 1 mg/L cefotaxime

or ceftazidime and further incubated overnight. Lactose-fermenting colonies were identified as *E. coli* using standard biochemical tests. Production of ESBL was confirmed by observing ≥5 mm of growth inhibition around a ceftazidime-containing or cefotaxime-containing disk in the presence of clavulanic acid on a Mueller–Hinton agar plate [14]. The colonies with negative confirmatory test findings for ESBL production were tested for the presence of plasmid-mediated AmpC-type β-lactamase genes as described above.

Molecular typing and genetic analyses

For all ESBL-producing and CMY-producing isolates of both clinical and food origin, PCR analyses to determine the type of ESBL gene carried by each isolate were performed. Primer sets to detect TEM-type, SHV-type and CTX-M-type ESBL genes as well as CMY genes were used [15,18]. Positive results were confirmed by sequencing of the products. In addition, we determined the phylogenetic groups of the *E. coli* clinical isolates and those of food origin by multiplex PCR analysis [19]. To determine the genetic relatedness of the study isolates, pulsed-field gel electrophoresis analysis was performed, using *Xba*I as a restriction endonuclease, and electrophoresing the genome on a CHEF III DR system (Bio-Rad, Hercules, CA, USA), on all isolates, as described previously [7]. Cluster analysis was performed by using the unweighted pair group method based on Dice coefficients. The results were interpreted according to the criteria proposed by Tenover *et al.* [20].

Results

In Pittsburgh, 2583 *E. coli* isolates were identified during the study period. Of these, 1183 (46%) were obtained from outpatient locations. In Seville, 2606 *E. coli* isolates were identified. Of these, 2062 (79%) were obtained from outpatient locations.

In Pittsburgh, 47 cases with ESBL-producing and CMY-producing *E. coli* were identified and enrolled. Eighty cases were identified and enrolled in Seville. They represented cases from a total of 125 patients, as two patients were enrolled for two separate episodes. Urinary tract and blood were the most common sites, accounting for 95 cases (75%) and 21 cases (14%), respectively. Other less frequent sites of infection included the respiratory tract (six; 5%), wounds (three; 2%), and others (two; 2%). The places of acquisition and types of β-lactamase produced by the *E. coli* isolates are summarized in Table 1 and Fig. 1. At both sites, among the hospital-acquired and healthcare-associated isolates, the majority of *E. coli* isolates produced either CTX-M-type or SHV-type ESBLs or CMYs. In Pittsburgh, the community-acquired isolates produced CTX-M-type ESBLs or CMYs. In Seville, most community-acquired isolates produced either CTX-M-type or SHV-type ESBLs.

We conducted 20 and 12 independent meat samplings in Pittsburgh and Seville, respectively. These involved seven local supermarkets in Pittsburgh and ten in Seville. Fig. 2 summarizes the results. ESBL-producing *E. coli* was frequently isolated from retail meat purchased in Seville. In Seville, eight (67%), seven (58%), three (25%) and one (9%) chicken, turkey, pork and ground beef samples, respectively, yielded a total of 55 clonally unrelated ESBL-producing *E. coli* isolates. They produced either CTX-M-type ESBLs (CTX-M-9 group, six isolates; CTX-M-1 group, four isolates; overall, 18%) or SHV-type ESBLs (SHV-12, 45 isolates; 82%). No CMY-producing *E. coli* isolate was identified from retail meat in Seville. In contrast, in Pittsburgh, only one chicken sample (and none of the other meat types) grew ESBL-producing *E. coli*, which

TABLE 1. Distribution of extended-spectrum β-lactamase (ESBL)-producing and CMY-type β-lactamase (CMY)-producing *Escherichia coli* according to the place of acquisition and the β-lactamase types

	Seville		Pittsburgh	
	No. of isolates	ESBL/CMY families	No. of isolates	ESBL/CMY families
Hospital-acquired	22	CTX-M-1 group: 9 CTX-M-9 group: 5 SHV-12: 8	18	CTX-M-9 group: 5 SHV-7: 1[a] CMY-2/32/33: 11 Unidentified: 2
Healthcare-associated	19	CTX-M-1 group: 4 CTX-M-9 group: 8 SHV-12: 6 Unidentified: 1	22	CTX-M-1 group: 5 CTX-M-2 group: 1 CTX-M-9 group: 2 SHV-7/12: 6 TEM-137: 1 CMY-2: 7
Community-acquired	39	CTX-M-1 group: 7 CTX-M-9 group: 15 SHV-12: 16 CMY-4: 1	6	CTX-M-1 group: 3 CMY-2: 3

Data are expressed as number of cases. The site of acquisition could not be determined for one CMY case in Pittsburgh.
[a]This isolate also produced CTX-M-type ESBL.

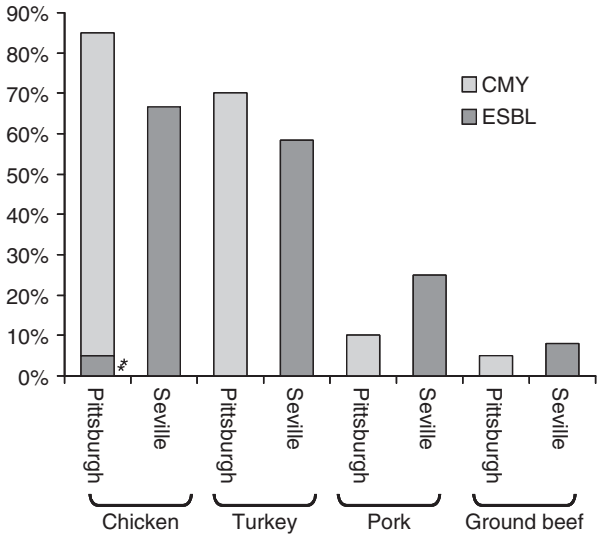

FIG. 2. Prevalence of extended-spectrum β-lactamase (ESBL)-producing or CMY-type β-lactamase (CMY)-producing *Escherichia coli* in retail meat at both sites. The retail chicken product with ESBL-producing *E. coli* in Pittsburgh also grew CMY-producing *E. coli* (*).

produced CTX-M-1 group ESBLs. However, CMY-2-producing *E. coli* isolates were identified in 17 (85%), 14 (70%), two (10%) and one (5%) chicken, turkey, pork and ground beef samples, respectively, in Pittsburgh.

Clonality was not observed among isolates from patients and retail meat at either site, except for the ESBL-producing *E. coli* isolate identified in a retail chicken product in Pittsburgh. This isolate showed a pulsed-field gel electrophoresis pattern indistinguishable from that of an isolate identified in a healthcare-associated patient. Among the *E. coli* clinical isolates and those of food origin from both sites, membership in a virulence-associated phylogenetic group (group B2 or D) of available isolates was similar for ESBL-producing *E. coli* (54/103 (52%) vs. 25/56 (45%), respectively). For CMY-producing *E. coli*, this was higher in clinical isolates than in meat isolates (21/23 (91%) vs. 17/34 (49%), respectively).

Discussion

In this analysis of ESBL-producing and CMY-producing *E. coli*, we observed a predominance of community-acquired and healthcare-associated cases at teaching hospitals in Pittsburgh and Seville. The occurrence of infections due to ESBL-producing and CMY-producing *E. coli* among community patients has serious implications for the empirical management of common infections, and warrants continued surveillance. The predominance of healthcare-associated acquisition of ESBL-producing and CMY-producing *E. coli* in Pittsburgh could be attributed, at least in part, to recent hospital admission and residence in nursing homes. Data regarding the prevalence of colonization with ESBL-producing *Enterobacteriaceae* among nursing home residents are scarce. Reports from the 1990s document colonization rates of 15% at baseline to as high as 46% in an outbreak setting [21,22]. A better understanding of the transmission dynamics of ESBL-producing and CMY-producing *E. coli* within these facilities and between them and acute-care hospitals is vital for the formulation of effective infection control strategies.

We isolated *E. coli* producing ESBLs and CMYs from a large proportion of retail meat samples in Seville and Pittsburgh, respectively. The ESBL and CMY gene contents of the *E. coli* isolates of food origin correlated well with the genes that were locally prevalent in clinical isolates, although we were surprised by the fact that SHV enzymes were more frequent than CTX-M enzymes. The importance of SHV-producing *E. coli* isolates as community pathogens has been recently analysed in Spain [23]. Contamination of retail meat, especially poultry, with *E. coli* producing CMYs is well documented [13,24]. Recovery of ESBL-producing *E. coli* from retail meat is less common, but has also been reported [25–28]. Numerous studies have reported the isolation of *E. coli* producing ESBLs and CMYs from food-producing animals [13,24]. Less is known about whether these animals actually serve as the source of the organisms causing disease in humans. Recent studies suggest that antibiotic-resistant *E. coli* strains isolated from retail poultry share similar phylogenetic and virulence markers with contemporary human strains [29], and that poultry isolates have higher virulence scores than beef and pork isolates [30]. Also, certain food habits have been found to be associated with faecal carriage of ESBL-producing *E. coli* [31].

Our study has several limitations. First, because the study was performed at single centres in Pittsburgh and Seville, the national trend could not be captured at either country to allow the drawing of strong conclusions of broader public health relevance. Second, the Pittsburgh site had limited numbers of specimens from outpatient locations. Third, retail foods other than meat were not cultured. Finally, we provided an ecological relationship between contamination of retail meat and colonization or infection due to ESBL-producing and CMY-producing *E. coli*, but we could not demonstrate a direct causal relationship.

In summary, the majority of infections due to ESBL-producing and CMY-producing *E. coli* were acquired in the community or in association with the healthcare system but outside hospitals, both in Pittsburgh and Seville. The majority of retail poultry samples contained *E. coli* producing ESBLs or CMYs at both locales. Our findings provide further circum-

stantial evidence that retail meat may serve as a source of ESBL-producing and CMY-producing *E. coli* isolates, which may then colonize the human intestine and cause infections, or serve as donors of ESBL and CMY genes to the human strains by means of conjugal transfer of resistance plasmids.

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Transparency Declaration

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References

1. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18: 657–686.
2. Bauernfeind A, Horl G. Novel R-factor borne β-lactamase of *Escherichia coli* confering resistance to cephalosporins. *Infection* 1987; 15: 257–259.
3. Bauernfeind A, Chong Y, Schweighart S. Extended broad spectrum β-lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. *Infection* 1989; 17: 316–321.
4. Jacoby GA. AmpC β-lactamases. *Clin Microbiol Rev* 2009; 22: 161–182.
5. Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *Lancet Infect Dis* 2008; 8: 159–166.
6. Pitout JD, Gregson DB, Church DL, Laupland KB. Population-based laboratory surveillance for AmpC β-lactamase-producing *Escherichia coli*, Calgary. *Emerg Infect Dis* 2007; 13: 443–448.
7. Sidjabat HE, Paterson DL, Qureshi ZA *et al.* Clinical features and molecular epidemiology of CMY-type β-lactamase-producing *Escherichia coli*. *Clin Infect Dis* 2009; 48: 739–744.
8. Hanson ND, Moland ES, Hong SG *et al.* Surveillance of community-based reservoirs reveals the presence of CTX-M, imported AmpC, and OXA-30 β-lactamases in urine isolates of *Klebsiella pneumoniae* and *Escherichia coli* in a U.S. community. *Antimicrob Agents Chemother* 2008; 52: 3814–3816.
9. Kang CI, Kim SH, Park WB *et al.* Bloodstream infections due to extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. *Antimicrob Agents Chemother* 2004; 48: 4574–4581.
10. Rodriguez-Bano J, Navarro MD, Romero L *et al.* Bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. *Clin Infect Dis* 2006; 43: 1407–1414.
11. Rodriguez-Bano J, Alcalá JC, Cisneros JM *et al.* Community infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. *Arch Intern Med* 2008; 168: 1897–1902.
12. Rodriguez-Bano J, Navarro MD, Romero L *et al.* Epidemiology and clinical features of infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 2004; 42: 1089–1094.
13. Carattoli A. Animal reservoirs for extended spectrum β-lactamase producers. *Clin Microbiol Infect* 2008; 14 (suppl 1): 117–123.
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. Wayne, PA: CLSI, 2006.
15. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002; 40: 2153–2162.
16. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008; 36: 309–332.
17. Friedman ND, Kaye KS, Stout JE *et al.* Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med* 2002; 137: 791–797.
18. Garcia Dde O, Doi Y, Szabo D *et al.* Multiclonal outbreak of *Klebsiella pneumoniae* producing extended-spectrum β-lactamase CTX-M-2 and novel variant CTX-M-59 in a neonatal intensive care unit in Brazil. *Antimicrob Agents Chemother* 2008; 52: 1790–1793.
19. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
20. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
21. Wiener J, Quinn JP, Bradford PA *et al.* Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA* 1999; 281: 517–523.
22. Trick WE, Weinstein RA, DeMarais PL *et al.* Colonization of skilled-care facility residents with antimicrobial-resistant pathogens. *J Am Geriatr Soc* 2001; 49: 270–276.
23. Rodríguez-Baño J, Alcalá J, Cisneros JM *et al. Escherichia coli* producing SHV-type extended-spectrum beta-lactamase is a significant cause of community-acquired infection. *J Antimicrob Chemother* 2009; 63: 781–784.
24. Li XZ, Mehrotra M, Ghimire S *et al.* β-Lactam resistance and β-lactamases in bacteria of animal origin. *Vet Microbiol* 2007; 121: 197–214.
25. Jouini A, Vinue L, Slama KB *et al.* Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *J Antimicrob Chemother* 2007; 60: 1137–1141.
26. Mesa RJ, Blanc V, Blanch AR *et al.* Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). *J Antimicrob Chemother* 2006; 58: 211–215.

27. Jensen LB, Hasman H, Agerso Y *et al.* First description of an oxyimino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. *J Antimicrob Chemother* 2006; 57: 793–794.
28. Warren RE, Ensor VM, O'Neill P *et al.* Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother* 2008; 61: 504–508.
29. Johnson JR, Sannes MR, Croy C *et al.* Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerg Infect Dis* 2007; 13: 838–846.
30. Johnson JR, McCabe JS, White DG *et al.* Molecular analysis of *Escherichia coli* from retail meats (2002–2004) from the United States National Antimicrobial Resistance Monitoring System. *Clin Infect Dis* 2009; 49: 195–201.
31. Rodríguez-Baño J, López-Cerero L, Navarro MD, Díaz de Alba P, Pascual A. Faecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli*: prevalence, risk factors, and molecular epidemiology. *J Antimicrob Chemother* 2008; 62: 1142–1149.