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Community-Wide Outbreaks of Clonally Related CTX-M-14 β-Lactamase-Producing *Escherichia coli* Strains in the Calgary Health Region

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***Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs) typically cause nosocomial infections. Previous surveillance in the Calgary Health Region showed that *Escherichia coli* strains producing ESBLs were common among community patients. During the period (2000 to 2002): 23 of 157 (15%) of the strains were positive for bla_{CTX-M} genes from the CTX-M-I group (CTX-M-1-like) and 87 of 157 (55%) of the strains were positive for bla_{CTX-M} genes from the CTX-M-III group (CTX-M-14-like). The objective of this study was to investigate the molecular epidemiology of these strains. The β-lactamases were characterized, and the genetic relatedness of the isolates was analyzed by digesting genomic DNA with the restriction endonuclease XbaI and by performing pulse-field gel electrophoresis (PFGE). PFGE revealed two closely related restriction patterns (clusters CTXM14A and CTXM14AR) among 67 (77%) CTX-M-14 producers. These strains from CTXM14A had nearly identical susceptibility patterns and were isolated most often from urine samples obtained at community sites during 2000 and 2001. Strains from the CTX-M-1-like and CTX-M-negative groups were unrelated to clusters CTXM14, CTXM14AR, and CTXM14NR. We conclude that clonally related strains of *E. coli* producing CTX-M-14 β-lactamases were responsible for a predominantly community-wide outbreak. Further studies are warranted to investigate whether community-onset diseases caused by ESBL-producing *E. coli* are related to a point source or transmission within the community.**

Organisms producing extended-spectrum β-lactamases (ESBLs) are important causes of nosocomial infections and have limited therapeutic options. Specific risk factors for acquisition identified previously included length of hospital stay, severity of illness, time in the intensive care unit (ICU), intubations and mechanical ventilation, urinary or arterial catheterization, and previous exposure to antibiotics (7). The majority of the patients infected with ESBL-producing organisms have been isolated from patients admitted to ICUs, but infections can also occur in almost any other area of the hospital (12). These organisms are also isolated with increasing frequency from patients in extended-care facilities (8, 33) and recently from those with community-onset disease (3, 21, 26, 30).

An interesting issue concerning the epidemiology of ESBL-producing organisms is the evolution, maintenance, and dissemination of resistance genes among bacterial populations in larger geographic regions (14). Various surveys have reported the frequency of ESBL producers from an area or region which most probably describes a transient situation that is characteristic for that region only at a specific point in time (4, 11, 13, 20). These studies have given some insight about the prevalence and global spread of different types of ESBLs. However, there are very limited data available about the spread of ESBL-producing bacteria in larger, well-defined geographic regions (14).

Recently, a family of ESBLs that preferentially hydrolyze cefotaxime, the CTX-M-β-lactamases, have been recognized and reported in the literature with increasing frequency and are causing infections in patients from various countries (5, 17). A previous study identified CTX-M β-lactamases produced by *Escherichia coli* as the predominant type of ESBL in the Calgary Health Region (CHR) (21). The CHR is a fully integrated, publicly funded, regional health system that provides health care to all of the residents of the cities of Calgary and Airdrie and approximately 20 nearby small towns, villages, and hamlets. During the 3-year period from 2000 to 2002, 232 ESBL-producing *E. coli* strains were isolated from 168 patients (157 were deemed to be CHR residents) with the overall frequency of 1.3% (232 of 17,846). Of the strains isolated from the CHR residents: 23 of 157 (15%) were positive for bla_{CTX-M} genes from the CTX-M-I group (CTX-M-1-like) and 87 of 157 (55%) were positive for bla_{CTX-M} genes from the CTX-M-III group (CTX-M-14-like). We suspected that a clonal outbreak had occurred among isolates from patients with community-onset disease but did not have adequate molecular information to confirm this. We sought here to further characterize the CTX-M enzymes involved and determine the molecular epidemiology of these strains.

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MATERIALS AND METHODS

Clinical isolates. The *Escherichia coli* strains were isolated at Calgary Laboratory Services by using standard techniques. Antimicrobial susceptibilities were

determined by using Vitek (Vitek AMS; bioMérieux Vitek Systems, Inc., Hazelwood, MO). The results were interpreted by using National Committee for Clinical Laboratory Standards criteria for broth dilution MICs (19). The presence or absence of an ESBL was determined according to the National Committee for Clinical Laboratory Standards ESBL screening and confirmation criteria (19). The quality control strains used for the present study were *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Klebsiella pneumoniae* ATCC 700603. Community-onset infections were diagnosed in individuals who were not hospitalized in the preceding 3 months or who were either outpatients or admitted patients with a first positive culture obtained within 48 h of hospital admission. Other hospitalized patients and all residents of nursing homes were deemed to have nosocomial infections.

β-Lactamase studies. Isoelectric focusing (IEF), which included cefotaxime hydrolysis and inhibitor profiles in polyacrylamide gels, was performed on freeze-thaw extracts as previously described (24). PCR amplification for $bla_{\text{CTX-Ms}}$, bla_{TEM}, bla_{OXA} and bla_{SHV} was carried out on the strains by using primers and PCR conditions described previously (22, 25). The genes responsible for the production of the CTX-M-14-like β-lactamases were amplified on a GeneAmp 9600 ThermoCycler instrument (Applied Biosystems, Norwalk, CO) under PCR conditions previously described (25) with the primers CTXM14F2 5′-GATGTA ACACGGATTGACC-3′ and CTXM14R1 5′-CGTTGTCGGGAAGATACGT G-3′. Automated sequencing was performed on the PCR products with the ABI Prism 3100 genetic analyzer (Applied Biosystems) as previously described by using Sequence Analysis software (25). The sequences of the different amplicons were compared to each other and to homologous sequences by using Sequence Navigator software. The nucleotide and the deduced protein sequences were analyzed by using the software available from the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Conjugation experiments. To determine whether the resistance was transferable, transconjugation experiments using the filter paper mating technique were performed with *E. coli* C600N (Nal^r) as recipient (24). Tranconconjugants were selected on LB agar (Difco, Detroit, MI) plates containing 12 μg of nalidixic acid and 64 μg of ampicillin/ml.

PFGE. All of the ESBL-producing *E. coli* were typed with pulsed-field gel electrophoresis (PFGE) after the extraction of genomic DNA and digestion with XbaI by using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA (31). The subsequent PFGE analyses were performed on a CHEF-MAPPER apparatus (Bio-Rad Laboratories, Hercules, CA). DNA relatedness was calculated based on the Dice coefficient, and isolates were considered to be genetically related if the Dice coefficient correlation was ≥80%, which corresponds to the "possibly related (four- to six-band difference)" criteria of Tenover et al. (32).

RESULTS

β-Lactamase studies. Of the strains isolated from the CHR residents 23 were positive for $bla_{\text{CTX-M}}$ genes from the CTX-M-I group (CTX-M-1-like) and 87 were positive for $bla_{\text{CTX-M}}$ genes from the CTX-M-III group. IEF revealed that isolates positive for $bla_{\text{CTX-M-14-like}}$ genes produced a single β-lactamase with a pI value of 8.1 that aligned with CTX-M-14. These enzymes hydrolyzed 0.75 μg of cefotaxime/ml in polyacrylamide gels and were inhibited by clavulanic acid in the overlay technique. PCR with CTXM14F and CTXM14R1 amplified a 941-bp amplicon, and sequence analysis of the products revealed 100% identity with the sequence of the $bla_{\text{CTX-M-14}}$ allele (15).

Analytical IEF of the isolates positive for $bla_{\text{CTX-M-1-like}}$ genes showed the presence of cefotaxime hydrolyzing enzymes with pIs of 9.0 in 21 of 23 and 8.7 in the remaining two strains. All of the isolates produced additional enzymes with a pI value of 5.4 that aligned with TEM-1, whereas 8 of 23 also produced a β-lactamase with a pI of 7.6 that aligned with SHV-1. PCR with primers for bla_{TEM} amplified a 971-bp fragment in all strains positive for $bla_{\text{CTX-M-1-like}}$ genes and with primers for bla_{OXA} amplified an 885-bp fragment in the strains (eight in all) with a β-lactamase of 7.6. Sequencing of these enzymes has been unsuccessful to date.

Strains representing the CTX-M-negative group produced different cefotaxime hydrolyzing β-lactamases with pI values ranging from 8.2 to 5.4 that are suggestive of the TEM, OXA, and SHV types of ESBLs. All of the β-lactamases produced by isolates from the CTX-M-I and CTX-M-negative groups were inhibited by clavulanate on IEF gels.

Conjugation experiments. We were unable to transfer resistance to ampicillin when we used strains producing CTX-M-14 β-lactamases as donors. These strains were representative of CTXM14A (five strains), CTXM14AR (four strains), and CTXM14NR (five strains).

PFGE. PFGE revealed two closely related restriction patterns (clusters CTXM14A [59 isolates] and CTXM14AR [8 isolates]) among 67 of 87 (77%) of CTX-M-14 producers (Fig. 1). These two clusters correspond to a cutoff value of 80% identity for PFGE. The remaining 20 CTX-M-14 producers (CTXM14NR) were not related to these two clusters (Fig. 1). The strains belonging to CTXM14A and CTXM14AR clusters had nearly identical susceptibility patterns (Table 1); 66 of 67 (99%) were resistant to ciprofloxacin (CIP) and 4 of 67 (6%) were resistant to trimethoprim-sulfamethoxazole ([SXT]), whereas 13 of 20 (65%) of the CTXM14NR strains were resistant to CIP and 6 of 20 (60%) were resistant to SXT ($P = 0.0001$ and $P = 0.008$, respectively, Fisher exact test) (Table 1). The majority of CTX-M-14-producing strains were isolated from urine samples obtained at community sites, and strains from cluster CTXM14A were more likely to be isolated from urine samples than strains from CTXM14AR and CTXM14NR (Table 1). Strains from CTXM14AR and CTXM14NR were more likely to be isolated from blood (Table 1). Of the 87 patients with an infection caused by a CTX-M-14-producing *E. coli*, 19 patients (10 from CTXM14A, 3 from CTXM14AR, and 6 from CTXM14NR) were admitted to a hospital within the preceding 12 months. No apparent clustering of patients in a certain area or center was evident. The CTXM14A cluster was responsible for an outbreak during July 2000 to September of 2001, with a solitary case in 2002 (Fig. 2). Strains from the CTXM14AR cluster appeared for the first time in May 2001, with a single case in 2002. The strains from CTXM14A accounted for a large increase in the total number of CTXM-14 producers from July 2000 to August 2001. Strains from CTXM14AR appeared after CTXM14A, with a seeming outbreak that was superimposed on the second "hump" of the former clone, and persisted briefly thereafter (Fig. 2). Strains producing ESBLs from the CTXM-I and CTX-M-negative groups were unrelated to these clusters when typed by PFGE (data not shown) and had different susceptibility patterns. The CTX-M-I group was significantly more resistant to SXT (16 of 23 strains [70%] versus 9 of 87 strains [10%]; $P < 0.0001$) and gentamicin (13 of 23 strains [57%] versus 5 of 87 strains [6%]; $P < 0.0001$) compared to CTX-M-14 strains, whereas the CTX-M-negative group was more sensitive to CIP (6 of 47 strains [13%]) compared to the CTX-M-positive strains (97 of 111 strains [88%]; $P < 0.0001$) (21).

DISCUSSION

Infections caused by ESBL-producing *Enterobacteriaceae* had mostly been described as nosocomially acquired (23) or nursing home related (6, 8, 33). Investigators have used mo-

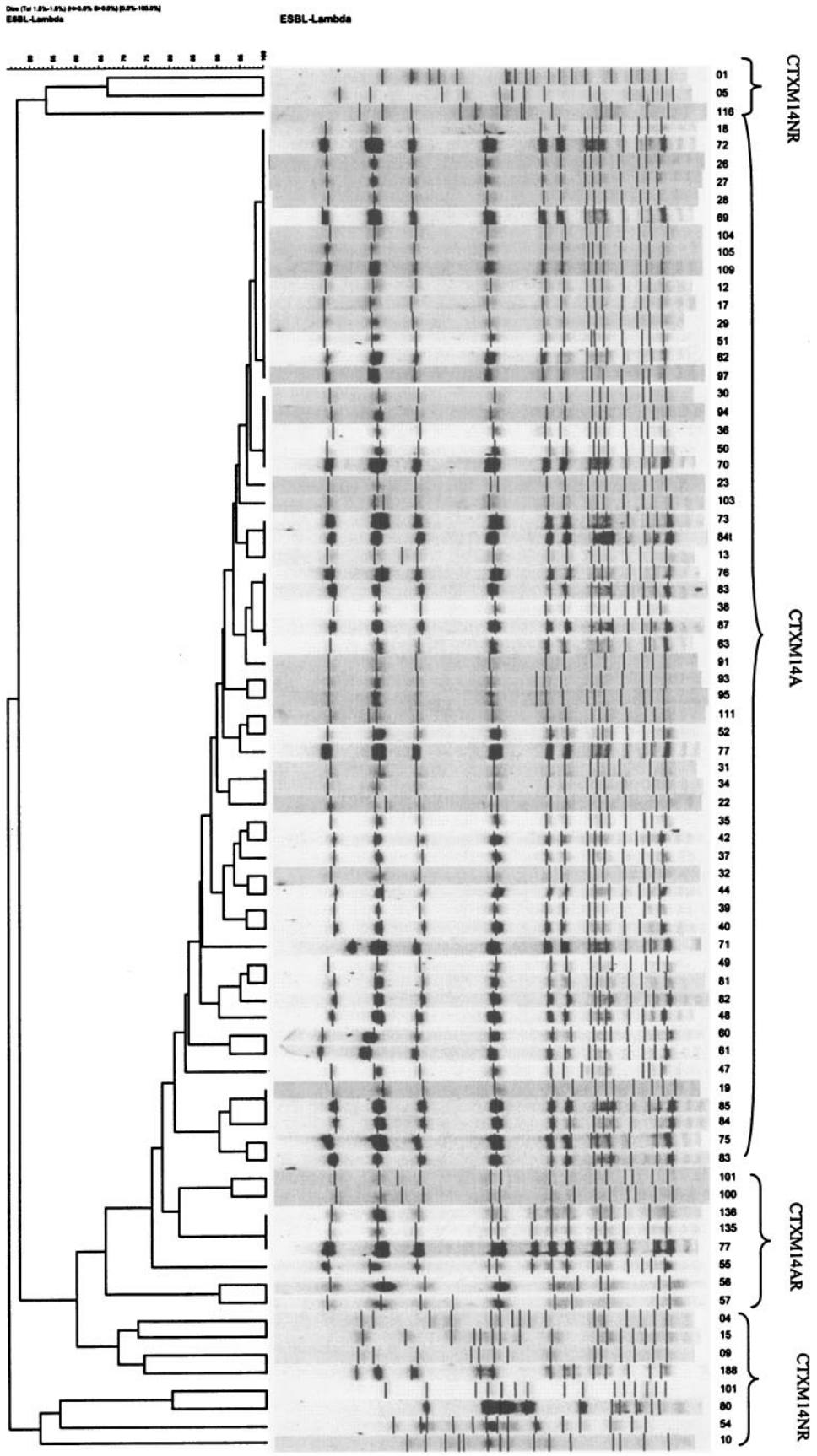

FIG. 1. PFGE patterns and dendrogram of *E. coli* strains producing CTX-M-14 β-lactamases isolated from the CHR.

TABLE 1. Antimicrobial susceptibilities, onset of infections, and isolation sites of CTX-M-14-producing *E. coli* strains from the CHR[a]

Strain (*n*)	No. of strains (%)								
	Resistance[b]			Onset of infection[c]			Site of isolation		
	GEN	SXT	CIP	Community	Hospital	Nursing home	Urine	Blood	Other sites
CTX-M-14 (87)	6 (7)	10 (12)	79 (91)	62 (72)	24 (28)	1 (1)	78 (90)	7 (8)	2 (2)
CTXM14A (59)	1 (2)	3 (5)	58 (98)	47† (80)	11† (19)	1 (2)	59† (100)	0† (0)	0 (0)
CTXM14AR (8)	1 (13)	1 (13)	8 (100)	4 (50)	4 (50)	0 (0)	4 (50)	4 (50)	0 (0)
CTXM14NR (20)	4* (20)	6* (30)	13* (65)	11 (55)	9 (45)	0 (0)	15 (75)	3 (15)	2 (10)

[a] CTXM14A and CTXM14AR indicate clusters with >80% similarity. CTXM14NR indicates strains not related to CTXM14A and CTXM14AR. *, $P < 0.05$ (significantly different from CTXM14A and CTXMAR [Fisher exact test]); †, $P < 0.05$ (significantly different from CTXMAR and CTXMNR [Fisher exact test]).
[b] GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin.
[c] Community-onset infections were diagnosed in individuals who did not have a hospitalization in the preceding 3 months and who were either outpatients or admitted patients who had first positive cultures obtained within 48 h of hospital admission. Other hospitalized patients and all residents of nursing homes were deemed to have nosocomial infections.

lecular methods such as PFGE to examine the molecular epidemiology of ESBL-producing strains involved in outbreaks of nosocomial infections (7). The molecular methods have shown that the majority of outbreaks often originate in an ICU and then spread to other parts of the hospital by various means, such as clonal dissemination of the ESBL-producing strains (28) or horizontal transmission of the plasmid encoding for the ESBL among nonrelated strains (9). This type of epidemiology has also been described in the nursing home setting (29, 33).

Recent data suggest that infections due to ESBL-producing organisms might be an emerging problem in the outpatient settings in different countries (1, 3, 18, 21, 26, 34). Very limited data are available about the molecular epidemiology of ESBL-producing *Enterobacteriaceae* isolated from patients with community-onset infections. A recent study from Spain described the clinical features and epidemiology of infections due to ESBL-producing *E. coli* in nonhospitalized patients (30). Their findings suggest that these strains are an emerging cause of urinary tract infections in this setting but no evidence of horizontal transmission was evident.

Our study describes aspects of the molecular epidemiology of ESBL-producing *E. coli* in a large well-defined geographic region. In the CHR, Calgary Laboratory Services receives all clinical specimens submitted for bacteriologic testing, including those from all hospitals, nursing homes, physicians' offices, and community collection sites (10). Our surveillance included all clinical specimens from hospital and community sites, and we restricted our study to *E. coli* strains isolated from CHR residents, since other ESBL-producing organisms are rare in our region (only seven patients with ESBL-producing *K. pneumoniae* were identified during this study period). Our results show that clonally related strains of *E. coli* producing CTX-M-14 β-lactamases (clusters CTXM14A and CTXM14AR) were responsible for an outbreak of community onset urinary

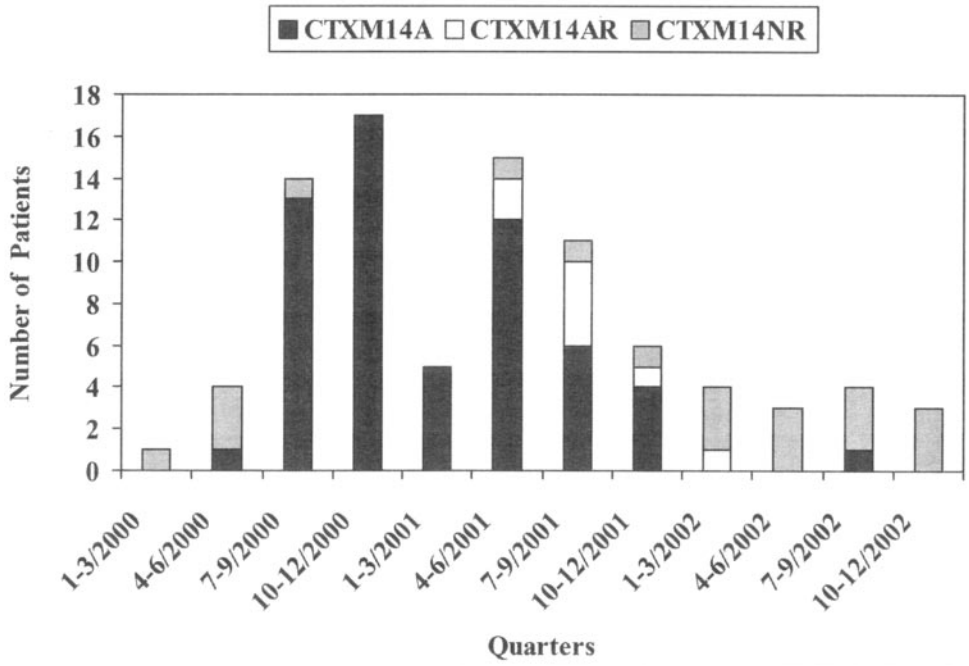

FIG. 2. Distribution of different clusters of *Escherichia coli* producing CTX-M-14 β-lactamases in the CHR from 2000 to 2002. CTXM14A and CTXM14AR (A-related) indicate clusters with >80% similarity. CTXM14NR indicates strains not related to CTXM14A and CTXM14AR.

tract disease during 2000 and 2001 in the CHR (Fig. 1 and 2). CTXM14AR strains were more likely to be hospital acquired than CTXM14A strains (Table 1) and appeared slightly later than the former clone; thus, CTXM14AR might represent a derivative of CTXM14A that emerged in hospitals after CTXM-14 was introduced there from the community. These clusters were absent among *E. coli* strains producing CTX-M-14 β-lactamases isolated during 2002 (only two strains belonging to CTXM14A and CTXM14AR were identified in 2002). This finding is suggestive of a common source type of outbreak that occurred during 2000 and 2001 (Fig. 2). To our knowledge, this is the first study to show an outbreak of clonally related strains of ESBL-producing *Enterobacteriaceae* that involved community patients.

A notable limitation to our study is the limited epidemiological data available. We did not have specific information regarding travel history, and food and/or water exposures. In addition, we were unable to explore any relationship between patients such as family contacts or temporal associations to investigate the possibility of a common source or transmission between individuals in the community. We have recognized the importance of such information and a prospective study to comprehensively explore the role of these risk factors is under way at our center. We postulate that a contaminated food- or waterborne source might have been responsible for this common source type of outbreak since *E. coli* O157:H7 food-related outbreaks occurred frequently during the summer months in the CHR (27). This type of seasonal variation has been well demonstrated for common source outbreaks associated with environmental and food-borne associated pathogens (2, 16).

In conclusion, the present study is novel in that it demonstrates that large clonal outbreaks of ESBL-producing organisms may arise in the community setting and illustrates the importance of performing molecular surveillance on antimicrobial-resistant pathogens isolated from community sources. The findings of our study support the view that ESBL-producing *E. coli* is an emerging pathogen in the community setting and warrant increased efforts in surveillance and the study of risk factors associated with the acquisition of these isolates. This will guide future prevention and control measures. The present study adds another dimension to the molecular epidemiology of organisms producing ESBLs and suggests that these strains will continue to present challenges for clinical microbiologists and clinicians alike.

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