Community origins and regional differences in plasmid-mediated fluoroquinolone resistant Enterobacteriaceae infections in children

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Running Title: Community Origins of PMFQR Enterobacteriaceae

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Abstract

Fluoroquinolones (FQs) are uncommonly prescribed in children, yet pediatric multidrug-resistant (MDR)-Enterobacteriaceae (Ent) infections often reveal FQ resistance (FQR). We sought to define the molecular epidemiology of FQR and MDR-Ent in children. A case-control analysis of children with MDR-Ent infections at 3 Chicago hospitals was performed. Cases were children with third-generation-cephalosporin-resistant (3GCR) and/or carbapenem-resistant (CR)-Ent infections. PCR and DNA analysis assessed *bla* and plasmid-mediated FQR (PMFQR) genes. Controls were children with 3GC and carbapenem susceptible-Ent infections matched by age, source and hospital. We assessed clinical-epidemiologic predictors of PMFQR Ent infection. Of 169 3GCR and/or CR Ent isolates from children (median age 4.8 years), 85 were FQR; 56 (66%) contained PMFQR genes. The predominant organism was *E. coli* and most common *bla* gene $bla_{\text{CTX-M-1 group}}$. In FQR isolates, PMFQR gene mutations included *aac6'1b-cr*, *oqxA/B*, *qepA*, and *qnrA/B/D/S* in 83%, 15%, 13% and 11% of isolates, respectively. FQR *E. coli* was often associated with phylogroup B2, ST43/ST131. On multivariable analysis, PMFQR *Ent* infections occurred mostly in outpatients (OR 33.1) of non-black-white-Hispanic race (OR 6.5). Residents of Southwest Chicago were >5 times more likely to have PMFQR-*Ent* infections than those in the reference region, while residence in Central Chicago was associated with a 97% decreased risk. Other demographic, comorbidity, invasive-device, antibiotic use, or healthcare differences were not found. The strong association of infection with MDROs showing FQR with patient residence rather than with traditional risk factors suggests that the community environment is a major contributor to spread of these pathogens in children.

Introduction

Multi-drug resistant (MDR) Enterobacteriaceae infections are associated with significant morbidity and mortality and are an emerging problem in children in the US during the last decade (1, 2). Globally, this has been attributed mainly to the rise in extended-spectrum beta lactamase (ESBL) producing Enterobacteriaceae (ESBL Ent) and carbapenem-resistant Enterobacteriaceae (CRE) (3, 4). Increasing resistance to several other classes of antibiotics was found in these studies; including to the fluoroquinolones, a class of antibiotics with limited indications for use in children (1, 2).

The CTX-M-ESBL harboring *Escherichia coli* are among the most common multi-drug resistant organisms (MDROs), often possessing resistance genes to other important antibiotic classes including aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim-sulfamethoxazole (5, 6). In adults, fluoroquinolone resistance in (FQR) Gram-negative bacteria has been linked to chromosomal and to plasmid-mediated resistance mechanisms and is thought to be associated mostly with the dramatic increase in use of these antibiotics during the 1980s(7, 8).

The reasons for increased FQR in children are unclear, and studies assessing the resistance determinants associated with the FQR phenotype in Enterobacteriaceae recovered from children are limited(9); therefore, we examined this population to determine which children had a higher likelihood of infections with organisms resistant to both beta-lactam and fluoroquinolone antibiotics.

We determined the genetic basis of FQR in beta-lactamase producing Enterobacteriaceae isolates from children cared for by multiple centers in the Chicago area and analyzed a subgroup

of children with infections with similar resistance determinants, namely those due to isolates containing genes encoding plasmid-mediated fluoroquinolone resistance (PMFQR) and ESBL mediated resistance to determine genotypes, host factors, and exposures leading to infection with MDR Enterobacteriaceae strains. We hypothesized that because of differences in healthcare delivery in urban settings, acquisition of PMFQR in children would be linked to geographic location and have environmental influences and community origins. Our analysis revealed that there are genetic and geospatial links to MDR in this pediatric population.

METHODS

Study Setting

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

Descriptive Study Design

Study Population

This study included patients aged 0 to 18.99 years who had clinical cultures positive for Enterobacteriaceae with 3GCR or CR and the suspected presence of a beta-lactamase gene based

on clinical laboratory testing. Additionally, isolates found to be concomitantly resistant to FQR were further characterized. Infections were diagnosed between January 1, 2011 and December 31, 2014 and only the first infection per patient was included. The study was approved by the institutional review boards of the three participating institutions and need for informed consent was waived.

Testing of Antibiotic Susceptibility in Enterobacteriaceae

The Hospitals A-C microbiology laboratories phenotypically analyzed presumed ESBL Ent, AmpC Ent and CR isolates via the Vitek 2 microbial identification system (*bioMérieux, Athens, GA)* or by the MicroScan WalkAway system (Siemens Healthcare Diagnostics, Tarrytown, NY). Screening for ESBL production involved testing with one or more of the following agents: aztreonam, ceftazidime, ceftriaxone, cefotaxime or cefpodoxime, based on guidelines of the Clinical and Laboratory Standards Institute (CLSI) (10). ESBL production was confirmed on the automated instruments or by disk diffusion assays (BBL; Becton, Dickinson and Company, Sparks, MD) or by measuring minimum inhibitory concentrations (MICs) of ceftazidime and cefotaxime in the presence and absence of clavulanic acid. A measurement of an increase in disk zone diameter of > 5 mm or a 4-fold reduction in the MIC of ceftazidime or cefotaxime in the presence of clavulanic acid served as confirmation of the ESBL phenotype (10).

The carbapenemase phenotype, per Centers for Disease Control and Prevention (CDC) criteria, included isolates that were non-susceptible to all 3GCs (cefotaxime, ceftazidime, or ceftriaxone) and resistant to one or more carbapenem (imipenem, meropenem, doripenem, or ertapenem) (11). Carbapenemase production was phenotypically confirmed by MBL E-test (bioMérieux, Athens, GA) or Modified Hodge Test, as appropriate (12, 13).

Determination of Beta-Lactam Resistance Mechanisms

Genomic DNA was extracted and purified from isolates using the DNeasy Blood & Tissue Kit (QIAGEN, Inc., Valencia, CA). To evaluate for the presence of *bla* genes in isolates, a DNA microarray based assay was performed (Check-Points, Check-MDR CT101 kit; Wageningen, The Netherlands). The CT101 microarray based assay can detect the following *bla* groups: CTX-M-1 group, CTX-M-2 group, CTX-M-8 and -25 group, CTX-M-9 group, SHV WT and SHV-type ESBL, TEM wild-type, and TEM-type ESBL, plasmid based AmpC cephalosporinases (pAmpC) (CMY II, ACC, FOX, DHA, ACT/ MIR) and carbapenemases (KPC and NDM) (14). When isolates were *bla* negative by the CT101 assay, a broader DNA microarray, (Check-Points, Check-MDR CT103XL kit) was performed. The CT103XL assay can detect the presence of additional ESBL genes (VEB, PER, BEL, GES) and carbapenemase genes (GES, GIM, IMP, SPM, VIM, and OXA-23, -24/40, -48, and -58) (15). The assays were performed as described in our laboratory previously (16).

Analysis of Determinants Yielding Fluoroquinolone Resistance

To investigate the presence of FQR determinants in MDR Enterobacteriaceae isolates, we analyzed the quinolone resistance-determining region (QRDR) located on the bacterial chromosome and assessed for PMFQR in strains found FQR by CLSI standards (10). Briefly, genus specific assays for mutations in *gyrA* and *parC* genes of the QRDR (in *E. coli, Klebsiella sp.*, and *Enterobacter sp.*) and for PMFQR were performed by polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequencing of amplicons (7). Extraction of genomic DNA followed by amplification and sequencing were performed using primers and methods as previously described (17-19). Specific PMFQR genes screened include *qnrA, qnrB, qnrD, qnrS,*

qepA, *oqxA* and *oqxB* and *aac6'-Ib-cr* and represent transmissible elements reported in Enterobacteriaceae (20, 21) .

Multilocus Sequence Typing (MLST)

Per protocol, eight *E. coli* housekeeping genes (*dinB, icdA, pabB, polB, putP, trpA, trpB and uidA*) and seven *Klebsiella* species (sp.) housekeeping genes *(rpoB, gapA, mdh, pgi, phoE, tonB, infB)* were amplified and sequenced as in prior studies (16, 22, 23). Alleles and sequence types (ST) were assigned for select isolates of varying genotypic profiles by the Pasteur MLST scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/).

Analysis of Plasmid Replicon Types and Phylogenetic Grouping

E. coli were assigned to four major phylogenetic groups (A, B1, B2 and D) using a well-established multiplex PCR-based method (24). Plasmids were typed, in select isolates with varying genotypic profiles, based on incompatibility groups corresponding to the nomenclature assigned by Carattoli *et al*. (25).

Analytic Study Design

We used a retrospective case–control study design to assess factors associated with infection due to beta-lactam, FQ resistant isolates in which we had detected a PMFQR gene. We chose to analyze FQR in detail because this class of antibiotics is uncommonly used in children, yet 50% of the isolates between 2011 and 2014 were FQR.

We selected as controls, children with infections due to bacteria susceptible to the antibiotics of interest. Specifically, the control group included children with infections that were susceptible to 3GC, carbapenem and FQ antibiotics to understand differences between children who acquired plasmid-mediated MDR Enterobacteriaceae infections and those who did not.

Only patients with clinical infections were included, as determined by study investigator case review and/or using standard criteria defined by the CDC National Healthcare Safety Network (26). Children serving as control subjects were identified using hospital electronic laboratory records (ELRs). Control patients were matched approximately 3:1 to the cases by age range, hospital, and specimen source.

Covariates

Several variables were analyzed as potential factors associated with FQR Ent infection based on known associations for acquisition in adults including (1) demographics (age, gender, race/ethnicity); (2) comorbid conditions (as defined by ICD-9 codes); (3) recent inpatient and outpatient healthcare exposures, including hospitalization and/or procedures in the previous 30 days; (4) all recent antibiotic exposures in the 40 days prior to culture; (5) presence, number, and type of invasive medical devices; and (6) the impact of location of patient residence in the Chicago area as assessed by dividing the metropolitan area into 7 regions using zip code level data, which included Chicago proper and its suburban areas (i.e. Northwest side and Northwest Suburbs, Southwest side and Southwest Suburbs, etc.). An eighth region included patients from other parts of Illinois or from other states.

Statistical Analysis

Case and control groups were examined for differences using parametric or non-parametric tests as appropriate for categorical and continuous variables; $P \leq 0.05$ was considered statistically significant unless otherwise specified. Variables with $p<0.1$ on bivariate analysis were included in multivariable analysis. Stepwise multiple logistic regression was used to assess the multivariable relationship between the covariates and the groups. The final multivariable logistic regression model included the simplest model with significant covariates ($p<0.05$) from

the stepwise selection process, with PMFQR Ent infection as the outcome variable. The simplest model was chosen based on a relatively small sample size and the effect of variables in the model. All analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Composition of Fluoroquinolone Resistance (FQR) Genes in Enterobacteriaceae

We assessed 169 *bla*-producing Ent isolates between 2011 – 2014 from Hospitals A, B, and C for the presence of FQR (Table 1). Of 169 Ent isolates, 85 (50%) were FQR of which 82 (96.4%) were available for further testing. The median age of children with FQR-Ent infections was 4.8 years. The predominant organism was *E. coli*, 65/82 (79%), and the predominant *bla* genotype found associated with FQR in Ent was $bla_{\text{CTX-M-1-group}}$ in 62% of cases. Within *E. coli*, FQR was most often associated with phylogroup B2 and ST43 (Pasteur scheme)/ST131 (Achtman scheme) harboring $bla_{\text{CTX-M-1-group}}$ in 47/63 (75%) cases.

FQR isolates were further characterized to understand resistance determinants associated with FQR in pediatric Ent isolates. Chromosomal mutations of the QRDR (*gyrA*/*parC*) were present in 71/79 (89.9%) of FQR isolates by DNA sequence analysis. Three isolates did not yield results. PMFQR genes were detected by PCR in 56/82 (66%); 53 (95%) were available for further analysis. The median case patient age was 6 years. PMFQR genes included *aac 6'1b-cr*, *oqx A/B*, *qepA*, and *qnr A/B/D/S* in 83%, 15%, 13% and 11% of isolates, respectively. PMFQR was found in combination with *gyrA* and/or *parC* mutations in 43/49 (88%) isolates, which is associated with high level resistance. The predominant *bla* genotype found associated with PMFQR was $bla_{\text{CTX-M-1-group}}$ in 76%, followed by $bla_{\text{SHV ESBL}}$ associations in 11%. Almost all (98%) PMFQR Ent were multi-drug resistant, e.g. resistant to $\geq$3 antibiotic classes.

Analysis of Factors Associated with PMFQR Enterobacteriaceae Infections in Children

The 53 cases of PMFQR Ent infection were matched by age range, hospital, and culture source to 131 controls with antibiotic-sensitive Ent infections. Significant factors associated with PMFQR Ent infection on bivariate analysis included: *E. coli* infection, race/ethnicity, infection diagnosed in an outpatient clinic, history of quinolone use, and residence in the Southwest region (hereafter referred to as the high-risk region) comprised of southwest Chicago and the southwestern Chicago suburbs (Table 2). Children with PMFQR infection were less likely to have infection with *Enterobacter* sp., a central venous catheter, neonatal intensive care unit admission at the time of infection diagnosis, or residence outside the "high-risk" region (comprised of the downtown Chicago area, near North side, Chicago loop, and North Chicago). Case-control differences in comorbid conditions; presence of respiratory, gastrointestinal, genitourinary, or overall count of foreign bodies; or recent prior healthcare exposure were not found.

We did not find evidence of significant effect modification during the model building stages nor did we find evidence of significant confounding; therefore, no additional covariates were added back to the final model after the stepwise selection process was completed, and the simplest model was used in the final regression model.

On multivariable analysis (Table 3), having infection diagnosed in the outpatient clinic setting was significantly associated with PMFQR Enterobacteriaceae infection (OR=33.1; 95% CI 7.1, 162.8; $p<0.001$). Being of a race or ethnicity other than white, black, or Hispanic was significantly associated with PMFQR Enterobacteriaceae infection (OR 6.5; 95% CI 1.7, 24.3; $p=0.006$). Interestingly, among children with Enterobacteriaceae infections, those residing in southwestern region of Chicago had more than five times the odds of having a PMFQR infection

compared to those living in the reference West Chicago region (OR 5.6; 95% CI 1.6, 19.2; p=0.006) after controlling for race and healthcare setting. In contrast, for children who resided in the downtown region, there was a 97% decrease in the odds of PMFQR infection in those living in this region compared to those residing in reference West Chicago region (OR 0.03; 95% CI .002, 0.33; p=0.005). No other regional associations were found.

To ensure these regional associations were unique to PMFQR containing isolates, we ran two additional case-control analyses specifically assessing whether regional differences within the Chicago metropolitan area were seen with 1) ESBL-producing strains that were sensitive to fluoroquinolones; and 2) *bla*$_{\text{CTX-M-1group}}$ ESBL-producing isolates (related to circulation of ST131 clonal *E. coli* strains). Regional differences in acquisition were not found in either analysis (data not shown).

DISCUSSION

Multi-drug resistant Enterobacteriaceae are a growing concern globally. Much of the propagation and spread of these organisms has been related to the ST131 *E. coli* strains and to high-risk clones containing IncFII plasmids and other genetic structures, such as transposons, integrons, and insertion sequences associated with multiple antibiotic resistance gene cassettes (27). Occasionally, beta-lactamase and other antibiotic resistance genes are transferred horizontally (28).

In our pediatric patients there was a predominance of ST131 *E. coli* harboring *bla*$_{\text{CTX-M}}$. We hypothesize that there is significant horizontal gene transfer between genera. This is very worrisome from a public health perspective since children, once colonized with MDR Enterobacteriaceae can remain colonized for months to years and could serve as reservoirs and

"silent disseminators" of MDROs (29, 30). Interestingly, the community focus of the MDROs in children is in stark contrast to the epidemiology of these bacteria in adults in Chicago, where MDR Enterobacteriaceae acquisition is highly linked to residence in long-term care facilities and to interfacility transfer (31, 32).

We found striking residential differences for children infected with PMFQR containing Enterobacteriaceae (PMFQR Ent) compared to children infected with antibiotic sensitive strains. PMFQR Ent contain plasmid-based resistance genes to fluoroquinolones, an antibiotic uncommonly used in children. In one Chicago region, the Southwest region, there was a substantial increase in odds of PMFQR Ent infection, and in the Downtown region, there was a significant decrease in the likelihood of PMFQR Ent infection (Figure 1). Our study included 3 major pediatric centers, none of which (and no major medical centers for children) is located in the "high-risk" Southwest region, yet all three centers diagnosed and treated patients with PMFQR Ent infections from that region. In contrast, Hospital B, the largest hospital in the region specifically dedicated to the care of children, is located in the downtown region, and therefore services many children residing in that area; yet this was the area of "lowest risk" for PMFQR Ent infection. This may reflect linkage to *bla*$_{\text{CTX-M}}$ harboring plasmids which are endemic in some communities, but the reservoirs are currently undefined.

Interestingly, we found that children with PMFQR Ent infection were more likely to present in the outpatient clinic setting than were those with antibiotic sensitive Ent infections – the opposite of expectation that MDROs are healthcare linked and suggesting that PMFQR Ent infections were community acquired and less severe.

A strong additional association on multivariable analysis was the higher likelihood of PMFQR Ent infection in those of non-white, non-black, and non-Hispanic race. This risk was statistically independent of residence, and strikingly none of the children located in the "high risk" southwest region with PMFQR Ent infection were of race "other", supporting the strong independence of these 2 risk factors. Due to the retrospective nature of the study, we were unable to gather further data on "other" race or ethnicity. We did not have travel data for the majority of children, although it is well documented that travel to certain countries can be associated with high rates of ESBL Ent acquisition, particularly in South and Southeast Asia (30, 33, 34). It is also well described that there is an increased risk of colonization of household members after return of the traveler who first acquired an ESBL Ent.(35, 36)

We did not find significant differences in comorbidities between cases and controls. This puts further importance on evaluating environmental sources for plasmid-possessing antibiotic resistance genes (32). Community-based environmental influences would include higher exposure risks in certain communities due to certain foods, livestock, animals, water sources, fertilizer, soil, and vegetation (37). For example, if there is a link to food exposures, such as restaurant chains that cook with high saturated fats, and additionally serve food animals that are fed antibiotics and hormones for growth effects, this exposure would increase the risk of acquisition of antibiotic-resistant bacteria, as well as obesity (38, 39). This in turn increases the risk of other diseases such as cardiovascular disease and diabetes (40). Some of the PMFQR genes, for example, oqxA and oqxB are multidrug efflux pumps named for their resistance to olaquinadox, which is used as a growth promoter on pig farms (41).

Studies in our region and nationally have suggested that an increased risk of exposure to antibiotics in children (42), as well as to antibiotic resistant bacteria, may be related to socioeconomic status and race (43, 44). While we did assess race, we did not formally compare differences between socioeconomic factors in the regions, as we did not have street or neighborhood level data on infected patients. However, in a general comparison of regional zip codes using Illinois census data, we did not find overall differences in the socioeconomic status of the "high risk" southwest region and neighboring regions such as the south and west regions.

We recognize that our study has limitations. This was a retrospective study designed to determine mechanisms of antibiotic resistance in Enterobacteriaceae recovered from children cared for at three centers in a single metropolitan area; this may potentially impact generalizability to other regions. Additionally, a plasmid-based origin of the recovered antibiotic-resistance genes is suggested by our DNA sequence analysis results, yet it is possible that some of these genes represent chromosomal resistance mechanisms. However, subsequent plasmid-replicon typing and DNA sequence analysis for a subgroup of bacteria support our findings of the DNA microarray. Our sample size was relatively small, which may allow for selection bias; however, the pooling of multicentered data from institutions of differing types serving diverse populations throughout the 3rd largest metropolitan area in the U.S. potentially lessens this bias. The smaller sample sizes in pediatric studies are related to the overall low prevalence of these organisms in children in most U.S. areas (1-3%), including in the Chicago and the Midwest region (29, 32, 34, 45, 46), although national trends indicate an increase in prevalence of these menacing organisms in pediatric populations during the last decade, suggesting they are an emerging threat that needs further evaluation (46-49).

In conclusion, we found that there is significant complexity and diversity in the determinants associated with beta-lactam and fluoroquinolone antibiotic resistance in children, and that pediatric MDR Enterobacteriaceae exhibited differences when compared to descriptions of strains circulating in adult patients in a region where such infections are endemic. We also describe, for the first time, the impact of residence on infection with MDR Enterobacteriaceae in children located in the same geographic area, however the reservoirs remain undefined. Future studies should focus on further molecular characterization of circulating strains and the environmental influences associated with these differences in regional acquisition. We anticipate that an imminent threat of the "silent dissemination" of multi-drug resistant Enterobacteriaceae in community settings is occurring in children. Local, federal, and international programs dedicated to this serious problem must focus on halting the spread of these menacing pathogens in our most vulnerable population, children.

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References

1. **Logan, L. K., N. P. Braykov, R. A. Weinstein, and R. Laxminarayan.** 2014. Extended-Spectrum ß-Lactamase–Producing and Third-Generation Cephalosporin-Resistant Enterobacteriaceae in Children: Trends in the United States, 1999–2011. Journal of the Pediatric Infectious Diseases Society. . doi: 10.1093/jpids/piu010.

2. **Logan, L. K., J. P. Renschler, S. Gandra, R. A. Weinstein, R. Laxminarayan, Centers for Disease Control, and Prevention Epicenters Program.** 2015. Carbapenem-Resistant Enterobacteriaceae in Children, United States, 1999-2012. Emerg. Infect. Dis. **21:**2014-2021. doi: 10.3201/eid2111.150548 [doi].

3. **Lukac, P. J., R. A. Bonomo, and L. K. Logan.** 2015. Extended-spectrum β-lactamase–producing Enterobacteriaceae in children: old foe, emerging threat. Clinical Infectious Diseases. **60:**1389-1397.

4. **Logan, L. K., and R. A. Weinstein.** 2017. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. J. Infect. Dis. **215:**S28-S36.

5. **Cantón, R., and T. M. Coque.** 2006. The CTX-M β-lactamase pandemic. Curr. Opin. Microbiol. **9:**466-475.

6. **Munoz-Price, L. S., L. Poirel, R. A. Bonomo, M. J. Schwaber, G. L. Daikos, M. Cormican, G. Cornaglia, J. Garau, M. Gniadkowski, and M. K. Hayden.** 2013. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. The Lancet Infectious Diseases. **13:**785-796.

7. **Robicsek, A., G. A. Jacoby, and D. C. Hooper.** 2006. The worldwide emergence of plasmid-mediated quinolone resistance. The Lancet Infectious Diseases. **6:**629-640.

8. **Mathers, A. J., G. Peirano, and J. D. D. Pitout.** 2015. The Role of Epidemic Resistance Plasmids and International High-Risk Clones in the Spread of Multidrug-Resistant Enterobacteriaceae. Clinical Microbiology Reviews. **28:**565-591. doi: 10.1128/CMR.00116-14.

9. **Medernach, R. L., and L. K. Logan.** 2018. The Growing Threat of Antibiotic Resistance in Children. Infect. Dis. Clin. North Am. **32:**1-17.

10. **Clinical and Laboratory Standards Institute.** 2010. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement (June 2010 Update). December 29, 2011.

11. **Centers for Disease Control and Prevention (CDC).** CDC - 2012 CRE Toolkit - Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE). **2015:**.

12. **Centers for Disease Control and Prevention.** 2013. Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae. .

13. **Birgy, A., P. Bidet, N. Genel, C. Doit, D. Decre, G. Arlet, and E. Bingen.** 2012. Phenotypic screening of carbapenemases and associated beta-lactamases in carbapenem-resistant Enterobacteriaceae. J. Clin. Microbiol. **50:**1295-1302. doi: 10.1128/JCM.06131-11 [doi].

14. **Bogaerts, P., A. M. Hujer, T. Naas, R. R. de Castro, A. Endimiani, P. Nordmann, Y. Glupczynski, and R. A. Bonomo.** 2011. Multicenter evaluation of a new DNA microarray for rapid detection of clinically relevant bla genes from beta-lactam-resistant gram-negative bacteria. Antimicrob. Agents Chemother. **55:**4457-4460. doi: 10.1128/AAC.00353-11 [doi].

15. **Cunningham, S., B. Johnston, S. Vasoo, J. Johnson, and R. Patel.** 2014. Abstr. Evaluation of Check-Points Check-MDR CT103 PCR-Microarray Kit for Detection and Classification of ESBL, AmpC and Carbapenemase Genes. 54th Interscience Conference on Antimicrobial Agents and Chemotherapy. **Washington, D.C.:**.

16. **Logan, L. K., A. M. Hujer, S. H. Marshall, T. N. Domitrovic, S. D. Rudin, X. Zheng, N. K. Qureshi, M. K. Hayden, F. A. Scaggs, A. Karadkhele, and R. A. Bonomo.** 2016. Analysis of beta-Lactamase Resistance Determinants in Enterobacteriaceae from Chicago Children: a Multicenter Survey. Antimicrob. Agents Chemother. **60:**3462-3469. doi: 10.1128/AAC.00098-16 [doi].

17. **Cano, M. E., J. M. Rodriguez-Martinez, J. Aguero, A. Pascual, J. Calvo, J. M. Garcia-Lobo, C. Velasco, M. V. Francia, and L. Martinez-Martinez.** 2009. Detection of plasmid-mediated quinolone resistance genes in clinical isolates of Enterobacter spp. in Spain. J. Clin. Microbiol. **47:**2033-2039.

18. **Hujer, K. M., A. M. Hujer, A. Endimiani, J. M. Thomson, M. D. Adams, K. Goglin, P. N. Rather, T. T. Pennella, C. Massire, M. W. Eshoo, R. Sampath, L. B. Blyn, D. J. Ecker, and R. A. Bonomo.** 2009. Rapid determination of quinolone resistance in Acinetobacter spp. J. Clin. Microbiol. **47:**1436-1442.

19. **Endimiani, A., A. M. Hujer, F. Perez, C. R. Bethel, K. M. Hujer, J. Kroeger, M. Oethinger, D. L. Paterson, M. D. Adams, M. R. Jacobs, D. J. Diekema, G. S. Hall, S. G. Jenkins, L. B. Rice, F. C. Tenover, and R. A. Bonomo.** 2009. Characterization of blaKPC-containing Klebsiella pneumoniae isolates detected in different institutions in the Eastern USA. J. Antimicrob. Chemother. **63:**427-437.

20. **Jacoby, G. A.** 2017. Plasmid-mediated quinolone resistance, p. 265-268. *In* Anonymous Antimicrobial Drug Resistance. Springer.

21. **Chen, X., W. Zhang, W. Pan, J. Yin, Z. Pan, S. Gao, and X. Jiao.** 2012. Prevalence of qnr, aac(6')-Ib-cr, qepA, and oqxAB in Escherichia coli Isolates from Humans, Animals, and Environment. Antimicrobial Agents and Chemotherapy. . doi: 10.1128/AAC.06191-11.

22. **Diancourt, L., V. Passet, J. Verhoef, P. A. Grimont, and S. Brisse.** 2005. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J. Clin. Microbiol. **43:**4178-4182. doi: 43/8/4178 [pii].

23. **Jaureguy, F., L. Landraud, V. Passet, L. Diancourt, E. Frapy, G. Guigon, E. Carbonnelle, O. Lortholary, O. Clermont, E. Denamur, B. Picard, X. Nassif, and S. Brisse.** 2008. Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. BMC Genomics. **9:**560-2164-9-560. doi: 10.1186/1471-2164-9-560 [doi].

24. **Bingen-Bidois, M., O. Clermont, S. Bonacorsi, M. Terki, N. Brahimi, C. Loukil, D. Barraud, and E. Bingen.** 2002. Phylogenetic analysis and prevalence of urosepsis strains of Escherichia coli bearing pathogenicity island-like domains. Infect. Immun. **70:**3216-3226.

25. **Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods. **63:**219-228. doi: http://dx.doi.org/10.1016/j.mimet.2005.03.018.

26. **Centers for Disease Control and Prevention (CDC).** 2017. The National Healthcare Safety Network (NHSN) Patient Safety Component Manual. .

27. **Canton, R., J. M. Gonzalez-Alba, and J. C. Galan.** 2012. CTX-M Enzymes: Origin and Diffusion. Front. Microbiol. **3:**110. doi: 10.3389/fmicb.2012.00110 [doi].

28. **Carattoli, A.** 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. **53:**2227-2238. doi: 10.1128/AAC.01707-08 [doi].

29. **Zerr, D. M., X. Qin, A. P. Oron, A. L. Adler, D. J. Wolter, J. E. Berry, L. Hoffman, and S. J. Weissman.** 2014. Pediatric infection and intestinal carriage due to extended-spectrum-cephalosporin-resistant Enterobacteriaceae. Antimicrob. Agents Chemother. **58:**3997-4004. doi: 10.1128/AAC.02558-14 [doi].

30. **Islam, S., R. Selvarangan, N. Kanwar, R. McHenry, J. D. Chappell, N. Halasa, M. E. Wikswo, D. C. Payne, P. H. Azimi, L. C. McDonald, and O. G. Gomez-Duarte.** 2017 Jun 15. Intestinal Carriage of Third-Generation Cephalosporin-Resistant and Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae in Healthy US Children. Journal of the Pediatric Infectious Diseases Societ. .

31. **Lin, M. Y., R. D. Lyles-Banks, K. Lolans, D. W. Hines, J. B. Spear, R. Petrak, W. E. Trick, R. A. Weinstein, M. K. Hayden, and Centers for Disease Control and Prevention Epicenters Program.** 2013. The importance of long-term acute care hospitals in the regional epidemiology of Klebsiella pneumoniae carbapenemase–producing Enterobacteriaceae. Clinical Infectious Diseases. **57:**1246-1252.

32. **Logan, L. K., L. A. Meltzer, J. B. McAuley, M. K. Hayden, T. Beck, N. P. Braykov, R. Laxminarayan, and R. A. Weinstein.** 2014. Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae Infections in Children: A Two-Center Case–Case–Control Study of Risk Factors and Outcomes in Chicago, Illinois. Journal of the Pediatric Infectious Diseases Society. **3:**312-319.

33. **Kuenzli, E., V. K. Jaeger, R. Frei, A. Neumayr, S. DeCrom, S. Haller, J. Blum, A. F. Widmer, H. Furrer, and M. Battegay.** 2014. High colonization rates of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli in Swiss Travellers to South Asia–a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC Infectious Diseases. **14:**528.

34. **Strysko, J. P., V. Mony, J. Cleveland, H. Siddiqui, P. Homel, and C. Gagliardo.** 2016. International travel is a risk factor for extended-spectrum β-lactamase-producing Enterobacteriaceae acquisition in children: A case-case-control study in an urban US hospital. Travel Medicine and Infectious Disease. **14:**568-571.

35. **Laupland, K. B., D. L. Church, J. Vidakovich, M. Mucenski, and J. D. Pitout.** 2008. Community-onset extended-spectrum β-lactamase (ESBL) producing Escherichia coli: importance of international travel. J. Infect. **57:**441-448.

36. **Paltansing, S., J. A. Vlot, M. E. Kraakman, R. Mesman, M. L. Bruijning, A. T. Bernards, L. G. Visser, and K. E. Veldkamp.** 2013. Extended-spectrum beta-lactamase-producing enterobacteriaceae among travelers from the Netherlands. Emerg. Infect. Dis. **19:**1206-1213. doi: 10.3201/eid.1908.130257 [doi].

37. **Silbergeld, E. K., J. Graham, and L. B. Price.** 2008. Industrial food animal production, antimicrobial resistance, and human health. Annu. Rev. Public Health. **29:**151-169.

38. **Riley, L. W., E. Raphael, and E. Faerstein.** 2013. Obesity in the United States - dysbiosis from exposure to low-dose antibiotics? Front. Public. Health. **1:**69. doi: 10.3389/fpubh.2013.00069 [doi].

39. **Cox, L. M., and M. J. Blaser.** 2015. Antibiotics in early life and obesity. Nature Reviews Endocrinology. **11:**182-190.

40. **Steinberger, J., S. R. Daniels, American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young), and American Heart Association Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism).** 2003. Obesity, insulin resistance, diabetes, and cardiovascular risk in children: an American Heart Association scientific statement from the Atherosclerosis, Hypertension, and Obesity in the Young Committee (Council on Cardiovascular Disease in the Young) and the Diabetes Committee (Council on Nutrition, Physical Activity, and Metabolism). Circulation. **107:**1448-1453.

41. **Hansen, L. H., S. J. Sørensen, H. S. Jørgensen, and L. B. Jensen.** 2005. The prevalence of the OqxAB multidrug efflux pump amongst olaquindox-resistant Escherichia coli in pigs. Microbial Drug Resistance. **11:**378-382.

42. **Goyal, M. K., T. J. Johnson, J. M. Chamberlain, T. C. Casper, T. Simmons, E. A. Alessandrini, L. Bajaj, R. W. Grundmeier, J. S. Gerber, S. A. Lorch, E. R. Alpern, and Pediatric Care Applied Research Network (PECARN).** 2017. Racial and Ethnic Differences in Antibiotic Use for Viral Illness in Emergency Departments. Pediatrics. . doi: e20170203 [pii].

43. **Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, and J. M. Townes.** 2007. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. Jama. **298:**1763-1771.

44. **Popovich, K. J., R. A. Weinstein, A. Aroutcheva, T. Rice, and B. Hota.** 2010. Community-associated methicillin-resistant Staphylococcus aureus and HIV: intersecting epidemics. Clinical Infectious Diseases. **50:**979-987.

45. **Zerr, D. M., S. J. Weissman, C. Zhou, M. P. Kronman, A. L. Adler, J. E. Berry, J. Rayar, J. Myers, W. L. Haaland, and C. D. Burnham.** 2017. The Molecular and Clinical Epidemiology of Extended-Spectrum Cephalosporin–and Carbapenem-Resistant Enterobacteriaceae at 4 US Pediatric Hospitals. Journal of the Pediatric Infectious Diseases Society. piw076.

46. **Logan, L. K., N. P. Braykov, R. A. Weinstein, and R. Laxminarayan.** 2014. Extended-spectrum β-lactamase–producing and third-generation cephalosporin-resistant Enterobacteriaceae in children: trends in the United States, 1999–2011. Journal of the Pediatric Infectious Diseases Society. **3:**320-328.

47. **Lukac, P. J., R. A. Bonomo, and L. K. Logan.** 2015. Extended-spectrum β-lactamase–producing Enterobacteriaceae in children: old foe, emerging threat. Clinical Infectious Diseases. **60:**1389-1397.

48. **Logan, L. K., J. P. Renschler, S. Gandra, R. A. Weinstein, R. Laxminarayan, Centers for Disease Control, and Prevention Epicenters Program.** 2015. Carbapenem-Resistant Enterobacteriaceae in Children, United States, 1999-2012. Emerg. Infect. Dis. **21:**2014-2021. doi: 10.3201/eid2111.150548 [doi].

49. **Logan, L. K., and R. A. Bonomo.** 2016. Metallo-β-lactamase (MBL)-producing Enterobacteriaceae in United States children*In* Anonymous Open forum infectious diseases. Oxford University Press.

CHARACTERISTICS OF FQR AND PMFQR ENTEROBACTERIACEAE

Variable [a]	FQR Ent	PMFQR Ent
Patient	n=82	n=53
Organism [b]		
E. coli	65 (79.3)	40 (75.5)
Klebsiella sp.	7 (8.5)	7 (13.2)
Proteus sp.	7 (8.5)	4 (7.5)
Enterobacter sp.	3 (3.7)	2 (3.8)
Other	0(0)	0 (0)
Source		
Urine	58 (70.7)	37 (69.8)
Respiratory	12 (14.6)	7 (13.2)
Abscess/Wound	4 (4.9)	3 (5.7)
Blood	3 (3.7)	3 (5.7)
Peritoneal/Abdomen	2 (2.4)	1 (1.9)
Central Nervous System	1 (1.2)	1 (1.9)
Other	2 (2.4)	1 (1.9)
Co-Antibiotic Resistance		
Trimethoprim/Sulfamethoxazole	59 (72.0)	38 (71.7)
Gentamicin	44 (53.7)	35 (66.0)
Amikacin	3 (3.7)	3 (5.7)
Carbapenem	1 (1.2)	1 (1.9)

Bla gene association [c]		
$CTX\text{-}M\text{-}1_{group}$	51 (62.2)	42 (79.2)
$CTX\text{-}M\text{-}9_{group}$	13 (15.9)	3 (5.7)
SHV_{ESBL}	9 (11.0)	6 (11.3)
VEB_{ESBL}	1 (1.2)	1 (1.9)
CMY_{AmpC}	2 (2.4)	1 (1.9)
ACT/MIR_{AmpC}	4 (4.9)	1 (1.9)
KPC_{CRE}	1 (1.2)	1 (1.9)
Mutation in QRDR[d]	71 (89.9)	43 (87.8)
Phylogenetic group of *E. coli*	n=65	n=40
B2	49 (75.4)	31 (77.5)
D	11(16.9)	6 (15.0)
A	4 (6.1)	3 (7.5)
B1	1 (1.5)	0 (0)

[a]Values represent n (%). Abbreviations: Ent, Enterobacteriaceae, FQR, Flouroquinolone resistant; PMFQR, Plasmid-mediated Fluoroquinolone resistant

[b] One isolate studied per patient

[c] Isolates may harbor one or more *bla* gene.

[d] 3 of the 82 isolates did not yield a result, calculation based on 79 isolates.

BIVARIATE ANALYSIS OF DEMOGRAPHICS AND FACTORS ASSOCIATED WITH PMFQR ENTEROBACTERIACEAE INFECTION

Characteristic[a]	PMFQR Infection	Non-PMFQR Infection[b]	p value
Patient	n=53	n=131	
Location at Diagnosis			<0.0001
Inpatient, non ICU	21 (39.6)	46 (35.1)	
Outpatient Clinic	16 (30.2)	7 (5.3)	
Emergency Room	4 (7.6)	23 (17.6)	
Pediatric ICU	11 (20.8)	37 (28.2)	
Neonatal ICU	1 (1.9)	18 (13.7)	
Region of Residence[d]			0.047
Downtown	1 (1.9)	19 (14.5)	
Northwest	6 (11.3)	14 (10.7)	
Far North	14 (26.4)	25 (19.1)	
West	12 (26.4)	36 (27.5)	

Southwest	10 (18.9)	8 (6.1)	
South	2 (3.8)	12 (9.2)	
Far South	3 (5.7)	8 (6.1)	
Other IL/Other states	3 (5.7)	9 (6.9)	
Recent Health Care			0.406
Inpatient Care	12 (22.6)	31 (23.7)	
Outpatient Care[e]	31 (58.5)	64 (48.9)	
No Recent Care	10 (18.9)	36 (27.5)	
Central venous line	9 (17.0)	44 (33.6)	0.025
Gastrointestinal	13 (24.5)	45 (34.4)	0.195
Genitourinary	14 (26.4)	37 (28.2)	0.802
Respiratory	11 (20.8)	38 (29.0)	0.253

[a] Values represent n (%) unless otherwise indicated.

[b] Non-PMFQR Infection were children with infections due to Enterobacteriaceae sensitive to extended spectrum cephalosporin and fluoroquinolone antibiotics.

[c] Other sources include abscess/wound, peritoneal/abdomen, or other organ systems

[d] Region of residence includes city region and neighboring suburbs, except for "Downtown", which includes downtown, near North side, loop, and Northside, all within city limits. "Other" includes other cities in Illinois not neighboring Chicago, and patients from other states.

[e] Includes extended-spectrum cephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefepime)

[f] Abbreviation PMFQR, Plasmid-mediated Fluoroquinolone Resistance; TMP-SMX, Trimethoprim-Sulfamethoxazole.

[g] Outpatient care includes care outside of routine child care visits and outpatient procedures.

MULTIVARIABLE ANALYSIS OF FACTORS ASSOCIATED WITH PMFQR ENTEROBACTERIACEAE INFECTIONS IN CHILDREN

Associated Factor with PMFQR infection[a]	OR	95% CI	p value
Outpatient Clinic Location at time of infection diagnosis	33.9	7.08, 162.8	<0.001
Race not white, black or Hispanic	6.5	1.70, 24.3	0.006
Home Residence in Southwest Region (SW Chicago and SW Suburbs)	5.6	1.6, 19.2	0.006
Home Residence in Downtown Region (Near North Side, Loop, Northside)	0.03	0.002, 0.33	0.005

[a] Abbreviations, SW, Southwest; Loop, Chicago Loop.

[b] Reference Region, West Region.