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ORIGINAL RESEARCH

The Clinical and Molecular Epidemiology of CTX-M-9 Group Producing Enterobacteriaceae Infections in Children

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ABSTRACT

Introduction: The pandemic of extended-spectrum beta-lactamase-(ESBL)-producing Enterobacteriaceae (Ent) is strongly linked to the dissemination of CTX-M-type-ESBL-Ent. We sought to define the epidemiology of infections in children due to an emerging resistance type, CTX-M-9-group-producing-Ent (CTX-M-9-grp-Ent).

Methods: A retrospective matched case-control analysis of children with CTX-M-9-grp-Ent infections who received medical care at three Chicago area hospitals was performed. Cases were defined as children possessing extended-spectrum cephalosporin-resistant (ESC-R) infections due to $bla_{\text{CTX-M-9}}$. PCR and DNA analysis assessed beta-lactamase (*bla*) genes, multi-locus sequence types (MLST) and phylogenetic grouping of *E. coli*. Controls were children with ESC-susceptible (ESC-S)-Ent infections matched one case to three controls

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by age, source, and hospital. The clinical-epidemiologic predictors of CTX-M-9-grp-Ent infection were assessed.

Results: Of 356 ESC-R-Ent isolates from children (median age 4.1 years), the CTX-M-9-group was the solely detected *bla* gene in 44 (12.4%). The predominant species was *E. coli* (91%) of virulent phylogroups D (60%) and B2 (40%). MLST revealed multiple strain types. On multivariable analysis, CTX-M-9-grp-Ent occurred more often in *E. coli* than other Ent genera (OR 7.4, 95% CI 2.4, 27.2), children of non-Black-White-Hispanic race (OR 7.4, 95% CI 2.4, 28.2), and outpatients (OR 4.5, 95% CI 1.7, 12.3), which was a very unexpected finding for infections due to antibiotic-resistant bacteria. Residents of South Chicago had a 6.7 times higher odds of having CTX-M-9-grp-Ent infections than those in the reference region (West), while residence in Northwestern Chicago was associated with an 81% decreased odds of infection. Other demographic, comorbidity, invasive-device, and antibiotic use differences were not found.

Conclusion: CTX-M-9-grp-Ent infection may be associated with patient residence and is occurring in children without traditional in-patient exposure risk factors. This suggests that among children, the community environment may be a key contributor in the spread of these resistant pathogens.

Keywords: Children; Drug resistance; *Enterobacteriaceae* infections; Epidemiology; Gram-negative bacteria

INTRODUCTION

The pandemic of multi-drug-resistant (MDR) Enterobacteriaceae remains one of the most significant public health threats of our time [1, 2]. MDR Enterobacteriaceae are associated with significant morbidity and mortality in infected individuals, and an increasing number of reports describe extra-intestinal and invasive infections with these organisms in children [3–5]. Many studies show that beta-lactamase (*bla*) genes harbored by Enterobacteriaceae, e.g., extended-spectrum beta-lactamases (ESBLs or bla_{ESBLs}) and carbapenemases (e.g., bla_{KPCs}), are the major contributors to this growing problem [6, 7]. Strains carrying *bla* genes often harbor additional antibiotic resistance determinants residing on mobile genetic elements, e.g., plasmids and transposons, which are capable of rapid dissemination [1, 7].

In the US and worldwide, the CTX-M-type ESBL-producing Enterobacteriaceae (CTX-M Ent) are the predominant ESBL-producing strains, with the clonal multi-locus sequence type (ST) 131 *E. coli* harboring CTX-M-15 (CTX-M-1 group) being the most commonly reported in both adult and pediatric studies [7, 8]. However, several other CTX-M types continue to circulate in Ent and are equally concerning, highly resistant pathogens that can be acquired in the community [9].

A notable example is the widespread CTX-M-9-group ESBL genes (e.g., $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-27}}$), which are the most common CTX-M genes circulating in Ent in some geographic regions [10]. A 2016 US study of the prevalence of CTX-M genes in Enterobacteriaceae found relative stability of $bla_{\text{CTX-M-15}}$ in *E. coli*. Nevertheless, an increase in the prevalence of $bla_{\text{CTX-M-14}}$ in *bla*-producing *E. coli* occurred (20.4% in 2014) compared with 2012 (16.0%) [11]. Despite this, much less is reported concerning

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the epidemiology of CTX-M-9-group infections. Unlike the clonal ST131 *E. coli* strains, CTX-M-9-group genes are associated with multiple strain types, and horizontal gene transfer via mobile genetic elements likely plays an important role in their dissemination [12]. While studies of ESBL-Ent in US children and internationally have reported infections due to CTX-M-9-group-producing Enterobacteriaceae (CTX-M-9-grp-Ent) [10, 13], pediatric studies have not focused on the clinical and molecular epidemiology and impact of infections specifically due to CTX-M-9-grp-Ent.

In previous investigations, the genetic basis for beta-lactam resistance in Enterobacteriaceae isolates recovered from children cared for by multiple centers in the Chicago area was determined [13]. Subsequently, subgroups of children with infections due to similar resistance determinants, (e.g., plasmid-mediated fluoroquinolone, ESBL, and carbapenem resistance genes) were analyzed to determine genotypes, host factors, and exposures associated with specific MDR Enterobacteriaceae strains [13, 14]. An analysis of children with plasmid-mediated fluoroquinolone-resistant (PMFQR)-Ent infections revealed that there are genetic and geospatial community links to MDR in our Chicago pediatric population [15]. Here, we report that CTX-M-9-grp-Ent infections in children are similarly linked to geographic location and that acquisition of CTX-M-9-grp-Ent in children demonstrates environmental influences and originates in the community.

METHODS

Study Setting

Hospital A contains a 115-bed children's hospital, which includes pediatric and psychiatric wards; a mother-newborn infant unit; and cardiac, neonatal, and pediatric intensive-care units (CICU, NICU, and PICU), located within a tertiary care academic medical center. Hospital B is a free-standing children's academic medical center comprised of 288 beds and provides quaternary care services, including bone marrow and organ transplantation. Hospital C is a 125-bed children's hospital within an academic medical center, has newborn infant and general pediatrics wards, and a NICU and PICU. The participating centers are all located within metropolitan Chicago.

Descriptive Study Design

Study Population

Patients included in this study were children < 21 years of age who were identified because they possessed extended-spectrum cephalosporin (ceftriaxone, ceftazidime, cefotaxime)-resistant (ESC-R) Enterobacteriaceae growing in clinical cultures and were suspected to harbor a transmissible *bla* gene conferring cephalosporin resistance. Infections diagnosed between 1 January 2011 and 31 December 2016 were included in the analysis; only the first infection per patient was included. Study approval was obtained from the institutional review boards of the participating institutions, and the need for informed consent was waived. All procedures followed were in accordance with institutional and national ethical standards, and the study was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments.

Antibiotic Susceptibility Testing in Enterobacteriaceae Isolates

The clinical microbiology laboratories of Hospitals A–C phenotypically analyzed ESC-R isolates by the Vitek 2 microbial identification system (bioMérieux, Athens, GA) or via the MicroScan WalkAway system (Beckman Coulter, Brea, CA). Following Clinical and Laboratory Standards Institute (CLSI) guidelines, one or more of the following antimicrobials, aztreonam, ceftriaxone, ceftazidime, cefotaxime, or cefpodoxime, were used to screen for ESBL production [16]. Techniques used to confirm ESBL production included automated instruments or disk diffusion assays (BBL; Becton, Dickinson and Co., Sparks, MD) to measure cefotaxime and ceftazidime susceptibility in the presence and absence of clavulanic acid. An increase in measure of a disk zone diameter of > 5 mm or a fourfold reduction in the minimum inhibitory concentration (MIC) of

cefotaxime and ceftazidime in the presence of clavulanic acid served as confirmation of the ESBL phenotype [16].

Molecular Analysis

Beta-Lactam Resistance Determinants

Genomic DNA from Enterobacteriaceae isolates was purified from isolates confirmed with an ESBL phenotype (DNeasy blood and tissue kit, Qiagen, Inc. Valencia, CA). DNA Microarray (Check-MDR CT101 and CT103XL; Check-Points, Wageningen, The Netherlands) and polymerase chain reaction (PCR) were performed to assess and confirm the presence of *bla* genes in isolates as previously described and according to manufacturer protocol [13, 17]. Isolates found to be positive solely for beta-lactamase genes belonging to CTX-M-9-grp were included in the analysis.

Nomenclature and Characterization

A well-established multiplex PCR-based method was used to assign *E. coli* to one of four phylogenetic groups (A, B1, B2, and D) [18]. Multi-locus sequence typing (MLST) [Pasteur website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/)] and DNA sequencing identified sequence types and PCR distinguished $bla_{\text{CTX-M-9-grp}}$ alleles in the ESBL-producing strains of *E. coli* and *Klebsiella* species as previously described [13, 19, 20].

Analytic Study Design

A retrospective case-control study design was used to assess factors associated with infection due to isolates in which we had detected a $bla_{\text{CTX-M-9-grp}}$ gene. Children serving as control subjects were identified using hospital electronic laboratory records (ELRs) and were matched 3:1 to the cases by age range, hospital, and specimen source, a design previously described [15, 21]. Only children diagnosed with clinical infections due to Enterobacteriaceae susceptible to extended-spectrum cephalosporins (ESC-S) were included, as determined by study investigator case review and/or using standard criteria defined by the CDC National Healthcare Safety Network [22].

Covariates

We analyzed several variables as potential factors associated with CTX-M-9-grp-Ent infection based on known associations for ESBL-producing Enterobacteriaceae acquisition in adults and children, including (1) demographics (age, gender, race/ethnicity); (2) comorbid conditions (as defined by ICD-9/ICD-10 codes); (3) recent inpatient and outpatient healthcare exposures, including hospitalization and/or procedures in the previous 30 days; (4) antibiotic exposures in the 40 days prior to culture [21]; (5) presence, number, and type of invasive medical devices; and (6) patient residence in the Chicago area as assessed by dividing the metropolitan area into seven regions using zip code level data, which included Chicago proper and its suburban areas (i.e., North Side and North suburbs, South Side and South suburbs, etc.). An eighth region was included for patients residing in other states or other parts of Illinois.

Statistical Analysis

To adjust for confounding at the design stage, one case with CTX-M-9 infection was matched to three controls with antibiotic-sensitive infections by the demographic variables of interval age range (0–1, 1–5, 6–12, > 13 years of age), infection source, and hospital, as matched in previously published studies [14, 15], to account for inter- and intra-facility variability in patient population and levels of care provided by the medical centers. The groups were examined for differences using chi-squared tests for categorical variables and analysis of variance for continuous variables. For the categorical variable region of residence, the West region was used as the control region. When significant differences were discovered, Fisher's exact and Wilcoxon rank sum tests were analyzed as appropriate. $P < 0.05$ was considered statistically significant unless otherwise specified. Potential confounders were included in a multivariable model if associated with the outcome in bivariate analysis at $P < 0.10$; they were retained if they changed the OR by $\geq 10\%$ or had a P value < 0.05. Potential interactions

among significant exposure variables were assessed by including an interaction term in the regression model, and statistical significance was determined with the use of the likelihood ratio test [15]. No interaction terms were found to be significant. Due to complexity of the model, sample size, and number of variables, stepwise multiple logistic regression was used to assess the multivariable relationship between the covariates and the groups. Briefly, starting with a null model where each variable was considered univariately, the variable with the smallest *P* value was added to the model and the remaining variables were reassessed one at a time. The procedure was repeated until there were no more statistically significant covariates ($P < 0.05$). A multivariable logistic regression model was used to examine all associated factors together. The parsimonious model selected by stepwise variable selection was considered the final model and included significant covariates from the stepwise selection process and CTX-M-9 Ent infection as the dichotomous outcome variable. All analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Of 356 pediatric Enterobacteriaceae isolates found to harbor 350 *bla* genes between 2011 and 2016, 44 (12.4%) were positive by DNA microarray and PCR amplification solely for $bla_{\text{CTX-M-9-grp}}$ genes. Most (40; 91%) CTX-M-9-grp-Ent infections and a significantly lesser fraction (78; 59%) of ESC-S infections were *E. coli*, $P < 0.001$ (Table 1). Urine was the most common source of CTX-M-9-grp-Ent (70%). The antimicrobial susceptibility testing results of CTX-M-9-grp-Ent are summarized in Table 2. The highest retained susceptibility was to amikacin, carbapenems, and piperacillin-tazobactam. Multi-drug resistance (resistance to ≥ 3 antibiotic classes) was found in 77% of case isolates.

Molecular Analysis

Of *E. coli*, 60% belonged to phylogenetic group D; the rest belonged to B2. Both are virulent phylogroups associated with extra-intestinal pathogenic *E. coli* infections. Of the CTX-M-9-group, the most common subtypes were CTX-M-14 (61%) and CTX-M-27 (34%). MLST revealed diverse strain types. Of the 10 ST types associated with 40 CTX-M-9 *E. coli*, 25% were ST8; ST43/ST131 and ST506 were the next most common (20% and 15%, respectively). Two novel *E. coli* strain types were also discovered to carry $bla_{\text{CTX-M-9-grp}}$ (data not shown).

Table 1 Characteristics of CTX M-9-grp extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and ESC-sensitive Enterobacteriaceae (ESC-S)

	CTX M-9-grp ESBL (*n* = 44)	ESC-S (*n* = 132)
Species, *n* (%)		
Klebsiella pneumoniae	3 (7)	23 (17)
Escherichia coli	40 (91)	78 (59)
Enterobacter cloacae	1 (2)	21 (16)
Other Enterobacteriaceae	0 (0)	10 (7)
Source, *n* (%)		
Urine	31 (70)	95 (72)
Respiratory	7 (16)	20 (15)
Blood	4 (9)	12 (9)
Other[a]	2 (5)	5 (4)

ESC extended-spectrum cephalosporin

[a] Other includes wound, abscess, abdominal, and peritoneal sources

Analysis of Factors Associated with CTX-M-9 Ent Infection

The 44 cases of CTX-M-9-grp-Ent infection were matched to 132 ESC-S controls. On bivariate analysis (Table 3) factors associated with higher likelihood of CTX-M-9-grp-Ent infection compared with ESC-S controls included: infection with *E. coli*, diagnosis in an outpatient clinic, absence of recent health care, residence in the South or Southwest regions (comprising South and Southwest Chicago and associated suburbs), and race "other" (non-White, non-Black, non-Hispanic). Children with CTX-M-9-grp-Ent

Table 2 Anatomical sites of isolation and antibiotic susceptibility patterns of CTX-M-9-grp-producing Enterobacteriaceae

Anatomical site[a]	No. of isolates[b]	% Susceptible (no. susceptible/no. available for testing)[b]										
		CTX	CAZ	FEP	P/T	CPM	GNT	TOB	AMK	FQ	TMP-SMX	TET	NIT
All	44	0	0	0	100 (31/31)	100	82	82	100 (40/40)	52 (22/42)	38 (16/42)	22 (6/27)	97 (30/31)
Urine	31	0	0	0	100 (21/21)	100	90	90	100 (28/28)	52 (15/29)	33 (10/30)	24 (6/25)	97 (30/31)
Respiratory	7	0	0	0	100 (4/4)	100	71	71	100	71	83 (5/6)	0 (0/1)	ND
Blood	4	0	0	0	100	100	50	50	100 (3/3)	50	25	0 (0/1)	ND
Other	2	0	0	0	100	100	50	50	100	0	0	ND	ND

CTX ceftriaxone, *CAZ* cefazolin, *FEP* cefepime, *P/T* piperacillin-tazobactam, *CPM* carbapenem (includes meropenem ertapenem or imipenem), *GNT* gentamicin, *TOB* tobramycin, *AMK* amikacin, *FQ* fluoroquinolones (includes ciprofloxacin and levofloxacin), *TMP-SMX* trimethoprim-sulfamethoxazole, *TET* tetracycline, *NIT* nitrofurantoin, *ATN* aztreonam, *ND* no data

[a] Respiratory includes bronchoalveolar lavage fluid, trachea, and sputum cultures. "Other" includes abscesses

[b] Susceptibility data not available for all antibiotics for all isolates

infections were less likely than controls to have infection with *Enterobacter* sp., diagnosis in the inpatient, non-ICU setting (i.e., general pediatric wards), residence in the Northwest region (comprised of Northwestern Chicago and the Northwestern suburbs), presence of a central venous line, respiratory comorbidities, and recent outpatient health care (including outpatient procedures).

During model building stages, we did not find evidence of a significant effect modification or significant confounding; therefore, additional covariates were not added back to the final model after the stepwise selection process was completed, and the simplest model with significant covariates was included in the final regression model.

In the multivariable regression analysis (Table 4), factors associated with CTX-M-9-grp-Ent infection included having infection due to *E. coli* (OR 7.4; 95% CI 2.4, 27.2; $P = 0.001$), being of "other" race or ethnicity (OR 7.4; 95% CI 2.4, 28.2; $P = 0.002$), and being diagnosed in the outpatient clinic setting (OR 4.5; 95% CI 1.7, 12.3; $P = 0.003$).

Remarkably, among children with Enterobacteriaceae infections, those residing in the South region of Chicago demonstrated more than six times the odds of having a CTX-M-9-grp-Ent infection compared with those living in the reference West Chicago region (OR 6.7; 95% CI 1.1, 37.9; $P = 0.03$) after controlling for race, infecting organism, and healthcare setting. In contrast, for children who resided in the Northwest region, there was an 81% decrease in the odds of CTX-M-9-grp-Ent infection compared with those residing in the reference West Chicago region (OR 0.19; 95% CI 0.04, 0.90; $P = 0.03$).

DISCUSSION

Our analysis is the first multi-center study to assess the clinical and molecular epidemiology of CTX-M-9-type ESBL-producing Enterobacteriaceae infections in children located in an urban setting. We found that *E. coli* was the most common infecting organism associated with $bla_{\text{CTX-M-9-grp}}$. The CTX-M-9 group is

Table 3 Bivariate analysis of demographics and factors associated with CTX-M-9-grp Enterobacteriaceae infections in children

Characteristic[a]	CTX-M-9-grp Ent infection	ESC-S Ent infection[b]	*P* value
Patient	*n* = 44	*n* = 132	
Age	4.14 (0–20.8)	4.48 (0–20.9)	0.11
Female	29 (65.9)	92 (69.7)	0.64
Race, White	15 (34.1)	38 (28.8)	0.51
Race, Black	3 (6.8)	29 (22.0)	0.03
Race, Hispanic	15 (34.1)	59 (44.7)	0.22
Race, non-White, non-Black, non-Hispanic	11 (25)	6 (4.6)	< 0.0001
Location at diagnosis			< 0.0001
Inpatient, non-ICU	7 (15.9)	42 (31.8)	0.04
Outpatient clinic	16 (36.4)	10 (7. 6)	< 0.001
Emergency room	9 (20.5)	23 (17.4)	0.65
Pediatric ICU	9 (20.5)	39 (29.6)	0.24
Neonatal ICU	3 (6.8)	18 (13.6)	0.22
Recent CTX[e]	9 (15.9)	33 (25)	0.21
Recent FQ[f]	3 (6.8)	4 (3.0)	0.27
Recent TMP/SMX[g]	5 (11.4)	18 (13.9)	0.68
Region of residence[c]			0.01
Recent health care			0.01
Inpatient care	11 (25)	34 (25.8)	0.92
Outpatient care[d]	10 (22.7)	59 (44.7)	0.01
No recent care	23 (52.3)	39 (29.6)	0.006
Foreign body present	19 (43.2)	75 (56.8)	0.12
Multiple foreign bodies present	13 (29.6)	49 (37.1)	0.36
Central venous line comorbidities	8 (18.2)	44 (33.3)	0.03
Cardiovascular	7 (15.9)	29 (22.0)	0.39
Endocrine	2 (4.6)	9 (7.0)	0.73
Gastrointestinal	12 (27.3)	32 (24.2)	0.69
Genitourinary	14 (26.4)	37 (28.0)	0.82
Hematology	6 (13.6)	20 (15.2)	0.81
Neurology	14 (31.8)	47 (35.6)	0.65
Respiratory	8 (18.2)	47 (35.6)	0.03

Table 3 continued

Characteristic[a]	CTX-M-9-grp Ent infection	ESC-S Ent infection[b]	*P* value
Renal	13 (29.6)	41 (31.1)	0.85
Multiple comorbidities	18 (40.9)	63 (47.0)	0.49

[a] Values represent *n* (%) unless otherwise indicated
[b] ESC-S Ent infections were children with infections due to Enterobacteriaceae sensitive to extended-spectrum cephalosporin antibiotics
[c] Region of residence includes city region and neighboring suburbs
[d] Outpatient care includes care outside of routine child care visits and outpatient procedures
[e] Abbreviation CTX includes extended-spectrum cephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefepime)
[f] FQ, fluoroquinolones (ciprofloxacin and levofloxacin)
[g] TMP-SMX, trimethoprim-sulfamethoxazole

Table 4 Multivariable analysis of factors associated with CTX-M-9-grp Enterobacteriaceae infections in children

Associated factors with CTX-M-9-grp Ent infection[a,c]	OR	95% CI	*P* value
Infection with *E. coli*	7.4	2.4, 27.2	0.001
Home residence in South region (South Chicago and South suburbs)[b]	6.7	1.1, 37.9	0.03
Race not White, Black or Hispanic	7.4	2.4, 28.2	0.002
Outpatient clinic location at time of infection diagnosis	4.5	1.7, 12.3	0.003
Home residence in Northwest region (NW Chicago and NW suburbs)	0.19	0.04, 0.90	0.03

[a] Ent, Enterobacteriaceae
[b] Reference region, West region
[c] Control group was children with Ent infections sensitive to extended-spectrum cephalosporins

thought to be the second most commonly community acquired ESBL genes in Enterobacteriaceae worldwide, and, similar to the high-risk clonal ST131 CTX-M-15 producing *E. coli*, are reported in people without significant healthcare exposure and are associated with multiple antibiotic gene cassettes leading to MDR [3, 9]. However, unlike the association of the CTX-M-1 group with ST131, we found that $bla_{\text{CTX-M-9-grp}}$ genes are carried by multiple strain types, including the identification of novel STs associated with pediatric infection.

Because of multiple associated strain types carrying $bla_{\text{CTX-M-9-grp}}$ genes, we hypothesized that significant horizontal gene transfer is occurring between genera. This observation is concerning as children once colonized with MDR Enterobacteriaceae can remain colonized for months to years, and young children (median age 4.1 years) were carrying organisms with multiple plasmids, capable of rapid dissemination and spread [13, 23, 24]. Additionally, we found novel ST types carrying $bla_{\text{CTX-M-9-grp}}$ genes. This portends a troublesome epidemiology suggesting the acquisition of these genes may be occurring in commensal or previously non-pathogenic strains of Enterobacteriaceae.

In our study, most (77%) CTX-M-9-grp-Ent isolates were phenotypically MDR. While the highest retained antibiotic susceptibility included piperacillin-tazobactam, clinicians should be cautioned in its use for serious, invasive infections (e.g., bacteremia). Recent randomized clinical trials have demonstrated inferiority of piperacillin-tazobactam to carbapenem therapy when used to treat ESC-R Ent bloodstream infections (including ESBL producers) and describe worse patient outcomes associated with piperacillin-tazobactam use as definitive therapy [25]. Of additional concern, susceptibility to oral agents was significantly reduced

leaving few oral options for step-down therapy for children (Table 2). The sole oral exception was nitrofurantoin (97% sensitivity), which can be used to treat uncomplicated lower urinary tract infection but should not be used for complicated, invasive infections or acute pyelonephritis because of the inability of the drug to attain therapeutic levels in the renal parenchyma and bloodstream [26].

The residential differences for children infected with CTX-M-9-grp-Ent compared with children infected with antibiotic-sensitive strains were striking. We found a substantial increase in odds of infection with the resistant strains in the South Chicago region and a significant decrease in odds of infection in the Northwest Chicago region. Our study included three major pediatric centers, none of which are located in the "high-risk" South region, yet all three centers diagnosed and treated patients with CTX-M-9-grp-Ent infections from these high-risk regions. The reservoirs associated with acquisition of CTX-M-9-grp-Ent in these regions are currently undefined.

Of additional concern, in a separate study of plasmid-mediated fluoroquinolone-resistant (PMFQR) Ent infections in children between 2011 and 2014, we found a substantial increase in odds of PMFQR Ent infection in the Southwest region, while in the Downtown region (comprising the downtown Chicago area, near North side, Chicago loop, and North Chicago), there was a significant decrease in the likelihood of PMFQR Ent infection [15]. These PMFQR Ent "high-risk" and "low-risk" regions neighbor the "high-risk" South region and "low-risk" Northwest region in the current study. The Southwest region was found to be associated with an increase in the odds of CTX-M-9-grp-Ent infection on bivariate analysis in this study, though this did not reach statistical significance on multivariable analysis once controlling for other variables.

Furthermore, we found that children with CTX-M-9-grp-Ent infection were significantly more likely to present in the outpatient clinic setting compared with those with antibiotic-susceptible Ent infections. This is surprising and striking as most infections caused by MDROs in the US are linked to healthcare settings [27]. While the majority of infections were associated with virulent extra-intestinal-pathogenic *E. coli* phylogenetic groups (B and D), these data suggest that CTX-M-9-grp-Ent infections in children are being acquired in the community and may be less severe at presentation as children presented more often in outpatient clinics. This is in stark contrast to the epidemiology of MDR Enterobacteriaceae infections reported in adults in Chicago, where acquisition has been strongly associated with residence in long-term care facilities and the interfacility transfer of patients [28].

The higher likelihood of CTX-M-9-grp-Ent infection in those of non-White, non-Black, and non-Hispanic race was an additional association discovered on multivariable analysis. This risk was statistically independent of residence, and only one of the children located in the "high risk" South region was of race "other," supporting the independence of the two risk factors for infection. We were unable to gather further data on "other" race or ethnicity because of the retrospective nature of the study, and we did not have travel data for the majority of children, which are important limitations. It is well described that travel to certain countries can be associated with high rates of ESBL Ent acquisition, particularly in South and Southeast Asia [29]. It is also well described that there is an increased risk of colonization when residing in a household with a family member with history of ESBL Ent or who acquired such a strain during travel to an endemic region [30].

Environmental influences originating in the community could include higher risks in certain populations due to specific exposures to foods, animals, water sources, fertilizer, soil, and vegetation [31]. We did not have data on companion pets for the majority of children and were unable to examine this risk factor, though pets may play a significant role in human acquisition of antibiotic-resistant bacteria [32]. Lastly, we did not find overall differences in a general comparison of socioeconomic status of the "high-risk" South region and neighboring regions such as the Southwest and West regions using regional zip codes and Illinois census data.

We recognize the limitations of our study. This was a retrospective study of children with

Enterobacteriaceae infections in a single metropolitan area, which may affect generalizability to other regions. While selection bias may result from small sample sizes, the pooling of multicentered data from institutions of differing types in the third largest US metropolitan area and serving diverse populations potentially lessens this bias. Moreover, our sample size is consistent with the overall low prevalence of these MDR Enterobacteriaceae children in the majority of the US (1–3%) including Chicago and the Midwest region [33]; however, it should be noted that national trends indicate an increase in the incidence and prevalence of pediatric MDR Enterobacteriaceae over the last decade, an emerging threat that needs further assessment [5, 34]. Finally, we did not compare these CTX-M-9-grp-Ent infections with those due to other *bla* genes; however, future comparative studies are planned.

CONCLUSION

We describe, for the first time, community origins of CTX-M-9-grp Enterobacteriaceae in children and the impact of residence on infection in children located in the same geographic area. The reservoirs associated with CTX-M-9-grp-Ent infections remain undefined. Future studies must focus on environmental influences associated with regional acquisition. "Silent dissemination" of community-acquired MDR Enterobacteriaceae is likely occurring in children outside of healthcare settings. Dedicated programs on a local, national, and global scale must focus on children and halting of spread of these dangerous pathogens.

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Data Availability. The data sets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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