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Analysis of β-Lactamase Resistance Determinants in *Enterobacteriaceae* from Chicago Children: a Multicenter Survey

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Multidrug-resistant (MDR) *Enterobacteriaceae* infections are increasing in U.S. children; however, there is a paucity of multicentered analyses of antibiotic resistance genes responsible for MDR phenotypes among pediatric *Enterobacteriaceae* isolates. In this study, 225 isolates phenotypically identified as extended-spectrum β-lactamase (ESBL) or carbapenemase producers, recovered from children ages 0 to 18 years hospitalized between January 2011 and April 2015 at three Chicago area hospitals, were analyzed. We used DNA microarray platforms to detect ESBL, plasmid-mediated AmpC (pAmpC), and carbapenemase type β-lactamase (*bla*) genes. Repetitive-sequence-based PCR and multilocus sequence typing (MLST) were performed to assess isolate similarity. Plasmid replicon typing was conducted to classify plasmids. The median patient age was 4.2 years, 56% were female, and 44% presented in the outpatient setting. The majority (60.9%) of isolates were *Escherichia coli* and from urinary sources (69.8%). Of 225 isolates exhibiting ESBL- or carbapenemase-producing phenotypes, 90.7% contained a *bla* gene. The most common genotype was the $bla_{\text{CTX-M-1}}$ group (49.8%); 1.8% were carbapenem-resistant *Enterobacteriaceae* (three bla_{KPC} and one bla_{IMP}). Overall, pAmpC ($bla_{\text{ACT/MIR}}$ and bla_{CMY}) were present in 14.2%. The predominant *E. coli* phylogenetic group was the virulent B2 group (67.6%) associated with ST43/ST131 (Pasteur/Achtman MLST scheme) containing the $bla_{\text{CTX-M-1}}$ group (84%), and plasmid replicon types FIA, FII, and FIB. *K. pneumoniae* harboring bla_{KPC} were non-ST258 with replicon types I1 and A/C. *Enterobacter* spp. carrying $bla_{\text{ACT/MIR}}$ contained plasmid replicon FIIA. We found that β-lactam resistance in children is diverse and that certain resistance mechanisms differ from known circulating genotypes in adults in an endemic area. The potential impact of complex molecular types and the silent dissemination of MDR *Enterobacteriaceae* in a vulnerable population needs to be studied further.

The dissemination of antibiotic-resistant *Enterobacteriaceae* during the last 2 decades has been rapid, resulting in a pandemic of infections associated with significant morbidity and mortality (1–3). The major driving force of antibiotic resistance within this family of Gram-negative bacteria is the β-lactamases (2, 4). Currently, more than 1,600 known β-lactamases are cataloged; a list that continues to expand (5).

Genes encoding β-lactamases (*bla*) may be chromosomal in origin; however, much of the global spread of β-lactam resistance is facilitated by mobile genetic elements (such as plasmids and transposons) harboring *bla* genes encoding extended-spectrum β-lactamases (ESBL), AmpC cephalosporinases (AmpC), and carbapenemases (e.g., *Klebsiella pneumoniae* carbapenemase [KPC] and New Delhi MBL [NDM]) conferring carbapenem resistance in *Enterobacteriaceae* (CRE) (2, 4, 6, 7). Many of these β-lactamase-producing organisms carry additional plasmid-borne genes against other classes of antibiotics rendering them multidrug resistant (MDR) (4, 7), i.e., resistant to three or more classes of antibiotics (8), leaving few, if any, antibiotics to treat these infections (9).

Recent studies describe the prevalence of MDR *Enterobacteriaceae* as increasing in the United States, including in children (10–12). However, few studies report the genetic determinants associated with MDR *Enterobacteriaceae* in pediatric populations, and there is a paucity of multicenter studies defining the molecular epidemiology of these organisms. Knowledge of the molecular epidemiology of MDR *Enterobacteriaceae* can have a profound effect on clinical practice, infection control measures, and public health policies for children. In this study, we sought to determine whether children cared for at three distinct institutions located in the same geographic area would be subject to similar antibiotic resistance threats. Understanding the composition and distribution of antibiotic resistance genotypes is a critical step in defining the impact of MDR *Enterobacteriaceae* infections in children and future treatment decisions.

MATERIALS AND METHODS

Study settings and population. Hospital A is a tertiary care medical center that includes a children's hospital composed of 115 pediatric beds (level III neonatal, cardiac surgery, and pediatric intensive-care units and gen-

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eral pediatric and psychiatric wards) and a mother-newborn infant unit. Hospital B contains a 125-bed children's hospital (general pediatrics and newborn infant, neonatal, and pediatric intensive care units). Hospital C is a 288-bed, academic free-standing children's hospital providing complex quaternary pediatric care, including solid organ and stem cell transplantation services. All of the hospitals are located in the Chicago area.

This study included patients 0 to 18.99 years of age found to have a positive culture for an *Enterobacteriaceae* with an ESBL and/or carbapenem-resistant phenotype due to a carbapenemase. Isolates were collected between January 2011 and April 2015, and only one isolate per patient per admission was included. The study was approved by the institutional review boards of the three participating institutions. The institutions joined the study at various time points during the study period.

Bacterial isolates and antibiotic susceptibility testing. The clinical microbiology laboratories of hospitals A, B, and C performed phenotypic identification and susceptibility testing of ESBL- and carbapenemase-producing isolates at the respective institutions using the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Tarrytown, NY). Based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI), screening β-lactam antibiotics for ESBL-producing bacteria included any one of the following: cefpodoxime, cefotaxime, ceftazidime, ceftriaxone, or aztreonam (13). ESBL production was confirmed by disk diffusion as necessary (BBL; Becton, Dickinson and Company, Sparks, MD) or on the Microscan instrument by comparing MICs of cefotaxime and ceftazidime with or without the addition of clavulanic acid. A 4-fold reduction in MIC or an increase in zone diameter of >5 mm associated with cefotaxime or ceftazidime in combination with clavulanic acid compared to the MIC of the antibiotic when tested alone confirmed an ESBL phenotype (13).

Isolates were considered to have a carbapenemase phenotype by Centers for Disease Control and Prevention criteria, if they were resistant to all expanded-spectrum cephalosporins (ceftriaxone, cefotaxime, or ceftazidime) and nonsusceptible to at least one carbapenem (ertapenem, meropenem, imipenem, or doripenem) (14). The presence of carbapenemases was phenotypically assessed using the Modified Hodge Test and/or the MBL Etest (bioMérieux, Athens, GA), as appropriate.

Analysis of *bla* genes. Genomic DNA was purified from bacterial isolates by using a DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA). A DNA microarray-based assay was performed to evaluate for the presence of *bla* genes in isolates (Check-MDR CT101; Check-Points, Wageningen, The Netherlands). The assay detects $bla_{\text{CTX-M-1}}$ group, $bla_{\text{CTX-M-2}}$ group, $bla_{\text{CTX-M-8 plus -25}}$ group, $bla_{\text{CTX-M-9}}$ group, and $bla_{\text{TEM-WT}}$ (wild type) and $bla_{\text{TEM-type}}$ ESBL genes, $bla_{\text{SHV-WT}}$ (wild type) and $bla_{\text{SHV-type}}$ ESBL genes, plasmid-based AmpC cephalosporinases (pAmpC; bla_{ACC}, $bla_{\text{ACT/MIR}}$, $bla_{\text{CMY II}}$, bla_{DHA}, and bla_{FOX}), and carbapenemases (bla_{KPC} and bla_{NDM}) (15). A more comprehensive DNA microarray (Check-MDR CT103XL; Check-Points) was performed on isolates identified as *bla* negative by the Check-MDR CT101 kit. The Check-MDR CT103XL kit includes additional ESBL (bla_{VEB}, bla_{PER}, bla_{BEL}, and bla_{GES}) and carbapenemase (bla_{VIM}, bla_{IMP}, bla_{GES}, bla_{GIM}, bla_{SPM}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-48}}$, and $bla_{\text{OXA-58}}$) targets (16). Experiments were performed according to the manufacturer's protocol.

Rep-PCR. To assess for clonal relatedness among strains of *E. coli*, *Klebsiella* spp., and *Enterobacter* spp., repetitive-sequenced-based PCR (rep-PCR) was performed using DiversiLab (bioMérieux, Athens, GA) *E. coli*, *Klebsiella*, and *Enterobacter* fingerprinting kits. Genomic DNA was extracted using an UltraClean microbial DNA isolation kit (MO BIO Laboratories, Carlsbad, CA), followed by PCR amplification and separation of rep-PCR amplicons by electrophoresis on microfluidic chips using the DiversiLab manufacturer's protocol for detection (Agilent Bioanalyzer 2100; Agilent Technologies, Inc., Santa Clara, CA) and analysis (DiversiLab online software). Isolates in which band patterns demonstrated >95% similarity (Pearson's correlation) were considered to represent the same strain type (17). Among isolates analyzed by rep-PCR, PCR amplification and sequencing were also performed to further characterize *bla* genes as revealed by microarray testing.

TABLE 1 Demographic and healthcare setting of Chicago children infected with MDR *Enterobacteriaceae*[a]

Variable	No. (%) of children (n = 225)
Median age in yrs (range)	4.2 (0.008–18.9)
Male	98 (44)
Race	
Hispanic	80 (36)
White	71 (32)
Black	34 (15)
Age <1 years	60 (27)
Healthcare setting	
PICU	59 (26)
NICU	15 (7)
Pediatric ward	52 (23)
Outpatient	99 (44)

[a] MDR, multidrug resistant, i.e., resistant to three or more antibiotic classes. "Outpatient" includes ambulatory healthcare and the emergency department. PICU, pediatric intensive care unit; NICU, neonatal intensive care unit. All values represent "number (%)" unless indicated otherwise in column 1.

MLST and *hsp60* sequencing. Gene amplification and sequencing of seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *tonB*, and *infB*) for *Klebsiella* spp. and eight housekeeping genes (*dinB*, *icdA*, *pabB*, *polB*, *putP*, *trpA*, *trpB*, and *uidA*) for *E. coli* were performed as previously described (18, 19), and allele and sequence types (STs) were determined by using the multilocus sequence typing (MLST) Pasteur website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). We used *hsp60* sequencing, which targets a single housekeeping gene, to further determine relatedness among *Enterobacter* spp. (20).

Phylogenetic analysis and plasmid replicon typing. A previously described multiplex PCR-based method was used to assign *E. coli* to one of the four major phylogenetic groups (A, B1, B2, and D) (21). From rep-PCR strain types, plasmid replicon typing was performed on representative isolates according to the scheme described by Carattoli et al. (22).

RESULTS

Characteristics of pediatric patients in the study population. *Enterobacteriaceae* strains phenotypically identified as ESBL- or carbapenemase-producing bacteria were recovered from 225 children during the study period. The median age was 4.2 years (range, 0.008 to 18.9 years), and 27% were younger than 1 year of age (Table 1). In this study, the majority (56%) were female, 36% were Hispanic, and 44% of the children presented in the outpatient setting.

Characteristics of bacterial isolates in the study population. Of the 225 isolates, the majority (60.9%) were *E. coli*, followed by 16.4% *Klebsiella* spp., 13.3% *Enterobacter* spp., 4.9% *Proteus* spp., 3.6% *Serratia* spp., and 0.9% other (including *Morganella* and *Citrobacter* spp.). The most common specimen source was urine (69.8%); 4.9% were from blood, 16.9% were from respiratory sources (sputum, tracheal aspirate and bronchoalveolar lavage), 2.7% were from wounds or abscesses, 2.2% were from peritoneal or abdominal sources, 0.4% were from the central nervous system, and 3.1% were from other sources.

Antimicrobial susceptibility testing. The antibiotic susceptibility testing of the 222 available isolates are summarized in Table 2. Overall, carbapenems and amikacin retained the greatest activity, with 98.2% of isolates susceptible to meropenem or imipenem and 97.1% susceptible to amikacin; 82.2% of urinary isolates were

TABLE 2 Anatomical sites of isolation and antibiotic susceptibility patterns of ESBL- and carbapenemase-producing *Enterobacteriaceae* isolates from children

Anatomical site[a]	No. of isolates[b]	% susceptible[c]											
		CTX	CAZ	FEP	P/T	CPM[d]	GNT	TOB	AMK	FQ	TMP-SMX	TET	NIT
All	222	2.5	3.7	4.5	79.1	98.2	56.2	45.0	97.1	50.8	60.4	42.6	81.8
Urine	155	2.8	4.5	3.8	86.7	98.1	54.3	44.8	97.4	43.1	37.5	43.5	82.2
Respiratory	38	2.6	2.6	2.8	64.3	97.3	44.7	45.9	97.4	73.7	56.8	60.0	0.0
Blood	10	0.0	0.0	0.0	33.3	100	55.6	55.6	100	55.6	33.3	0.0	0.0
Wound	7	0.0	0.0	28.6	100	100	57.1	28.6	100	28.6	57.1	0.0	ND
Other	7	0.0	0.0	0.0	69.0	100	71.4	57.1	100	57.1	28.6	50.0	ND
Abd/Perit	4	0.0	0.0	0.0	100	50.0	0.0	50.0	100	100	25.0	ND	ND
CNS	1	0.0	0.0	0.0	ND	100	100	100	100	0.0	100	ND	ND

[a] "Respiratory" includes bronchoalveolar lavage fluid, trachea, and sputum cultures. "Wound" includes abscess and wound cultures. "Abd/Perit" includes intra-abdominal and peritoneal fluid cultures. CNS, central nervous system cultures.
[b] That is, the number of isolates with antibiotic susceptibility data available.
[c] CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; P/T, piperacillin/tazobactam; CPM, carbapenems (includes imipenem, meropenem, and ertapenem); GNT, gentamicin; TOB, tobramycin; AMK, amikacin; FQ, fluoroquinolones (includes ciprofloxacin and levofloxacin); TMP-SMX, trimethoprim-sulfamethoxazole; TET, tetracycline; NIT, nitrofurantoin. ND, no data.
[d] Carbapenem resistance was due exclusively to the CPE isolates.

susceptible to nitrofurantoin. Isolates had relatively high rates of resistance (~40 to 60%) to gentamicin, tobramycin, trimethoprim-sulfamethoxazole, tetracycline, and fluoroquinolones (ciprofloxacin and levofloxacin, see Table 2).

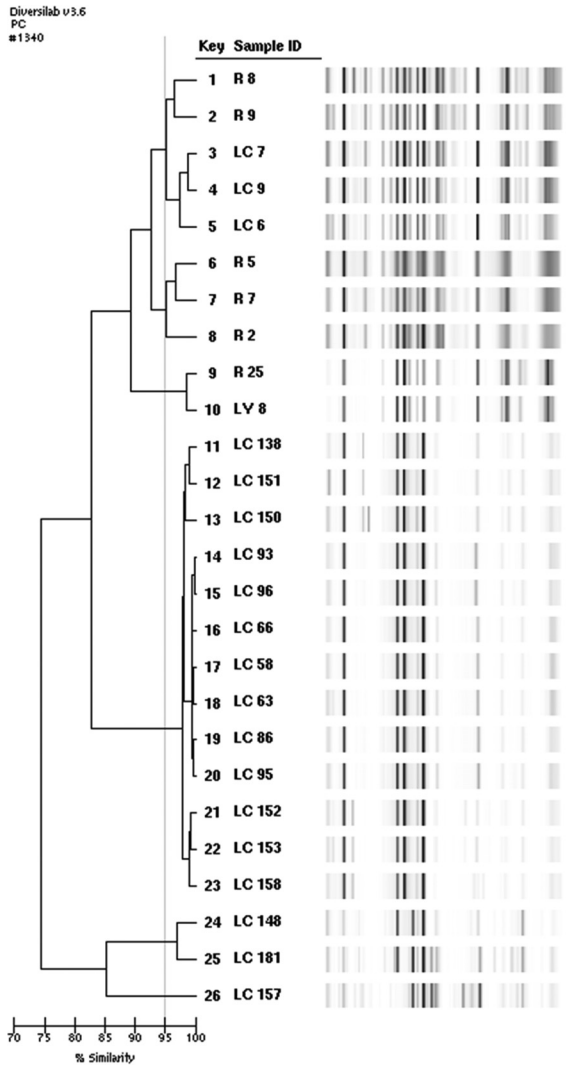
Composition of *bla* genes in *Enterobacteriaceae* isolates. Table 3 summarizes the *bla* genes detected by DNA microarray testing. Molecular characterization revealed that 90.7% of isolates contained an ESBL, AmpC, or carbapenemase gene, and in some isolates more than one *bla* was found (238 *bla* genes in 225 isolates). CTX-M-type ESBLs were the most common *bla* genes detected; they were found in 152 of 225 *Enterobacteriaceae* isolates (67.6%). Approximately half (49.8%) belonged to the $bla_{\text{CTX-M-1}}$ group, which contains $bla_{\text{CTX-M-15}}$, the gene most frequently associated with pandemic CTX-M *E. coli* strains (1, 23). Additionally, $bla_{\text{TEM-}}$ and $bla_{\text{SHV-type}}$ ESBL genes were found in 5.3 and 16.4% of isolates, respectively, and only 0.4% isolates contained $bla_{\text{PER-type}}$ ESBL genes.

bla_{AmpC} cephalosporinase genes comprised the resistance determinants detected in 32/225 (14.2%) of isolates, of which 7 (3.1%) were $bla_{\text{CMY-type}}$ genes and 25 (11.1%) were $bla_{\text{ACT/MIR-type}}$ AmpC genes. bla_{CMY} genes were predominantly identified in *E. coli* (6 of 7), whereas the majority of 25 ACT/MIR genes were recovered from *Enterobacter* spp. (84%), with 16% in *E. coli* isolates. $bla_{\text{ACT/MIR}}$ in *Enterobacter* spp. were often associated with $bla_{\text{SHV-type}}$ ESBL genes, 12/21 (57%), and coexistence of ESBL and AmpC genes were found in 21 of 32 (65.6%) *Enterobacteriaceae* isolates. bla_{AmpC} genes were not detected in *Proteus* or *Serratia* spp.; however, a $bla_{\text{CMY-like}}$ gene was found in one *Citrobacter freundii* isolate, which represents an intrinsic chromosomal bla_{AmpC} gene specific to *Citrobacter* spp., based on DNA sequence

TABLE 3 *bla* genes detected in the collection of *Enterobacteriaceae* isolates from Chicago children

bla gene (no.) in *Enterobacteriaceae* samples ($n = 225$)[a]	% detection of *bla* genes in isolates (no. of isolates) by organism[b]						
	All (225)	*E. coli* (137)	*Klebsiella* spp. (37)	*Enterobacter* spp. (30)	*Proteus* spp. (11)	*Serratia* spp. (8)	Other (2)
Genes encoding ESBL (202)							
CTX-M-1 group (112)	49.8	81.3	10.7	3.6	4.5	0.0	0.0
CTX-M-9 group (36)	16.0	83.3	11.1	2.8	2.8	0.0	0.0
CTX-M-2 group (4)	1.8	25.0	25.0	0.0	50.0	0.0	0.0
TEM (12)	5.3	66.7	8.3	8.3	16.7	0.0	0.0
SHV (37)	16.4	16.2	29.7	40.5	0.0	13.5	0.0
PER (1)	0.4	100	0.0	0.0	0.0	0.0	0.0
VEB (0)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Genes encoding AmpC (32)							
ACT/MIR (25)	11.1	16.0	0.0	84.0	0.0	0.0	0.0
CMY (7)	3.1	85.7	0.0	0.0	0.0	0.0	14.3[c]
Genes encoding carbapenemases (4)							
KPC (3)	1.3	25.0	75.0	0.0	0.0	0.0	0.0
IMP (1)	0.4	0.0	100	0.0	0.0	0.0	0.0
Total *bla* genes (238)							

[a] *bla*, β-lactamase gene, ESBL, extended-spectrum β-lactamases; AmpC, AmpC cephalosporinases; KPC, *Klebsiella pneumoniae* carbapenemase; IMP, active on imipenem.
[b] Isolates may contain more than one *bla* gene; 9.3% ($n = 21$) of the isolates were *bla* gene negative. Wild-type, narrow-spectrum *bla* genes were not included in the totals.
[c] This value represents an intrinsic chromosomal bla_{AmpC} gene specific to *Citrobacter* spp., which was picked up by the DNA microarray (Check-Points) as a $bla_{\text{CMY-II-like}}$ gene.



Phylogroup	ESBL gene	MLST
B2	CTX-M-1 group	ST43
B2	CTX-M-9 group	ST43
B2	Negative	ST43
B2	CTX-M-1 group	ST43
B2	Negative	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-9 group	ST506
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-9 group	ST506
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-1 group	ST43
B2	CTX-M-9 group	ST43
B2	CTX-M-1 group	ST4
B2	Negative	ST4
B2	TEM 238S	ST455

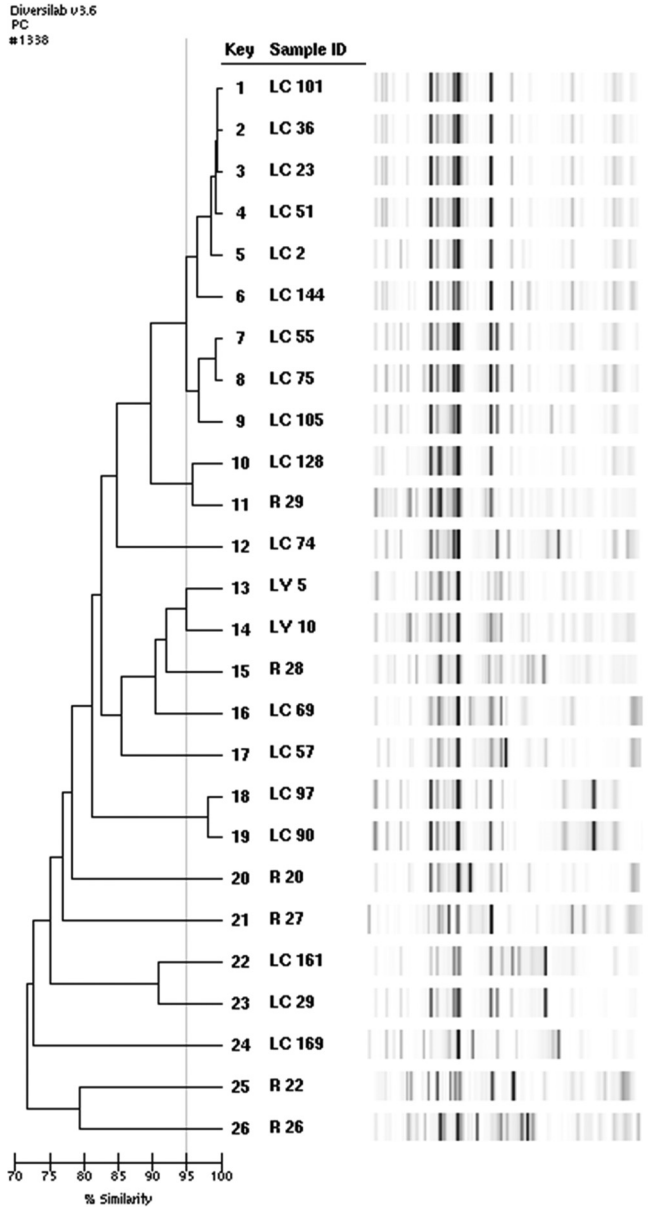
FIG 1 Genetic relatedness in representative ESBL *E. coli* isolates from Chicago children. Isolates in which band patterns demonstrated >95% similarity (Pearson's correlation) were considered to be clonal and of the same strain type. Phylogroup, phylogenetic group; ESBL, extended-spectrum β-lactamase; MLST, multilocus sequence type, Pasteur scheme. ST43 is equivalent to ST131 on the Achtman's scheme. MLST was performed on select isolates from rep-PCR strain types. The CTX-M-1 group of note contains CTX-M-15, associated with the pandemic CTX-M type ESBL in *E. coli*. The CTX-M-9 group of note contains CTX-M-9 and CTX-M-14, the second most common circulating CTX-M type ESBL. LC 157 is a novel ST type.

analysis. Carbapenemases were detected in four isolates (1.8%), three of which were identified as bla_{KPC} and one *K. pneumoniae* contained a bla_{IMP} metallo-β-lactamase gene. The bla_{KPC} genes were carried by two *K. pneumoniae* and one *E. coli* strains, and all were identified by DNA sequencing to be bla_{KPC-2}. Other ESBL, pAmpC, or carbapenemase *bla* genes were not detected in carbapenemase-containing isolates.

Rep-PCR, MLST, *bla* and *hsp60* gene sequencing, and plasmid replicon typing. Rep-PCR was performed on all *E. coli*, *Klebsiella* spp. and *Enterobacter* isolates. We identified 40 different strain types of *E. coli* by rep-PCR. A dendrogram of the most highly represented rep-PCR profiles is shown in Fig. 1, representing 32.5% of the isolates. These corresponded to phylogroup B2. A subset of isolates with rep-PCR profiles related to one or more other strains were further studied by MLST, *bla* gene sequencing and replicon typing, and the majority were found to correspond to ST43 (ST131 in Achtman's MLST scheme), carry $bla_{CTX-M-15}$, and contain plasmids of incompatibility types of replicon FIA, FII, and FIB. The *E. coli* strain harboring a bla_{KPC-2} gene belonged to phylogroup D, was identified as a novel sequence type (ST701), and had a distinct fingerprint pattern.

With respect to *K. pneumoniae* isolates, the rep-PCR profiles were mainly unrelated (see Fig. S1 in the supplemental material). Plasmid replicon typing revealed that the bla_{KPC-2}-producing *K. pneumoniae* isolates contained plasmid replicon types I1 and A/C, and MLST showed that neither belonged to the ST258 lineage (ST22 and ST29). The *K. pneumoniae* containing the bla_{IMP} gene was identified in ST253. Genetic relatedness in *Enterobacter* strains by rep-PCR (Fig. 2) correlated well with the *hsp*60 sequencing results and have been presented previously (24).

Phylogenetic grouping of *E. coli*. Phylogenetic groups of *E. coli* include four main groups (A, B1, B2, and D), and groups B2 and D are most often associated with severe clinical disease attributed to increased virulence factors (21). Of 136 *E. coli* isolates



ESBL gene	AmpC gene
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
-	ACT/MIR
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
SHV 238S+240K	-
SHV 238S+240K	ACT/MIR
SHV 238S+240K	ACT/MIR
CTX-M-1 group	-
CTX-M-1 group	-
-	-
-	ACT/MIR
-	ACT/MIR
-	ACT/MIR
SHV 238S+240K	-
SHV 238S+240K	-
-	ACT/MIR
-	ACT/MIR
SHV 238S+240K	ACT/MIR
CTX-M-9 group	-
CTX-M-1 group	-
TEM 164S	-
SHV 238S+240K	ACT/MIR

FIG 2 Genetic relatedness of β-lactamase-carrying *Enterobacter* isolates from Chicago children. A dash indicates no β-lactamase gene was detected. Isolates in which band patterns demonstrated >95% similarity (Pearson's correlation) were considered clonal and of the same strain type. ESBL, extended-spectrum β-lactamase. AmpC, AmpC cephalosporinase; MLST, multilocus sequence type, Pasteur scheme. The term "ACT/MIR" means the gene may be an ACT- or MIR-type AmpC cephalosporinase gene but was not further differentiated by DNA microarray (Check-Points).

tested, 119 (87.5%) were phylogenetic group B2 or D, with 67.6% and 19.1% belonging to B2 and D, respectively. One result was indeterminate (B2/D). Of all the *E. coli* strains, 78/136 (57.4%) were associated with $bla_{\text{CTX-M-1}}$ group and 19.1% were associated with $bla_{\text{CTX-M-9}}$ group. Most (65/78, 83.3%) belonged to the B2-*E. coli* $bla_{\text{CTX-M-1}}$ group. Only 14 (10.3%) and 3 (2.2%) of the strains were of phylogroups A and B1, respectively.

DISCUSSION

In this unique survey, we linked resistance phenotypes with the genetic determinants of antibiotic resistance in *Enterobacteriaceae* isolates from children. The children from which these isolates were recovered were from three different centers located in the same city. As a result, we have an important "snapshot" on the molecular epidemiology of this emerging problem. A recent national study of trends of ESBL-producing *Enterobacteriaceae* in children using antimicrobial susceptibility data from 300 U.S. laboratories reported that the prevalence of the ESBL phenotype in *Enterobacteriaceae* isolated from children more than tripled during the study period, from 0.28% in 1999 to 0.92% in 2011, with the largest increases occurring in young children ages 1 to 5 years and in the intensive care unit setting (although the increase was seen in all age groups and health care settings) (11). This is consistent with our patient demographics. We also found that the most common circulating strain in children was phylogenetic group B2, multilocus sequence type 43 (ST43) *E. coli* harboring

$bla_{CTX\text{-}M\text{-}1}$ group ESBLs, which contain $bla_{CTX\text{-}M\text{-}15}$, the predominant ESBL type disseminating globally. The pandemic, MDR, CTX-M-producing *E. coli* strains are of a large clonal lineage possessing the FimH30 allele (of the type 1 fimbriae *fimH* adhesin gene) and belong to the virulent phylogenetic group B2 (associated with extraintestinal pathogenic *E. coli*) and ST43 of the Pasteur MLST scheme, which is specific to the H30 subclone of the ST131 of the Achtman's MLST scheme (25).

The *E. coli* ST43/ST131 CTX-M strains are diverse due to a broad range of plasmids carrying a variety of resistance determinants; however, these strains commonly carry genes associated with resistance to fluoroquinolones, concomitant resistance to aminoglycosides, and trimethoprim-sulfamethoxazole (TMP-SMX) (26). In the United States, *E. coli* ST43 and ST131 are discovered more commonly among health care-associated strains; however, there are increasing reports of community acquisition globally. These isolates are associated with serious infections, especially of the urinary tract and bloodstream, and have significant attributable morbidity and mortality (1, 23). In our pediatric population, we found that of 136 *E. coli* isolates, 68% were phylogenetic group B2, and 84% of those strains harbored $bla_{CTX\text{-}M\text{-}1}$ group ESBL genes, an observation consistent with adult and Chicago area data (25–27). We also found significant coresistance with 51, 56, and 60% of isolates displaying fluoroquinolone, aminoglycoside, and TMP-SMX resistance, respectively.

The rise of ESBL-producing *Enterobacteriaceae* in the pediatric community is important for many reasons. There are few drugs available and approved to treat infections with these organisms in children (9, 23), and children who become colonized with MDR *Enterobacteriaceae* may be colonized for prolonged periods, even up to 4 years, thus potentially serving as ongoing, silent sources of spread (23, 28, 29). Although the predominance of $bla_{CTX\text{-}M\text{-}type}$ ESBL genes in *E. coli* in our population was consistent with data from adults, the genotypic information from other studied genera was different. Our study was performed in a region that is endemic for KPC-producing *Enterobacteriaceae* strains, but we did not find evidence of the ST258 *K. pneumoniae* which harbors the bla_{KPC} gene ($bla_{KPC\text{-}2}$ or $bla_{KPC\text{-}3}$) in our patient population (30). The bla_{KPC}-containing *K. pneumoniae* isolates that we identified were ST22 and ST29 and carried $bla_{KPC\text{-}2}$. Our findings are consistent with single-center pediatric studies suggesting that U.S. children with carbapenemase-producing *Enterobacteriaceae* may be more commonly infected with endemic strain types circulating within their institution or region, which may differ from adult studies where long-term-care facilities have been shown to be a significant reservoir of KPC-containing *K. pneumoniae* affecting acute care hospitals via interfacility transfer (10, 29, 31). Furthermore, recent national phenotypic data suggest that carbapenem-resistant *Enterobacteriaceae* infections are increasing in U.S. children and that the genetic makeup of these carbapenem-resistant pathogens likely differs from adults (12).

Our finding of a bla_{IMP} metallo-β-lactamase (MBL)-producing *K. pneumoniae* in our population likely represents a "sentinel event," and cases of MBLs in U.S. children have only recently been described, including infections associated with New Delhi MBL (NDM) (32). Knowing the molecular mechanisms of β-lactam resistance in *Enterobacteriaceae* is extremely valuable since it may impact treatment decisions and infection control procedures such as patient isolation, cohorting, and environmental cleaning methodologies. The rapid global dissemination of carbapenemase genes, such as the bla_{KPC}, bla_{NDM}, bla_{VIM}, and bla_{IMP} MBL genes, as well as the $bla_{CTX\text{-}M}$ ESBL genes, is due to the successful integration and transfer of mobile genetic elements in Gram-negative bacteria. Local and regional surveillance identifying patients carrying these organisms can help prevent intra- and interfacility spread (33).

Our AmpC cephalosporinase data are unique in that the most common transmissible bla_{AmpC} genes thought to be circulating in U.S. children and adults are the $bla_{CMY\text{-}2}$ genes, which are most often found in *E. coli* (23, 34); however, in our isolates, the most common bla_{AmpC} genes identified were $bla_{ACT/MIR\text{-}type}$ genes in *Enterobacter* isolates (84%), which were associated with $bla_{SHV\text{-}type}$ ESBL genes in 40.5% of cases. Typing of a subset of *Enterobacter* isolates revealed $bla_{ACT\text{-}16}$ and $bla_{ACT\text{-}17}$ genes and plasmid replicon type FIIA. Our data suggest that for *Enterobacter*, the dissemination of ESBLs may be related to specific mobile genetic elements, i.e., "promiscuous plasmids" carrying *bla* genes, rather than predominant circulating clonal types (Fig. 2). Of note, 6% of isolates in this study were found to contain only bla_{AmpC} genes; however, all were phenotypically identified as ESBL producers. We emphasize this as the treatment of ESBL and AmpC producers is different. For example, one might consider cefepime therapy in the treatment of infections due to AmpC-positive isolates, whereas for ESBL producers, cefepime is discouraged, and many institutions place patients with ESBL-producing *Enterobacteriaceae* infections under isolation precautions. Further detailed characterization of these strains is necessary to delineate whether "silent dissemination" of plasmid-mediated AmpC is occurring in the pediatric population at a greater frequency than previously recognized (28, 35, 36).

We recognize the limitations of our study. The design is a retrospective cohort study of resistance mechanisms in *Enterobacteriaceae* recovered from children located at three centers in a single geographic region, which may impact generalizability to other regions. Isolates were collected on the basis of phenotypic resistance suggestive of ESBL and carbapenemase production; therefore, no antibiotic-sensitive strains were collected. In addition, whereas a plasmid-based location of the *bla* genes by the majority of isolates is supported by DNA sequence analysis, some of these genes may represent chromosome-based mechanisms of resistance. Further studies are ongoing to assess plasmid localization of the determinants uncovered during the study. We did perform DNA sequence analysis for a subset of isolates and plasmid replicon typing in order to further strengthen our DNA microarray results.

In conclusion, our study represents the first multicentered U.S. study of the molecular epidemiology of ESBL-, AmpC-, and carbapenemase-producing *Enterobacteriaceae* isolates from children cared for at pediatric acute care facilities within a single metropolitan area. We found that the characterization of plasmid-mediated β-lactam resistance in children is complex and diverse and that the molecular characteristics in pediatric isolates exhibit differences compared to strain types circulating in adults in an area where such infections are endemic. This diversity and complexity must be further studied to assess the potential impact of various molecular types in pediatric infections and the imminent threat of silent dissemination of these dangerous bacteria within a vulnerable population. Our study also highlights the unique challenges that will be faced when developing strategies to control the spread of MDR organisms in children: having novel strain types as carri-

ers of carbapenemase genes portends an even more complex molecular epidemiology.

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