Prevalence and Molecular Characterization of Extended-Spectrum- β -Lactamase-Producing *Enterobacteriaceae* in a Pediatric Patient Population

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Very little is known about the prevalence and composition of various types of extended-spectrum β -lactamases (ESBL) in pediatric patients. The aims of this study were the following: (i) to determine the prevalence of ESBLs among *Enterobacteriaceae* in a tertiary-care pediatric population; (ii) to characterize the genetic composition of the identified ESBL enzymes; and (iii) to determine the relative prevalence of CTX-M enzymes and *Escherichia coli* ST131 strains among ESBL-producing isolates in the same pediatric patient population. Among the 1,430 *Enterobacteriaceae* isolates screened for elevated MICs to cefotaxime and/or ceftazidime from pediatric patients during a 1-year period, 94 isolates possessed at least one ESBL gene. CTX-M was the most commonly isolated ESBL type, consisting of 74% of all ESBLs versus 27% TEM and 24% SHV enzymes. Sequence analysis and probe-specific real-time PCR revealed that the majority (80%) of the CTX-M-type ESBLs were CTX-M-15 enzymes, followed by CTX-M-14 (17%) and CTX-M-27 (2.8%). Multilocus sequence typing (MLST) and repetitive PCR analyses revealed that the relative prevalence of ST131 among ESBL-producing *E. coli* isolates is 10.2%. This study highlights the growing problem of ESBL resistance in pediatric *Enterobacteriaceae* isolates and demonstrates a transition toward the predominance of CTX-M-type enzymes among ESBL-producing *Enterobacteriaceae* organisms causing pediatric infections.

In the past 2 decades, there has been an explosive increase in the prevalence of high-level resistance to beta-lactam antibiotics in members of Gram-negative *Enterobacteriaceae* due to the presence of extended-spectrum β -lactamase (ESBL) enzymes (5). ESBLs are bacterial enzymes that confer resistance to broad-spectrum cephalosporins, including oxymino-beta-lactam antibiotics (29). Importantly, ESBL-producing organisms are associated with infections that result in poor clinical outcomes, delayed initiation of appropriate antibacterial therapy, longer hospital stays, and greater hospital expenses (26). Additionally, there has been a rapid and widespread dissemination of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing ESBLs in the community setting. As a result, these organisms have become important pathogens of both community-onset and hospital-associated infections on a global scale (21).

Recently, studies have shown that CTX-M-type ESBLs have replaced TEM- and SHV-type ESBLs in Europe, Canada, and Asia as the most common ESBL type among various members of the *Enterobacteriaceae* (5). Presently, CTX-M β -lactamases include more than 90 different enzymes that are classified into five groups based on their amino acid identities and include the CTX-M-1, -2, -8, -9, and -25 groups (33). Some of the CTX-M enzymes have specific geographical distributions: CTX-M-9 and CTX-M-14 are in Spain and North America and CTX-M-1 is in Italy, whereas multiple reports have shown that CTX-M-15 is the most widely distributed CTX-M enzyme worldwide (20, 22, 29). There have been several reports of outbreaks caused by CTX-M-producing organisms in hospital settings, such as cancer centers, geriatric wards, and hospitalized nursing home patients (29). Additionally, molecular epidemiology studies revealed that one specific *E. coli* clone, ST131, has been tightly associated with the production of CTX-M enzymes (28). This ST131 clone is a pandemic clone that

is associated predominantly with community-onset urinary tract infections (UTIs) worldwide (32).

Although the United States initially appeared to have been spared from the CTX-M-type ESBL epidemic, a study by Jorgensen et al. in 2007 indicated that CTX-M-type ESBLs had emerged in *E. coli* and other species of *Enterobacteriaceae* in the United States as well (21). Furthermore, recent studies from Philadelphia, Chicago, and Pittsburgh identified a high prevalence of CTX-M-producing *E. coli* ST131 in adult patient populations (23, 27, 34).

ESBL-mediated resistance in *Enterobacteriaceae* is a leading cause of serious infections in adults, neutropenic cancer patients, and patients with underlying diseases (1, 10, 12, 13). However, despite extensive studies of ESBL-producing organisms in adult patients both in hospital and community settings, there is a dearth of information on the development and spread of ESBL-mediated resistance, specifically CTX-M, in Gram-negative infections in pediatric populations. It is critical to better define the prevalence and molecular composition of resistant Gram-negative organisms in the health care settings for neonates, infants, and children to adopt best-practice infection control measures and aid in the appropriate choice of empirical antimicrobial coverage for infections in these pediatric populations.

The aims of this study were the following: (i) to determine the

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prevalence of ESBLs among *Enterobacteriaceae* in a tertiary-care pediatric population, (ii) to characterize the genetic composition of the identified ESBL enzymes, and (iii) to determine the relative prevalence of CTX-M enzymes and *E. coli* ST131 strains among ESBL-producing isolates in the same pediatric patient population.

MATERIALS AND METHODS

Setting. Texas Children's Hospital (TCH) is a pediatric tertiary health care facility located within the Texas Medical Center (Houston, TX). This study included all pediatric patients at TCH diagnosed with culture-positive *Enterobacteriaceae*, including *E. coli*, *Klebsiella* species (*Klebsiella oxytoca* and *Klebsiella pneumoniae*), *Enterobacter* species (*Enterobacter cloacae* and *Enterobacter aerogenes*), *Serratia marcescens*, *Morganella morganii*, *Proteus mirabilis*, and *Citrobacter freundii* infections during a 1-year period from October 2010 to September 2011. This study was approved by the Baylor College of Medicine Institutional Review Board.

Isolation and antibiotic susceptibility testing. A total of 2,101 *Enterobacteriaceae* isolates identified from patient specimens at the diagnostic microbiology laboratory of Texas Children's Hospital were prospectively collected in this study. These bacterial isolates were identified in the laboratory as a part of routine clinical care. Only one isolate per patient was included. Clinical samples were processed in the TCH Diagnostic Microbiology Laboratory according to standard operating procedures. Antibiotic susceptibility testing was determined by Etests on Mueller-Hinton agar for the determination of the MIC and by the use of the Vitek 2 instrument (bioMérieux, Durham, NC). The susceptibilities of all isolates to ampicillin, amoxicillin-clavulanic acid, amikacin, tetracycline, nitrofurantoin, sulfamethoxazole-trimethoprim, gentamicin, levofloxacin, norfloxacin, cefazolin, ceftriaxone, cefepime, and meropenem were determined by Vitek 2. The susceptibilities to cefoxitin (CTX), cefotaxime, ceftazidime (CAZ), piperacillin-tazobactam, and ciprofloxacin were determined by Etest. The Etest MIC values were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (8).

Resistance genotyping. All *Enterobacteriaceae* isolates with a MIC of ≥ 2 $\mu\text{g/ml}$ for CTX or ≥ 8 $\mu\text{g/ml}$ for CAZ were identified as potential ESBL producers as specified by the CLSI (8). During the beginning of the study in 2010, the CLSI standard, M100-S20 (8), was used for interpretation of the cephalosporin susceptibility results. In 2011, CLSI revised the cephalosporin breakpoints, lowering the ceftazidime breakpoints to ≥ 2 $\mu\text{g/ml}$. However, to maintain consistency in the study, screening for possible ESBL producers using the 2010 M100-S20 breakpoints was continued for the rest of the study period. For those isolates that met the criteria for possible ESBL production, a panel for the amplification of the *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} genes was applied. Crude genomic DNA was extracted from the isolates by heat lysis. Briefly, two colonies were suspended in 100 μl of distilled water, and the cells were lysed by heating at 95°C for 10 min. Cellular debris was removed by centrifugation, and the DNA was then subjected to PCR using specific primer pairs to screen for β -lactamase genes as described previously (21, 31). The LightCycler 480 DNA SYBR green I Master (Roche Diagnostics) was used for SYBR green detection with the following amplification protocol: 94°C for 10 min; 35 cycles consisting of 94°C for 10 s, 57°C for 30 s (62°C for *bla*_{SHV}), and 72°C for 30 s; and finally a melting curve analysis. The quantity of the amplified product was monitored by detection of fluorescence energy emitted at 530 nm by SYBR green. A melting curve analysis was then performed as recommended by the manufacturer. Melting curve analyses allowed confirmation of the specificity of the amplified product and allowed for differentiation of β -lactamase groups. Samples with amplicons having melting temperatures at the correct temperature were scored as positive for the respective target genes. A subset of amplicons showing the expected molecular mass was further sequenced; sequences were then analyzed using the BLAST software available from the National Library of Medicine (<http://www.ncbi.nlm.nih.gov/blast>).

Phylogenetic analysis. The phylogenetic grouping of the *E. coli* isolates was determined according to the presence of the *chuA* and *yjaA* genes

TABLE 1 Antimicrobial susceptibility data of 200 *Enterobacteriaceae* isolates included in the study

Antibiotic	Drug susceptibility (no. of isolates/no. tested [%])		
	Susceptible	Intermediate	Resistant
Amikacin	197/200 (98.5)	3/200 (1.5)	1/200 (0.5)
Ampicillin	0/200 (0)	0/200 (0)	200/200 (100)
Ceftazidime	12/200 (6)	12/200 (6)	176/200 (88)
Cefotaxime	10/200 (5)	17/200 (8.5)	171/200 (85.5)
Cefepime	155/200 (77.5)	5/200 (2.5)	50/200 (25)
Gentamicin	126/200 (63)	5/200 (2.5)	66/200 (33)
Levofloxacin	146/200 (73)	9/200 (4.5)	41/200 (20.5)
Meropenem	200/200 (100)	0/200 (0)	0/200 (0)
Nitrofurantoin	115/200 (57.5)	43/200 (21.5)	42/200 (21)
Tetracycline	128/200 (64)	2/200 (1)	70/200 (35)
Tobramycin	124/200 (62)	32/200 (16)	44/200 (22)
Trimethoprim-sulfamethoxazole	96/200 (48)		104/200 (52)

and TspE4.C2 using a multiplex PCR developed by Clermont et al. that allows the identification of the four major phylogenetic groups (groups A, B1, B2, and D) (6). The strains were designated phylogenetic group B2 (*chuA* present, *yjaA* present), D (*chuA* present, *yjaA* absent), B1 (*chuA* absent, TSPE4.C2 present), or A (*chuA* absent, TSPE4.C2 absent).

Rep-PCR. Clonal relatedness among *E. coli* isolates was determined using DiversiLab semiautomated repetitive PCR (Rep-PCR) typing as previously described (30). DNA samples from the isolates were amplified using the DiversiLab *E. coli* kit for DNA fingerprinting by following the manufacturer's instructions (bioMérieux, Inc., St. Laurent, Quebec, Canada) and a GeneAmp 9700 ThermoCycler instrument. Detection of Rep-PCR products was performed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA). Analysis of the resulting DNA fingerprint patterns was performed with DiversiLab software, version 3.3. ST131 was further confirmed by PCR for the *pabB* and *trpA* alleles as recently described by Dhanji et al. and Clermont et al. (7, 11).

MLST. Multilocus sequence typing (MLST) was carried out on all *E. coli* isolates according to the protocol and primers specified previously (37). Gene amplification and sequencing were performed by using specific primers for the 7 standard housekeeping genes (*adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) specified at the *E. coli* MLST web site (<http://mlst.ucc.ie/>). Allelic profile and ST determinations were performed per the scheme at the *E. coli* MLST web site.

RESULTS

During the study period, a total of 2,101 *Enterobacteriaceae* isolates were collected prospectively from pediatric patients with UTIs (1,598) and sterile-site infections (503) who were seen at Texas Children's Hospital. Only one isolate per patient per admission was included for further analysis, resulting in the inclusion of 1,430 isolates. These isolates were screened for elevated MICs to cefotaxime or ceftazidime (>2 and 8 $\mu\text{g/ml}$, respectively). Of the 1,430 isolates that were screened, 200 (13.9%) isolates met the criteria for elevated MIC to either cefotaxime or ceftazidime and possible ESBL production. The molecular resistance profiles of these 200 isolates were further characterized using molecular methods. The isolates were derived from multiple specimen sources: 124 from urine, 14 from blood, 13 from wound or abscess, and 49 from respiratory specimens (tracheal aspirate, sputum, and bronchoalveolar lavage).

Antibiotic susceptibility data. Results of the *in vitro* antimicrobial susceptibility testing of the 200 *Enterobacteriaceae* isolates that were included in this study are shown in Table 1. One hun-

FIG 1 Prevalence of ESBLs among *Enterobacteriaceae* isolates.

dred percent and 98.5% of the isolates were susceptible to meropenem and amikacin, respectively, whereas only 62 and 63% were susceptible to gentamicin and tobramycin. Although only 6 and 5% of the isolates were susceptible to ceftazidime and cefotaxime, respectively, 77.5% of the isolates were susceptible to cefepime. Furthermore, 73% of the isolates were observed to be susceptible to the quinolones tested (levofloxacin and norfloxacin).

Prevalence of ESBLs. The 200 isolates that met the criteria for elevated MICs to cefotaxime or ceftazidime and therefore possible ESBL production were characterized by real-time PCR to determine whether they carried any known ESBL genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}). Ninety-four of the 1,430 *Enterobacteriaceae* strains included in this study carried at least one ESBL gene, resulting in an overall prevalence of 7%. Furthermore, 78% of the strains carrying ESBL genes were isolated from urine, in contrast to the 12% of strains isolated from blood. The majority (72%) of the 94 ESBL strains were *E. coli* (68), 12 were *Klebsiella* strains, 10 were *Enterobacter* strains, and the remaining 4 isolates belonged to other species of the *Enterobacteriaceae*.

***bla* gene composition of ESBL-producing strains.** Molecular

characterization using *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}-specific primers revealed that CTX-M-type ESBLs were the most common ESBLs among the pediatric isolates. The *bla*_{CTX-M} gene was detected in 70 of the 94 ESBL-producing isolates (74%). Only 26 isolates (27%) carried *bla*_{TEM}, and only 23 isolates (24%) carried *bla*_{SHV}. Twenty-four (25.5%) isolates encoded multiple *bla* genes. As shown in Fig. 1, the majority (95.7%) of the CTX-M-positive isolates were *E. coli*. In contrast, only 2 *Klebsiella* sp. isolates (16.6%) and 1 *Enterobacter* sp. isolate (10%) were CTX-M positive. TEM ESBLs were identified in 18 *E. coli*, 4 *Klebsiella*, 3 *Enterobacter*, and 1 *M. morganii* strains. SHV ESBLs were identified in 9 *E. coli*, 9 *Klebsiella*, 4 *Enterobacter*, and 1 *C. freundii* isolates. Additionally, 18 of the 94 ESBL-producing isolates (19.1%) co-produced TEM- and/or SHV-type β-lactamases along with CTX-M enzymes. All three determinants were detected in 2 *K. pneumoniae* isolates. Molecular characterization of isolates carrying *bla*_{TEM} and *bla*_{SHV} revealed that all of the TEM-positive isolates encoded *bla*_{TEM-14}, and of the 9 SHV-positive isolates, 6 isolates (67%) produced an SHV-12-type enzyme and 3 isolates (33%) produced *bla*_{SHV-32} enzymes (data not shown).

Sequencing and typing of CTX-M-positive ESBLs. The 70 isolates carrying *bla*_{CTX-M} were further characterized by sequencing the complete *bla*_{CTX-M} gene. Sequence analysis revealed that of the 70 CTX-M-positive isolates, 56 isolates (80%) produced a CTX-M-15-type enzyme, 12 (17.1%) isolates carried *bla*_{CTX-M-14}, and 2 (2.85%) isolates carried *bla*_{CTX-M-27} enzymes (Table 2). In addition, 100% of the CTX-M-15 enzymes were identified in *E. coli*, and these isolates all were resistant to both cefotaxime and ceftazidime. Interestingly, 89.2% of the CTX-M-15-producing isolates had cefepime MICs exceeding the CLSI susceptibility breakpoint of 8 μg/ml, whereas only 11.3% of the other CTX-M-producing isolates had MICs elevated above the CLSI recommendation for cefepime (Table 2).

Phylogenetic characterization. Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) (15). In this study, the phylogenetic groups of

TABLE 2 Antimicrobial susceptibility data of *Enterobacteriaceae* isolates producing CTX-M-type ESBLs

Species and CTX-M type produced	No. of isolates with CTX-M type/total no. of isolates (%)	No. (%) of isolates resistant to ^a :						
		CIP	AMK	CAZ	CTX	FEP	SXT	MEM
<i>E. coli</i>								
CTX-M-15	56/70 (80)	45 (80.3)	1 (1.7)	56 (100)	56 (100)	50 (89.2)	48 (85.7)	0
CTX-M-14	9/70 (12.9)	3 (33.3)	0	9 (100)	8 (88.8)	1 (11.3)	6 (66.8)	0
CTX-M-27	2/70 (2.85)	1 (50)	0	2 (100)	0	0	1 (50)	0
ST131								
CTX-M-15	7/56 (12.5)	6 (85.7)	0	7 (100)	7 (100)	7 (100)	7 (100)	0
Non-ST131								
CTX-M-15	44/56 (78.5)	34 (77.2)	0	40 (91)	38 (86.3)	14 (29.5)	38 (88)	0
ST394								
CTX-M-15	3/56 (5.35)	3 (100)	0	3 (100)	3 (100)	2 (66.7)	3 (100)	0
ST393								
CTX-M-15	2/56 (3.57)	2 (100)	0	2 (100)	2 (100)	1 (50)	2 (100)	0
<i>Klebsiella</i> sp.								
CTX-M-14	2/2 (100)	1 (50)	0	2 (100)	1 (50)	1 (50)	2 (100)	0
<i>Enterobacter</i> sp.								
CTX-M-14	1/1 (100)	0	0	1 (100)	0	0	0	0

^a CIP, ciprofloxacin; AMK, amikacin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; SXT, trimethoprim-sulfamethoxazole; and MEM, meropenem.

FIG 2 Representative dendrogram based on DiversiLab Rep-PCR showing the genetic relatedness of CTX-M-15-producing *E. coli* isolates, their phylogenetic characteristics, and selected resistance genes.

the *E. coli* isolates were determined by a triplex PCR based on the detection of the *chuA* and *yjaA* genes and DNA fragment TSPE4.C2 as described by Clermont et al. (6). Analyses of the *E. coli* isolates by this method revealed that B2 and A groups were the most common (45.5 and 20.5%, respectively), followed by B1 and D group strains (11.7 and 10.6%, respectively). Furthermore, CTX-M-15-producing *E. coli* strains mostly belonged to the phylogroups B2, D, and A, while phylogroups D and B2 were common for the CTX-M-14-producing isolates (data not shown).

Rep-PCR and MLST analysis. Multilocus sequence typing based on nucleotide sequence analyses of housekeeping genes and repetitive sequence-based PCR (Rep-PCR) have been increasingly used to investigate epidemiological and phylogenetic relationships among *E. coli* strains. The CTX-M-15-producing *E. coli* isolates were typed by the DiversiLab semiautomated Rep-PCR technique to assess the similarity between the strains, and a subset of these isolates underwent MLST to determine their sequence types (ST). Figure 2 is a representative dendrogram of Rep-PCR showing similarity among members of a subset of CTX-M-15-producing *E. coli* isolates along with their MLST and phylogenetic profiles. The isolates analyzed by the DiversiLab system showed that 7 of the CTX-M-15-producing *E. coli* isolates (12.5%) were highly related to the ST131 control strain, with >92% similarity, and were arranged in two related DiversiLab profiles. Additionally, 2 of these 7 isolates, TCH01 and TCH64, were identical to the ST131 control strain, with >98% similarity. Furthermore, as shown in Fig. 2, MLST analyses of these isolates showed 100% concordance in identifying the ST131 clone as the most prevalent ST. The prevalence of ST131 clones was further confirmed using PCR detection of the *trpA* and *pabB* alleles as described previously (7, 11). Consistent with the Rep-PCR and MLST data, ST131-specific single-nucleotide polymorphisms (SNPs) were detected in both *pabB* and *trpA* alleles in only 12.5% of the isolates (data not shown). Thus, the results indicate that the relative prevalence of ST131

among ESBL-producing *E. coli* isolates is 10.2%. Interestingly, in addition to ST131, MLST identified 2 and 3 isolates that belonged to the clonal groups ST393 and ST394, respectively, both belonging to phylogenetic group D.

DISCUSSION

The prevalence of CTX-M-type ESBLs among clinical isolates has increased substantially in the past several years (5). Despite this increase in CTX-M-mediated resistance, this is the first study in the United States to specifically determine the prevalence of CTX-M-type ESBLs and to characterize the molecular composition of these CTX-M-type enzymes, which were isolated from a pediatric patient population.

A total of 1,430 *Enterobacteriaceae* isolates collected from a pediatric patient population during a 1-year time period were evaluated for the production of ESBL enzymes. The results showed that 94 clinical isolates produced at least one ESBL enzyme, an overall prevalence of 7%; the majority (72%) of the ESBL enzymes were found in *E. coli* isolates. Importantly, CTX-M-type enzymes accounted for 74% of all ESBLs. These results are in contrast to the historic trend in the United States in which TEM- and SHV-type enzymes produced by *Klebsiella pneumoniae* had been the primary ESBLs associated with infections. These results do, however, reflect the global trend toward a pandemic spread of CTX-M-type ESBLs in various *Enterobacteriaceae*.

Molecular characterization of the 94 ESBL-positive isolates showed that 70 isolates produced an CTX-M-type ESBL. The *bla*_{CTX-M} gene was detected in various *Enterobacteriaceae*, including *E. coli*, *Klebsiella* species, *Enterobacter* species, *Morganella* species, and *Citrobacter* species; however, the significant majority of ESBLs were detected in *E. coli* isolates. Probe-specific real-time PCR and sequence analysis revealed that the majority (80%) of the CTX-M-type ESBLs were classified as CTX-M-15, the same enzyme that is associated with the global CTX-M pandemic; this is

the first study to demonstrate such a high prevalence of CTX-M-15 enzymes (60% of all ESBLs) among pediatric ESBL-producing isolates in the United States.

In the limited number of pediatric studies conducted across the globe, the trend has been toward an increased diversity of CTX-M determinants compared to what has been reported in adult centers. *E. coli* harboring CTX-M-15 and -14 have consistently been reported as the predominant ESBL types in clinical isolates from adult centers worldwide (14, 40, 41). Whereas the observation among pediatric clinical isolates worldwide has varied, with CTX-M-15 being predominant in Tanzania, Switzerland, and Tunisia (3, 19), CTX-M-3 was predominant at a children's hospital in Taiwan (39), and a wide diversity of CTX-M enzymes was observed in children from Bolivia and Peru (25). In the present study, CTX-M-15, CTX-M-14, and CTX-M-27 enzymes were identified, further highlighting the diversity of the CTX-M groups in pediatric populations. CTX-M-27 enzymes have been reported from farm animals in Asia but are rarely observed in human beings (4, 35, 36, 38). While a diverse mix of CTX-M enzymes was detected in our study, the majority of isolates produced CTX-M-15; thus, the globally prevalent CTX-M-15 enzyme may represent an increasing threat to pediatric populations in the United States as well.

This study also showed that most ESBL-producing *E. coli* isolates were of phylogroup B2, followed by phylogroups A, B1, and D. The presence of isolates of phylogroups A and B1, which have been reported as human commensal or animal strains (9, 16), indicates that animals are important reservoirs for some of the cephalosporin-resistant *E. coli* isolates observed in children. Additionally, in contrast to a previous report which showed that *bla*_{CTX-M-15}-containing isolates exclusively belonged to phylogroups B2 and D (9), our study showed that *bla*_{CTX-M-15}-containing isolates were also part of B2, D, and A phylogroups. Furthermore, *bla*_{CTX-M-14}-containing isolates in our study belonged to groups D and B2.

A particularly interesting result of this study was the finding that ST131 was prevalent among phylogroup B2 *bla*_{CTX-M-15}-positive isolates in children. ST131 was identified by MLST and DiversiLab Rep-PCR in 12.5% of the CTX-M-15-producing *E. coli* isolates, and the overall prevalence of ST131 among ESBL-producing *E. coli* isolates was 10.2%. Clonal outbreaks of CTX-M-15-producing *E. coli* corresponding to ST131 have already been reported in adult populations worldwide (17, 24, 28, 32). Given the rapid dissemination and virulence potential of ST131, our results suggest that the high prevalence of CTX-M-15-producing *E. coli* among children is due in part to clone ST131. Interestingly, our study also identified ST393 and ST394 by MLST analyses. Previous reports from Canada and Spain have identified CTX-M-14-producing ST393 isolates as causes of extraintestinal infections (2, 18). However, to our knowledge, CTX-M-15 strains harboring ST393 and ST394 and belonging to phylogroup D have not yet been described in children in the United States. Further molecular characterization of the resistance plasmids and evaluation of the clinical impact of these clones is warranted to obtain better insights into the molecular epidemiology of CTX-M-15 in pediatric *E. coli*.

In conclusion, this study demonstrates a much higher overall prevalence of ESBL-producing *Enterobacteriaceae* in pediatrics than has been previously reported in the United States, with CTX-M-type enzymes being the predominant ESBLs. Additionally,

many of these isolates were associated with community-acquired UTIs. The increasing prevalence of CTX-M-15-harboring *E. coli* ST131 strains in children as demonstrated by this study has important clinical and public health implications due to the risk of treatment failure. The emergence of a variety of CTX-M-positive *E. coli* isolates in pediatric populations poses a serious threat, as β -lactams are often the first line of therapy for UTIs and fluoroquinolones are not routinely used in these populations. The results of this study demonstrate a need for heightened awareness regarding the increasing frequency of these highly resistant isolates and their potential impact on both community- and hospital-acquired pediatric infections.

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