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Xuan Qin
University of Washington - Seattle Campus

Danielle M. Zerr
University of Washington - Seattle Campus

Scott J. Weissman
University of Washington - Seattle Campus

Janet A. Englund
University of Washington - Seattle Campus

Donna M. Denno
University of Washington - Seattle Campus

See next page for additional authors

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Authors
Xuan Qin, Danielle M. Zerr, Scott J. Weissman, Janet A. Englund, Donna M. Denno, Eileen J. Klein, Phillip I. Tarr, Justin Kwong, Jennifer R. Stapp, Luis G. Tulloch, and Emmanouil Galanakis

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Prevalence and Mechanisms of Broad-Spectrum β-Lactam Resistance in *Enterobacteriaceae*: a Children's Hospital Experience▽

Xuan Qin,[1,3]* Danielle M. Zerr,[2,3] Scott J. Weissman,[2,3] Janet A. Englund,[2,3] Donna M. Denno,[2,3] Eileen J. Klein,[2,3] Phillip I. Tarr,[4] Justin Kwong,[1] Jennifer R. Stapp,[1] Luis G. Tulloch,[3] and Emmanouil Galanakis[1]

Microbiology Laboratory, Department of Laboratory Medicine, Children's Hospital and Regional Medical Center, Seattle, Washington[1]; Department of Pediatrics, University of Washington,[2] and University of Washington School of Medicine,[3] Seattle, Washington; and Departments of Pediatrics and Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri[4]

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The objective of this study was to investigate the trends and patterns of resistance in β-lactamase-producing members of the family *Enterobacteriaceae* in a children's hospital over a 9-year period (1999 to 2007). Clinically significant isolates of the *Enterobacteriaceae* were screened for patterns of broad-spectrum resistance to β-lactams. The strains likely to be resistant were subsequently confirmed by an inhibitor-based disc test. The plasmid-mediated resistance determinants in these isolates were identified by PCR and by in vitro transformation, which successfully reproduced the AmpC phenotype unrestricted by the species of the host organisms. Among 8,048 *Enterobacteriaceae* isolates belonging to the four chromosomal *ampC*-negative or -nonfunctional genera, 86 (1.07%) isolates (56 *Escherichia coli* isolates, 22 *Klebsiella* species isolates, 1 *Proteus mirabilis* isolate, and 7 *Salmonella* species isolates) exhibited broad-spectrum β-lactam resistance patterns. These organisms collectively produced three classes of β-lactamases, including class A extended-spectrum β-lactamases (n = 47), class C or AmpC β-lactamases (n = 36, including 4 isolates that produced both class A and class C enzymes), and class A or B carbapenem-hydrolyzing β-lactamases (n = 3). The proportion increased from 0.46% during the first 3 years to 1.84% during the last 3 years (relative risk [RR], 4.04; 95% confidence interval [CI], 2.28 to 7.42; $P < 0.001$). The increase was mainly due to the emergence of a plasmid-mediated bla_{CMY-2} β-lactamase, the incidence of which increased from 0.11% during the first 3 years to 0.96% during the last 3 years (RR, 9.11; 95% CI, 2.76 to 30.1; $P = 0.001$). Class A-type resistance increased slightly during the study period, from 0.35% during the first 3 years to 0.85% during the last 3 years (RR, 2.42; 95% CI, 1.15 to 5.07; $P = 0.02$). A *Proteus mirabilis* strain was documented to possess a novel bla_{DHA} determinant. Of special concern, three carbapenemase-producing isolates were identified between 2003 and 2006. The infections caused by resistant isolates of the *Enterobacteriaceae* mainly affected hospitalized patients with underlying conditions; however, 19 (22%) episodes were of community onset in otherwise well children. The rate of resistance to broad-spectrum β-lactams among isolates of the *Enterobacteriaceae* is increasing in children in both hospital- and community-acquired settings, and the resistance is driven largely by plasmid-mediated AmpC β-lactamases. These data have important implications for empirical antimicrobial strategies targeting serious pediatric infections. Further study of this problem is warranted.

The ever increasing variety of β-lactamases produced by isolates of the family *Enterobacteriaceae* raises concerns about our dependence on β-lactam drugs and the emergence of panresistant species. Rapidly emerging β-lactamases include extended-spectrum β-lactamases (molecular class A β-lactamases and, to a lesser extent, class D β-lactamases in the *Enterobacteriaceae*), AmpC β-lactamases (class C), and carbapenem-hydrolyzing β-lactamases (mainly those of classes B, A, and D) (2, 4, 12, 18, 20). *Escherichia coli* and *Klebsiella* spp. producing class A extended-spectrum β-lactamase (ESBL) enzymes have been well documented in the United States, but the emergence of bla_{CTX-M} determinants is a recent finding (2, 16). Plasmid-encoded bla_{CMY}, bla_{DHA}, and bla_{ACC} determinants producing class C enzymes are being described with increasing frequencies.

Class A ESBLs and class C (AmpC) β-lactamases hydrolyze extended-spectrum cephalosporins, but AmpC β-lactamases are able to actively hydrolyze cephamycins and are resistant to inhibition by clavulanate or other β-lactamase inhibitors in vitro (4, 18, 20). Carbapenemases have a broader range activity than class A or class C β-lactamases and affect the activities of carbapenems as well as those of cephalosporins (4, 13, 18, 20). The spread of most broad-spectrum β-lactamases is facilitated by transferable and transconjugable plasmids, which often carry other resistance genes by means of their integron architecture (12). Genes encoding ESBLs and carbapenemases are located on plasmids, while historically, genes encoding AmpC β-lactamases have primarily been located on the chromosomes of certain genera of the family *Enterobacteriaceae* (17). Recently, however, *ampC* genes were documented to episomalize into plasmids and disseminate into various species of the family *Enterobacteriaceae* (29). The plasmid-borne *ampC* gene is often constitutively expressed and may confer high-level resistance to β-lactams (20).

Resistant isolates of the *Enterobacteriaceae* have recently

* Corresponding author. Mailing address: Microbiology Laboratory, Department of Laboratory Medicine, 6P-1, Children's Hospital and Regional Medical Center, 4800 Sand Point Way, NE, Seattle, WA 98105-0371. Phone: (206) 987-2586. Fax: (206) 987-3840. E-mail: xuan.qin@seattlechildrens.org.

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emerged as a problem in adults, both in hospital settings and in community settings. The *Enterobacteriaceae* are also major pathogens in neonates, infants, and children, although little is known about the broad-spectrum β-lactamase-producing strains in this specific age group (7, 9, 19, 25–26, 28). The spread of β-lactam-resistant *Enterobacteriaceae* in children is of particular importance, since fluoroquinolones are not approved for use in this age group and are not considered first-line agents for use in this age group. Given the clinical impact of *Enterobacteriaceae* during childhood, we set out to investigate the epidemiology of highly resistant organisms and the molecular determinants of resistance in a pediatric hospital setting.

MATERIALS AND METHODS

Setting. Children's Hospital and Regional Medical Center (CHRMC) in Seattle, WA, is a 250-bed tertiary-care pediatric hospital with 13,000 patient admissions annually. The study included specimens collected over a 9-year period (from January 1999 to December 2007). The study was approved by the CHRMC Institutional Review Board.

Isolation and antibiotic susceptibility testing. In total, 8,048 isolates belonging to only five species, *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, and *Salmonella enterica*, were included. These isolates met the commonly used criteria (10) for clinically significant isolates (an organism isolated from a sterile body site or a significant quantity of growth from nonsterile sites, such as urine, wounds, or tracheal aspirates) and received a full microbiological evaluation, including the performance of antibiotic susceptibility tests. Organisms isolated from the same patient at intervals of >7 days were included. The majority of specimens were obtained to evaluate specific illnesses. In 2006, surveillance for multiresistant *E. coli* and/or *Klebsiella* spp. from patients hospitalized with cancer or for bone marrow or solid organ transplantation was initiated. In these cases, a stool or rectal swab sample for culture was obtained upon admission and periodically (<1 per month) after extended hospitalization.

Of the 8,048 total isolates, 86 were further characterized for their broad-spectrum β-lactam resistance phenotypes. Clinical samples were processed in the CHRMC Microbiology Laboratory according to standard operating procedures. Antibiotic susceptibility was determined by the standard disc diffusion method on Mueller-Hinton agar or by determination of the MIC by use of the Vitek system (11, 13, 24). The zones of inhibition were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) (3). The susceptibilities of all isolates to ampicillin, amoxicillin-clavulanic acid, aztreonam, cefazolin, cefuroxime, cefotetan (determined only with the Vitek system), ceftazidime, ceftriaxone, cefepime, meropenem, piperacillin-tazobactam, ciprofloxacin, gentamicin, and sulfamethoxazole-trimethoprim were determined; susceptibility to nitrofurantoin was tested only for the urinary isolates.

Characterization of β-lactamase-producing strains. On the basis of the criteria of the CLSI, the paired disc diffusion method was applied on Mueller-Hinton agar by using multiple discs with ceftazidime (30 μg), cefotaxime (30 μg), ceftazidime-clavulanic acid (30/10 μg), cefotaxime-clavulanic acid (30/10 μg), and cefpodoxime (30 μg); and tests with cefepime (30 μg), imipenem (10 μg), and/or meropenem (10 μg) were often repeated to rule out the presence of potential carbapenemase producers. ESBL production was defined as an increase in the zone of inhibition of ≥5 mm with either ceftazidime or cefotaxime discs when they were tested in combination with discs containing clavulanic acid (3, 11). The class C β-lactamase resistance or AmpC phenotype was defined as a change in the diameter of the zone of inhibition of <5 mm between discs containing the aforementioned drugs in combination with discs containing clavulanate, in addition to a likely susceptible zone of inhibition around the cefepime disc. Isolates *E. coli* ATCC 25922 and *K. pneumonia* ATCC 700603 were used as control strains.

PCR for β-lactamase genes. For those isolates that met the criteria for either the ESBL resistance or the AmpC phenotype, a five-gene panel for the amplification of the bla_{CMY}, bla_{DHA}, bla_{ACC}, bla_{TEM}, and bla_{CTX-M} genes was applied. The primers for bla_{IMP} and bla_{KPC} were used only with those isolates that exhibited resistance to carbapenems. The specific PCR primers and the previously described assay methods used and are listed in Table 1. A subset of amplicons showing the expected molecular mass was further sequenced, and a search for the homology of the sequences with the sequences of specific genetic determinants was performed.

TABLE 1. Specific primers used for molecular amplification and partial sequencing of the *bla* genes

bla gene	Oligonucleotide sequence	Product length (bp)	Reference or source
bla_{CMY-2}	TGG CCA GAA CTG ACA GGC AAA TTT CTC CTG AAC GTG GCT GGC	461	21
bla_{DHA}	AAC TTT CAC AGG TGT GCT GGG T CCG TAC GCA TAC TGG CTT TGC	405	21
bla_{ACC}	AAC AGC CTC AGC AGC CGG TTA T TTC GCC GCA ATC ATC CCT AGC	346	21
bla_{TEM}	GAA AGG GCC TCG TGA TAC GC TCA TCC ATA GTT GCC TGA CTC C	1,005	1
bla_{CTX-M}	CGA TGT GCA GTA CCA GTA A TTA GTG ACC AGA ATC AGC GG	585	2
bla_{IMP}	GTA CAG TCT ATG CCT CG GTA MGT TTC AAG AGT GAT GC	750	This study
bla_{KPC}	GCT ACA CCT AGC TCC ACC TTC ACA GTG GTT GGT AAT CCA TGC	988	This study

Plasmid content and transformation. Plasmid DNA was extracted from the isolates of *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *Salmonella* spp. that tested positive for bla_{CMY} by use of a QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany). The *E. coli* competent cells (Qiagen EZ kit) were transformed, and the transformants were selected on Luria-Bertani agar containing ceftazidime (10 μg/ml) (GlaxoSmithKline, Lisbon, Portugal) (9). The *E. coli* transformants were reexamined both phenotypically for the reproducibility of the susceptibility patterns and genotypically for the presence of the bla_{CMY} determinants compared to the phenotypes and genotypes of the corresponding host organisms.

Clinical variables. Clinical data were collected from the medical records of children receiving care at CHRMC. The clinical variables included gender, age, the duration of hospitalization, the number of previous hospitalizations, the history of the patient's antibiotic regimen prior to sampling, the presence of catheters, underlying conditions, the site of sampling, the setting of the sampling, and the outcome of infection.

Statistics. Statistical analysis was performed with the EpiInfo software program (version 3.4; Centers for Disease Control and Prevention). The proportions between groups were compared by the chi-square test. Relative risks (RRs; with Taylor series 95% confidence intervals [CIs]) were calculated.

RESULTS

Prevalence of β-lactamase-producing *Enterobacteriaceae*. Eighty-six (1.07%) isolates of the five specified species from 79 patients met the criteria of the CLSI (according to either the zone of inhibition or the MIC) for further drug susceptibility testing to determine the mechanisms of β-lactam resistance (Table 2). The isolates were derived from multiple specimen types (53 from urine specimens; 9 from blood specimens; 8 from stool specimens; 3 from wound or abscess specimens; 2 from peritoneal fluid specimens; and 1 each from cerebrospinal fluid, eye swab, genital swab, tracheal aspirate, and gastrointestinal fluid specimens). In three cases, the same strain was isolated from more than one site (only one strain from each of these patients was used), while more than one species was isolated from four urine specimens and a single blood culture (all bacterial species from these patients were included). Two earlier isolates had no recorded links to patients. The frequency of resistant organisms increased significantly during the 9-year study period ($P < 0.001$, chi-square test for trend). The proportion increased from 0.46% during the first 3 years to 1.84% during the last 3 years (RR, 4.04; 95% CI, 2.28 to 7.42; $P < 0.001$). This increase was observed both for *E. coli* isolates, the prevalence of which increased from 0.41% to 1.82% (RR, 4.67; 95% CI, 2.16 to 9.25; $P < 0.001$), and for non-*E. coli*

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TABLE 2. Characterization of gene determinants in 86 isolates of the *Enterobacteriaceae* exhibiting resistance to broad-spectrum β-lactams[a]

Isolate and resistance determinant	All isolates		AmpC-producing strains		ESBL-producing strains		Carbapenemase-producing strains	
	No. of strains that met the CLSI criteria/total no. tested (%)	No. of strains isolated from urine	No. of strains that met the CLSI criteria/total no. tested (%)	No. of strains isolated from urine	No. of strains that met the CLSI criteria/total no. tested (%)	No. of strains isolated from urine	No. of strains that met the CLSI criteria/total no. tested (%)	No. of strains isolated from urine
Escherichia coli	56/6,097 (0.9)	44	22/56 (39.2)	16	32/56 (57.1)	28	2/56 (3.6)	0
bla_{CMY}			19	14				
bla_{DHA}			2	1				
bla_{TEM}			2	2	21	20		
bla_{CTX-M}					10	10		
bla_{IMP}							2	
Negative for all determinants			1	1	4	4		
Missing isolates					1			
Klebsiella pneumoniae	17/941 (1.8)	11	6 (35.3)	4	10 (58.8)	7	1 (5.9)	0
bla_{CMY}			3	2				
bla_{DHA}			3	2				
bla_{TEM}			2	2	3	2		
bla_{CTX-M}			1	1	2	2	1	
bla_{KPC}								
Missing isolates					5	3		
Klebsiella oxytoca	5/398 (1.3)	1	0 (0)		5 (100)	1	0 (0)	
bla_{TEM}					2	1		
Negative for all determinants					1			
Missing isolates					2			
Proteus mirabilis	1/397 (0.25)	1	1 (100)	1	0 (0)		0 (0)	
bla_{DHA}			1	1				
Salmonella spp.	7/215 (3.3)	0	7/7 (100)		0 (0)		0 (0)	
bla_{CMY}			7					
Total	86/8,048 (1.1)	57	36 (41.9)	21	47 (54.6)	36	3 (3.5)	0

[a] The resistant isolates were from among 8,048 isolates of the *Enterobacteriaceae* isolated from clinical specimens at CHRMC from 1999 to 2007. More than one determinant may be present in some isolates.

isolates, the prevalence of which increased from 0.62% to 1.89% (RR, 3.06; 95% CI, 1.01 to 9.25; $P = 0.04$).

Class A ESBL-type resistance. Overall, 47 (0.58%) isolates were identified as ESBL producers, and 39 of these were available for molecular characterization. Genes encoding bla_{TEM} were detected in 26 isolates, and those encoding bla_{CTX-M} were detected in 13 isolates; 5 of these isolates contained both determinants, and 5 isolates were negative for both determinants (Table 2). The rate of class A-type resistance increased from 0.35% during the first 3 years to 0.85% during the last 3 years (RR, 2.42; 95% CI, 1.15 to 5.07; $P = 0.02$) (Fig. 1). The ESBL producers often demonstrated coresistance to gentamicin (45%), ciprofloxacin (46%), and sulfamethoxazole-trimethoprim (85%). Coresistance to all three agents occurred in 10% of the ESBL producers. Of the class A ESBL producers, 40% showed susceptibility to cefepime in vitro, although the clinical utility of this finding remains to be determined.

Class C AmpC-type resistance. AmpC-type resistance was observed in 36 (0.45%) isolates. The frequency of AmpC-type resistance increased sharply over the study period, from 0.11% during the first 3 years to 0.96% during the last 3 years (RR, 9.11; 95% CI, 2.76 to 30.1; $P < 0.001$) (Fig. 1). Among these isolates, 29 were positive for bla_{CMY}, 6 were positive for bla_{DHA}, and 1 remaining *E. coli* isolate was negative for all three *ampC* determinants (bla_{CMY}, bla_{DHA}, and bla_{ACC}) (Table 2). Two *E. coli* isolates and one *K. pneumoniae* isolate contained dual resistance determinants (bla_{CMY} and bla_{TEM}), and a single *K. pneumoniae* isolate contained triple resistance determinants (bla_{DHA}, bla_{TEM}, and bla_{CTX-M}). Sequencing of the bla_{CMY} amplification products revealed bla_{CMY-2}-like cod-

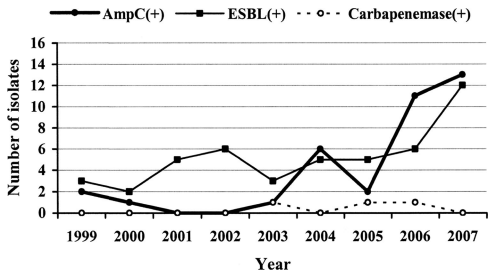

FIG. 1. Number of *Enterobacteriaceae* strains producing class A ESBLs, class C AmpC, or carbapenem-hydrolyzing β-lactamases. (+), positive.

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ing sequences in all 29 bla_{CMY}-positive isolates. Coresistance to other classes of antibiotics, such as gentamicin (17%), ciprofloxacin (19%), and sulfamethoxazole-trimethoprim (39%), appeared to be less pronounced in AmpC producers than in ESBL producers.

Our first bla_{CMY}-possessing isolate was a *K. pneumoniae* strain isolated in 2003 from the urine of an 18-year-old female patient with neurogenic bladder. Several months later, two bla_{CMY}-possessing *Salmonella* serovar 4,[5],12:i:− strains were isolated from the stools of two children; these were related to a food-borne outbreak in King County, WA, in the spring of 2004. Later in 2004, two *E. coli* strains were isolated from two children with prolonged hospitalizations: one isolate from the urine of a child with myelomeningocele and one isolate from a wound of a child with neuroblastoma. Following this increase in the incidence of bla_{CMY}-possessing isolates, AmpC-type resistance became more common than the previously dominant class A ESBL-type resistance (Fig. 1).

Plasmids carrying bla_{CMY} that were extracted from *E. coli* and *Salmonella* isolates were transferable in vitro. The AmpC phenotype and its corresponding genetic determinants were reproducible in *E. coli* transformants obtained with a Qiagen EZ kit by susceptibility testing and PCR. The cotransfer of antibiotic resistance to other antibiotics, such as gentamicin and sulfamethoxazole-trimethoprim, was not observed in the 15 *E. coli* transformants positive for bla_{CMY} obtained with the Qiagen EZ kit.

Isolates carrying both class A and class C resistance determinants. Four organisms (two *K. pneumoniae* isolates and two *E. coli* isolates) contained both class A and class C determinants. These organisms, which were isolated from cultures of urine from unrelated patients, exhibited multidrug resistance phenotypes. Two patients had histories of recurrent urinary tract infections (UTIs), and the resistant isolates (one *E. coli* isolate and one *K. pneumoniae* isolate, both of which carried bla_{CMY} and bla_{TEM}) were each associated with a single episode of infection. The *K. pneumoniae* strain was sensitive only to carbapenems and was isolated from a child who had undergone a urological procedure. An *E. coli* isolate that carried bla_{CMY} and bla_{TEM} and that was also gentamicin resistant was isolated from a third patient who had undergone bone marrow transplantation. The last patient had multiple congenital anomalies, and both an *E. coli* isolate (carrying bla_{TEM} and bla_{CTX-M}) and a *K. pneumoniae* isolate (carrying bla_{DHA}, bla_{TEM}, and bla_{CTX-M}) were isolated from the patient. These two isolates were susceptible to both carbapenems and ciprofloxacin.

Resistance to carbapenems. Two *E. coli* and one *K. pneumoniae* strains were found to be resistant to all β-lactams, including carbapenems. The two *E. coli* strains were found to contain the bla_{IMP-4} gene, and the *K. pneumoniae* strain was positive for bla_{KPC-2}. The two *E. coli* strains did not appear to be related. They were isolated 1 year apart (in December 2003 and December 2004, respectively), and the patients' hospital stays did not overlap. In addition, the patterns of susceptibility of the two isolates to aminoglycosides and quinolones were different. The *K. pneumoniae* strain containing bla_{KPC-2} was isolated in the fall of 2006 and was susceptible only to tigecycline and colistin. Both of the children from whom IMP-4-producing *E. coli* strains were isolated had previously been seen in other institutions (one in Alaska and the other in Mexico), and their antibiotic treatment histories were not available. The child from whom the KPC-2-producing *K. pneumoniae* strain was isolated had arrived in Seattle shortly after a prolonged hospitalization in the northeastern United States.

Patients. The infected patients ranged in age from 4 days to 25.5 years (median, 5.5 years). Among the infants aged <1 year, resistant isolates were more common in males (male-to-female ratio, 1.8:1), but beyond infancy, females were overrepresented (female-to-male ratio, 2.2:1; $P < 0.05$). The majority of the patients had chronic underlying conditions (60/79, or 76%), including urological, neurological, and hematological diseases requiring multiple hospitalizations, indwelling catheters, and multiple antibiotic regimens. Seventeen patients had severe urogenital diseases, 16 had leukemia, 4 had solid tumors, 5 had myelomeningocele, and 5 had undergone transplantation. Of the 79 cases, UTIs appeared to be the most common clinical manifestation (53 isolates were obtained from urine specimens). Urine was the most common source of *E. coli* (77%) and *K. pneumoniae* (65%) isolates (Table 2). The proportion of *E. coli* isolates obtained from urine was high regardless of the determinant of resistance (14/19 isolates [73%] from urine for bla_{CMY} versus 20/21 isolates [95%] from urine for bla_{TEM} and 10/10 isolates [100%] from urine for bla_{CTX-M}; Table 2). A similar pattern was observed for the *K. pneumoniae* isolates. Only one of the five *K. oxytoca* strains was isolated from urine. All seven *Salmonella* isolates were associated with gastroenteritis, and three of these isolates were linked with food-borne outbreaks. The child from whom the KPC-2-producing *K. pneumoniae* strain (a blood isolate) was obtained was the only patient who appeared to have died as a direct result of the infection.

Resistance in the community setting. Community-acquired infections were suggested in 19 (22.1%) cases on the basis of the patients' clinical histories. Seven of these children were otherwise well and presented with nontyphoidal *Salmonella* gastroenteritis over a period of 3 years. The *Salmonella* serotypes isolated were *S. enterica* serotypes Heidelberg, Minnesota, Newport, and Typhimurium and *Salmonella* serovar 4,[5],12:i:−; and all isolates contained plasmid-encoded bla_{CMY-2}. *Salmonella* serotype Heidelberg was isolated from both the stool and the blood of a single child. The remaining 12 children infected with resistant isolates of the *Enterobacteriaceae* presented with community-acquired UTIs which were caused by *E. coli* in 10 cases (bla_{TEM}, $n = 3$; bla_{CMY}, $n = 3$; bla_{CTX}, bla_{DHA}, and bla_{TEM}-bla_{CTX}, $n = 1$ each) and by *K. pneumoniae* in 2 cases (bla_{TEM}, $n = 1$; bla_{DHA}-bla_{TEM}-bla_{CTX}, $n = 1$). Only four children in this category had a clear history of antibiotic treatment.

Persistent colonization and recurrent infection. Persistent colonization was found in five hematology-oncology patients and one patient with short bowel syndrome. The longest period of persistent colonization and recurrent infection (of both urine and blood) documented to date was in the patient with short bowel syndrome, who was colonized by an *E. coli* isolate containing bla_{TEM} for more than 4 years. No single case of clearance was documented by surveillance cultures. These six cases with persistent colonization included all three patients who were infected by the carbapenemase producers. Two of the six patients were also simultaneously colonized by vanco-

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mycin-resistant enterococci. Overall, recurrent infections were documented in at least 14 patients, 10 of whom had recurrent UTIs, 3 of whom had chronic wound infections, and 1 of whom had recurrent bloodstream infections. Two different isolates were recovered from one patient. Among the 14 patients with recurrent infections, 6 were hematology-oncology patients and the remaining 8 had renal insufficiency or other chronic medical conditions. Among the 15 organisms causing recurrent infections, 8 produced class A β-lactamases (bla_{TEM}, $n = 4$; bla_{CTX}, $n = 2$; bla_{TEM}-bla_{CTX}, $n = 2$) and 5 produced class C β-lactamases (bla_{CMY}, $n = 3$; bla_{DHA}, $n = 1$; bla_{DHA}-bla_{TEM}-bla_{CTX}, $n = 1$); no genetic determinant was found in 2 isolates.

DISCUSSION

We found a statistically significant increase in the rate of resistance among isolates of the family *Enterobacteriaceae* over a 9-year study period. The resistance patterns were characterized by a modest increase in the prevalence of ESBL-producing strains; by the sporadic, although worrisome, occurrence of carbapenemase-producing strains; and by the sharp increase in the incidence of plasmid-mediated AmpC-producing strains, driven by the emergence of a plasmid containing bla_{CMY-2}. This is the first surveillance study of resistance among isolates of the *Enterobacteriaceae* in the pediatric population of which we are aware. Our findings suggest that plasmid-mediated β-lactamase-producing isolates of the family *Enterobacteriaceae* are of increasing concern in pediatrics, both in the hospital setting and in the community setting.

In our study, class A ESBL-producing *Enterobacteriaceae* mainly possessed the bla_{TEM} and the bla_{CTX-M} genes. *Enterobacteriaceae* harboring transferable bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{OXA} genes have been reported in clinical isolates worldwide (23, 25–26, 28, 30) and in commensal isolates in healthy children in Bolivia and Peru (19). Among the various ESBL enzymes, those of the CTX-M type have become predominant worldwide in both hospital and community settings (2, 4).

Plasmid-mediated AmpC resistance has recently been reported in clinical isolates of the *Enterobacteriaceae* worldwide (6, 7, 9, 22, 23, 30–32). Few population-based studies have reported on the frequency or clinical impact of these isolates, and very little is known about the associated epidemiology in pediatrics. In our study, the incidence of AmpC β-lactamase producers increased sharply due to the emergence of a bla_{CMY-2}-containing plasmid, and they appeared to be present in a wider range of *Enterobacteriaceae* isolates than the class A β-lactamases were.

In our study, the most frequent determinants of resistance in *E. coli* isolates were bla_{CMY-2} ($n = 19$), bla_{TEM} ($n = 21$), and bla_{CTX-M} ($n = 10$). Although we did not detect epidemiological links between the patients, the homogeneity suggests the potential transmission of bacterial strains between patients. The application of techniques for the determination of strain relatedness may be warranted in future investigations in order to reveal potential routes of transmission.

This study produced several novel or unusual findings, including the isolation of a *P. mirabilis* strain containing a bla_{DHA} determinant from a pediatric patient; the finding of three carbapenemase-producing isolates of *Enterobacteriaceae*, especially IMP-4-producing *E. coli* isolates, in pediatric patients; and the isolation of organisms with multiple determinants of broad-spectrum resistance from a pediatric population. The emergence of carbapenemase-producing *Enterobacteriaceae*, especially bla_{KPC} carriers, has already been reported in New York City and was subsequently reported in Europe and on other continents (5, 12, 20); but few cases have been reported in pediatric patients. Similarly, reports of organisms with multiple determinants of resistance are rare. In our study, six *E. coli* strains each contained two class A resistance determinants (bla_{TEM} and bla_{CTX-M}), while two *E. coli* strains and two *K. pneumoniae* strains each contained both class A (bla_{TEM} or bla_{CTX-M}) and class C (bla_{CMY} or bla_{DHA}) resistance determinants. *K. pneumoniae* strains harboring genes for both class A (bla_{SHV}) and class C (bla_{DHA}) resistance determinants were recently reported in adults in Belgium (27). We are unaware of reports of such organisms in pediatric patients. Genes encoding β-lactamases are frequently transferred on large plasmids together with genes encoding resistance to aminoglycosides, sulfonamides, doxycycline, and chloramphenicol (26). Multidrug resistance in the *Enterobacteriaceae* has crucial clinical implications in pediatrics, as it renders the empirical regimens (ampicillin plus gentamicin or ceftriaxone/cefotaxime) for the treatment of septicemia in children ineffective. Multidrug resistance trends require close monitoring, and the epidemiology of such resistance requires a better understanding so that empirical antimicrobial approaches may be effective, especially in the setting of critical illness.

The persistent nature of colonization with broad-spectrum β-lactam-resistant organisms was demonstrated in six patients in our study who were monitored periodically by the use of surveillance cultures. This has previously been demonstrated by others (14). We now routinely perform rectal swab surveillance cultures for patients undergoing bone marrow and solid organ transplantation to rule out the presence of vancomycin-resistant enterococci and broad-spectrum β-lactam-resistant *Enterobacteriaceae*. These data are then available to inform the empirical management of invasive infections in these patients.

Resistant isolates of the *Enterobacteriaceae* have historically been of nosocomial origin, and several factors have been shown to contribute to their emergence (4, 8, 9, 11–15, 28). However, community-acquired infections are increasingly recognized (2, 4, 14, 15, 23) and were quite common in our study, in which eight children with UTIs caused by resistant *Enterobacteriaceae* had no record of a previous hospital stay and/or a prolonged antibiotic treatment history. Further research on multidrug-resistant *Enterobacteriaceae* isolates from the community setting is warranted.

Our findings demonstrate the growing problem of broad-spectrum plasmid-mediated β-lactam resistance in pediatric *Enterobacteriaceae* isolates. The emergence of class C plasmid-mediated bla_{CMY} genes among diverse genera of the *Enterobacteriaceae* and the isolation of carbapenemase-producing organisms from children are of concern. The emergence of such a variety of resistant organisms poses a significant threat to pediatric patients, for whom β-lactams are the mainstay of empirical therapy for critical illness and quinolones are not commonly used as first-line therapies. We believe that the judicious use of antimicrobials and the implementation of infection control principles are important for the prevention of the further spread of resistance in *Enterobacteriaceae* both in

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the hospital and in the community. It is likely that such interventions are possible and will be most effective only when the overall resistance rate is still low. The broad-spectrum plasmid-mediated β-lactam resistance trends in pediatric *Enterobacteriaceae* isolates require continued close monitoring, and further study of the epidemiology of such resistance appears to be warranted so that potential interventions that may be used to halt the spread of resistance may be developed.

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ERRATUM

Prevalence and Mechanisms of Broad-Spectrum β-Lactam Resistance in *Enterobacteriaceae*: a Children's Hospital Experience

Xuan Qin, Danielle M. Zerr, Scott J. Weissman, Janet A. Englund, Donna M. Denno, Eileen J. Klein, Phillip I. Tarr, Justin Kwong, Jennifer R. Stapp, Luis G. Tulloch, and Emmanouil Galanakis

Microbiology Laboratory, Department of Laboratory Medicine, Children's Hospital and Regional Medical Center, Seattle, Washington; Department of Pediatrics, University of Washington, and University of Washington School of Medicine, Seattle, Washington; and Departments of Pediatrics and Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri

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