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Previous Antibiotic Exposure Increases Risk of Infection with Extended-Spectrum-β-Lactamase- and AmpC-Producing *Escherichia coli* and *Klebsiella pneumoniae* in Pediatric Patients

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The objective of this study was to determine whether antibiotic exposure is associated with extended-spectrum-beta-lactamase- or AmpC-producing *Escherichia coli* or *Klebsiella pneumoniae* infections in children. We collected extended-spectrum-beta-lactamase- or AmpC-producing *E. coli* or *K. pneumoniae* isolates and same-species susceptible controls from normally sterile sites of patients aged ≤21 years, along with associated clinical data, at four free-standing pediatric centers. After controlling for potential confounders, the relative risk of having an extended-spectrum-beta-lactamase-producing isolate rather than a susceptible isolate was 2.2 times higher (95% confidence interval [CI], 1.49 to 3.35) among those with antibiotic exposure in the 30 days prior to infection than in those with no antibiotic exposure. The results were similar when analyses were limited to exposure to third-generation cephalosporins, other broad-spectrum beta-lactams, or trimethoprim-sulfamethoxazole. Conversely, the relative risk of having an AmpC-producing versus a susceptible isolate was not significantly elevated with any antibiotic exposure in the 30 days prior to infection (adjusted relative risk ratio, 1.12; 95% CI, 0.65 to 1.91). However, when examining subgroups of antibiotics, the relative risk of having an AmpC-producing isolate was higher for patients with exposure to third-generation cephalosporins (adjusted relative risk ratio, 4.48; 95% CI, 1.75 to 11.43). Dose-response relationships between antibiotic exposure and extended-spectrum-beta-lactamase-producing or AmpC-producing isolates were not demonstrated. These results reinforce the need to study and implement pediatric antimicrobial stewardship strategies, and they indicate that epidemiological studies of third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates should include resistance mechanisms when possible.

Emerging antibiotic resistance is a serious threat to global public health. Multidrug resistance in *Enterobacteriaceae* specifically is a growing concern due to the continual increase in rates of resistance, the rapid emergence of new mechanisms of resistance, and a limited pipeline of new antibacterial agents (1, 2).

Antibiotic use promotes antibiotic resistance by selecting for antibiotic-resistant organisms and/or by disrupting the antibiotic-susceptible flora within individuals (3). Multiple studies of adult patients have demonstrated an association between prior antibiotic exposure and infection with extended-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* species (4–10), but there are fewer data from pediatric settings. Additionally, very few studies have specifically examined the role of antibiotic use in the development of AmpC-producing infections (11–16). Although recent data suggest that AmpC-producing *Enterobacteriaceae* may be increasingly prevalent within pediatric settings, the epidemiology of AmpC-producing infections in pediatrics has not been well characterized (17).

The objective of this study was to investigate the relationship between prior antibiotic exposure and subsequent ESBL- and AmpC-producing *E. coli* and *Klebsiella pneumoniae* infections in pediatric patients. We also sought to examine differential risks in hospitalization for treatment of the infection between pediatric patients with ESBL- or AmpC-producing isolates and those with susceptible isolates.

MATERIALS AND METHODS

Setting and institutional review. This prospective surveillance study involved four hospitals, referred to as "West," "Midwest 1," "Midwest 2," and "East." The Institutional Review Board at each hospital approved the study protocol.

Subjects and study isolates. Between 1 September 2009 and 30 September 2013, participating hospitals collected all extended-spectrum-cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates recovered from urine or other normally sterile sites during routine clinical care of both hospitalized and outpatient children ≤21 years of age. These candidate

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resistant case isolates included those nonsusceptible to ceftriaxone, cefotaxime, ceftazidime, cefepime, or aztreonam. For each resistant case isolate, three subsequent same-species isolates that were susceptible to the aforementioned agents were collected; these isolates will be referred to hereinafter as susceptible controls. Each hospital used its routine clinical microbiological methods to preliminarily classify isolates as susceptible or resistant. Isolates were archived at −70°C and shipped to the coordinating center quarterly. The date of isolate collection for both cases and controls represented the index date.

Coordinating center methods for confirmation and further characterization of study isolates. (i) Overview. Upon arrival from participating laboratories, candidate resistant isolates and control isolates were further evaluated at the coordinating center using standardized methods to confirm species and antibiotic susceptibility and to characterize resistance phenotype (ESBL versus AmpC producing) and genotype as described below.

(ii) Identification. Study isolates were identified to the species level using the Vitek card for identification of Gram-negative organisms (GN ID card; bioMérieux).

(iii) Antibiotic susceptibility testing. Antibiotic susceptibility was determined by disk diffusion. All isolates were tested for susceptibility to ampicillin, amoxicillin-clavulanic acid, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, meropenem, piperacillin-tazobactam, ciprofloxacin, gentamicin, and sulfamethoxazole-trimethoprim. The cephalosporin breakpoints recommended by CLSI in 2010 (18) were applied to all candidate resistant isolates.

(iv) Phenotypic characterization. The class A ESBL phenotype was characterized using paired disk diffusion and Etests (19, 20). The class C AmpC phenotype was identified using cefepime and cefoxitin disks and Etest strips (bioMérieux) containing cefotetan with and without cloxacillin (21, 22). Control strains included the CLSI-recommended strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 and a laboratory-characterized *E. coli* strain containing $bla_{\text{CMY-2}}$ (19).

(v) Resistance genotyping. All study isolates (cases and controls) were tested by PCR using primer sets for genes encoding common extended-spectrum cephalosporinases, including class A CTX-M and extended-spectrum TEM and SHV, as well as class C CMY, DHA, and FOX (see Table S1 in the supplemental material) (19, 23–25). Because narrow-spectrum bla_{SHV}-type ampicillinases are ubiquitous chromosomal traits among *K. pneumoniae*, all isolates of this species were screened for the presence of extended-spectrum bla_{SHV} variants (typically associated with IS*26* elements) using a combination of primers, as previously described (19, 26). Assembly and alignment of nucleotide sequences were performed to type the genetic determinants as previously described (19). Given the high prevalence of narrow-spectrum TEM among *Enterobacteriaceae*, sequencing of TEM amplicons was carried out only in case isolates with no resistance determinants detected.

Clinical data. Demographic and clinical data were collected from the medical records of cases and controls using standardized case report forms. Data on underlying medical conditions were collected and categorized using the strategy developed by Feudtner et al. (see Table S2 in the supplemental material) (27). Additionally, we added vesicoureteral reflux and neurogenic bladder (categorized as urologic) and neurogenic bowel (categorized as gastrointestinal) to our data collection form, as these conditions were not included in the strategy of Feudtner et al. For patients contributing urine isolates, symptom and culture data (collection method, etc.) were collected. Patients were characterized as likely having a urinary tract infection (UTI) if the culture was considered clinically significant (i.e., met standard microbiology laboratory criteria for susceptibility testing) (28) and/or the patient had symptoms of a UTI (presence of fever, abdominal/flank pain, vomiting, change in color or odor of urine, change in continence pattern, hematuria, dysuria, or frequency/urgency). All documented exposures to systemic (i.e., oral or intravenous) inpatient and outpatient antibiotic treatment and prophylaxis in the year prior to the index date were collected. Outpatient antibiotic and prophylaxis data were collected from orders or prescriptions from pharmacy records or from clinical chart notes. These data were recorded by the calendar month of exposure using the case report form. Inpatient antibiotic treatment exposures were obtained from the Pediatric Health Information System (PHIS) database, and the antibiotic administered, route of administration, and calendar date of receipt were recorded. The PHIS database is an administrative database that contains comprehensive inpatient data from 45 free-standing children's hospitals across the United States, including the 4 participating hospitals. The PHIS hospitals include the largest children's hospitals in America. Participating hospitals provide deidentified data that were subjected to rigorous reliability and validity checks before being incorporated into the database.

Antibiotic exposure. For statistical analyses, we grouped antibiotic exposure (whether prophylactic or treatment) into the following nonmutually exclusive categories: (i) any agent, denoting any antibiotic; (ii) broad-spectrum beta-lactams, including *(a)* the third-generation cephalosporins ceftriaxone, cefotaxime, ceftazidime, cefdinir, cefixime, and cefpodoxime, *(b)* carbapenems, and *(c)* cefepime and beta-lactam/beta-lactamase inhibitor combinations; (iii) fluoroquinolones, including ciprofloxacin, moxifloxacin, and levofloxacin; (iv) aminoglycosides, including gentamicin, tobramycin, and amikacin; (v) trimethoprim-sulfamethoxazole (TMP-SMX); and (vi) anaerobic agents, including beta-lactam/beta-lactamase inhibitor combinations, carbapenems, cefoxitin, clindamycin, metronidazole, moxifloxacin, and tigecycline.

A breakdown of antibiotic exposure by each category and/or individual antibiotic is provided in Table S3 in the supplemental material.

Statistical analyses. Isolates demonstrating both ESBL and AmpC phenotypes and/or those with both class A and class C genes detected were excluded from all analyses.

We first assessed distributional characteristics in demographic and clinical variables between the cases and controls. The Kruskal Wallis test was used for continuous variables, and chi-square was used for categorical variables; a Mantel-Haenszel approach with stratification by hospital was applied when the sample size was sufficiently large at each hospital.

To evaluate the association between prior antibiotic exposure (both any exposure and by the individual categories described above) and subsequent infection with a resistant isolate, we used multinomial logistic regression, as the outcome of interest was case status with three categories: ESBL producing, AmpC producing, and susceptible (controls). The association was quantified by a relative risk ratio (RRR) estimate. We selected potential confounders *a priori*, including age, sex, previous hospitalization in the past year, presence of an indwelling device (categorized as central venous catheter, urinary catheter without central venous catheter, or other), immunosuppression (defined in Table 1), and underlying medical conditions. As we were primarily interested in examining those medical conditions known to confer increased risk of UTI or infection overall, we initially planned to focus on urologic conditions and malignancy versus other diagnoses. For the analyses, end-stage renal disease was removed from the original urologic category and recategorized as "other" due to the difference in pathophysiology from the other urologic conditions. We also examined neuromuscular and gastrointestinal conditions, given their relatively high frequencies in our data set (see Table S2 in the supplemental material). Preliminary analyses demonstrated that neuromuscular and urologic conditions were highly correlated (76% of those with a neuromuscular condition also had a urologic condition, mostly neurogenic bladder). Similarly, half of the gastrointestinal conditions were neurogenic bowel and 60% of those with a gastrointestinal condition also had a urologic condition. Based on these findings, we formulated a categorical variable with the mutually exclusive categories of "urologic," "malignancy without urologic condition," and "other condition without urologic or malignancy." We initially intended to include international travel as a confounder but later excluded this variable due to poor data quality at the majority of sites. Reassuringly, there was no evidence of an association between international travel and previous antibiotic use at the site where

TABLE 1 Demographic and clinical information of patients with resistant versus control isolates

Characteristic	No. (%) unless otherwise noted				
	All patients (n = 1,204)	ESBL cases (n = 210)	AmpC cases (n = 94)	Controls (n = 900)	P value[a]
Hospital					0.91
West	417 (35)	73 (35)	32 (34)	312 (35)	
Midwest 1	306 (25)	51 (24)	27 (29)	228 (25)	
Midwest 2	168 (14)	27 (13)	15 (16)	126 (14)	
East	313 (26)	59 (28)	20 (21)	234 (26)	
Species					0.38
E. coli	1,058 (88)	178 (85)	89 (95)	791 (88)	
K. pneumoniae	146 (12)	32 (15)	5 (5)	109 (12)	
Median age (range) (yr)	5.2 (0.1–21.9)	4.3 (0.1–20.4)	7.7 (0.1–20.6)	5.2 (0.1–21.9)	0.02
IQR	1.4, 12.2	0.9, 10.5	1.9, 13.5	1.4, 12.5	
Female	976 (81)	154 (73)	73 (78)	749 (83)	0.006
Hispanic ethnicity	165 (14)	25 (13)	20 (21)	120 (14)	0.18
Race					<0.001
Caucasian	740 (64)	110 (56)	65 (70)	565 (66)	
African-American	270 (23)	29 (15)	19 (20)	222 (26)	
Asian	101 (9)	50 (25)	3 (3)	48 (6)	
Native American	14 (1)	6 (3)	0 (0)	8 (1)	
Pacific Islander	15 (1)	3 (2)	5 (5)	7 (1)	
More than one race	13 (1)	0 (0)	1 (1)	12 (1)	
Site of culture					<0.001
Urine	1,110 (92)	186 (89)	84 (89)	840 (93)	
Blood	77 (6)	15 (7)	7 (7)	55 (6)	
Other[b]	17 (1)	9 (4)	3 (3)	5 (1)	
Onset[c]					<0.001
Community associated	573 (48)	67 (32)	25 (26)	481 (53)	
Healthcare associated	503 (42)	107 (51)	58 (62)	338 (38)	
Hospital associated	128 (10)	36 (17)	11 (12)	81 (9)	
Hospitalization (in last yr)	357 (30)	91 (43)	48 (51)	218 (24)	<0.001
Medical condition category					<0.001
Urologic[d]	317 (26)	72 (34)	40 (43)	205 (23)	
Malignancy	53 (4)	17 (8)	4 (4)	32 (3)	
Other condition	197 (16)	44 (21)	20 (22)	133 (15)	
No condition	634 (53)	77 (37)	29 (31)	528 (59)	
History of Transplantation	62 (5)	18 (9)	9 (10)	35 (4)	<0.001
Immunosuppression (in last yr)[e]	137 (11)	36 (17)	17 (18)	84 (9)	<0.001
Device type					<0.001
Central venous catheter	135 (11)	40 (19)	11 (12)	84 (9)	
Foley catheter	26 (2)	9 (4)	2 (2)	15 (2)	
Other device	120 (10)	32 (16)	18 (19)	70 (8)	
No device	922 (77)	128 (61)	63 (67)	731 (81)	
Other antibiotic susceptibilities[f]					
Nonsusceptible to cip	231 (19)	150 (71)	18 (19)	63 (7)	<0.001
Nonsusceptible to gent	172 (14)	103 (49)	26 (28)	43 (5)	<0.001
Nonsusceptible to TMP-SMX	434 (36)	151 (72)	46 (49)	237 (26)	<0.001
Nonsusceptible to TMP-SMX and cip	171 (14)	115 (55)	16 (17)	40 (4)	<0.001
Nonsusceptible to all three	79 (7)	61 (29)	7 (7)	11 (1)	<0.001
Susceptible to all three	685 (57)	15 (7)	41 (44)	629 (70)	<0.001

[a] Generated comparing 3 categories of outcome for case status: ESBL producing, AmpC producing, and susceptible (controls).
[b] Other sites of infection include the following: in ESBL cases, peritoneal fluid (n = 4), bone (n = 3), and surgical wound (n = 2); in AmpC cases, peritoneal fluid (n = 2) and cerebrospinal fluid (CSF) (n = 1); and in controls, peritoneal fluid (n = 4) and CSF (n = 1).
[c] Definitions of onset are as follows: community associated, culture obtained in an outpatient setting or ≤48 h after hospital admission from an otherwise healthy patient without hospitalization in the previous year; healthcare associated, culture obtained in an outpatient setting or ≤48 h after hospital admission from a patient who had been hospitalized in the previous year and/or had a chronic medical condition requiring frequent health care or prolonged/recurrent antibiotic courses; and hospital associated, culture obtained >48 h after hospital admission or <48 h after hospital discharge from a patient without signs or symptoms of infection on admission.
[d] Diagnoses included in the urologic category are congenital urological abnormality, neurogenic bladder, and vesicoureteral reflux.
[e] Immunosuppressants included antineoplastic agents, high-dose glucocorticoids (≥2mg/kg of body weight), tumor necrosis factor inhibitors, calcineurin inhibitors, and mycophenolate mofetil.
[f] cip, ciprofloxacin; gent, gentamicin; TMP-SMX, trimethoprim-sulfamethoxazole.

this variable was captured most completely and systematically (data not shown).

Next, we used multinomial logistic regression to explore whether a dose-response relationship existed between antibiotic exposure in the 90 days prior to infection and having a resistant isolate. The model was constructed using the same set of potential confounders as listed above. Since we only had calendar month of receipt for outpatient antibiotic use, we assigned each documented outpatient antibiotic course to count as 10 days of antibiotic use, as this is a common treatment duration for many pediatric indications (29, 30; http://www.cdc.gov/getsmart/community/for-hcp/outpatient-hcp/pediatric-treatment-rec.html). If the outpatient antibiotic course was identified as prophylaxis, the antibiotic was considered to be given every day between the start and stop month. The distribution of the data limited the categories of antibiotic exposure we could examine in multivariable analysis. Most patients (60%) had no antibiotic exposure in the preceding 90 days, while ~11% had 1 to 15 days, ~8% had 16 to 30 days, ~7% had 31 to 60 days, and ~14% had 61 to 90 days. Based on clinically meaningful cut points and the distribution of the data, we divided days of antibiotic exposure into 3 categories: 0 days, 1 to 30 days, and 31 to 90 days of use. The referent category was 1 to 30 days of antibiotic use. A dose-response relationship would be supported if both the RRR comparing 0 days of antibiotic exposure to 1 to 30 days of antibiotic exposure was significantly less than 1 and the RRR comparing 31 to 90 days of exposure to 1 to 30 days of exposure was significantly greater than 1 (31).

Finally, we used multivariable logistic regression to evaluate the odds of being hospitalized after the identification of infection among case and control patients that were not already hospitalized when their index isolate was collected (i.e., hospital-acquired cases were excluded). We controlled for age, sex, previous hospitalization, any indwelling device, any underlying medical condition, immunosuppression (as defined in Table 1), species, and hospital in this model.

Statistical analyses were performed using Stata (version 12.1; Stata Corp., College Station, TX). We considered a two-tailed P value of <0.05 significant.

RESULTS

A total of 304 case isolates, including 210 ESBL- and 94 AmpC-producing isolates, and 900 susceptible control isolates were included in this study (12 controls were missing due to errors in collection or failure to meet eligibility criteria). Overall, *E. coli* and *K. pneumoniae* accounted for 88% and 12% of isolates, respectively (Table 1). Urine was the source of 92% of the isolates, and 99% of these met the criteria for likely UTI. An ESBL determinant was detected in 91% of the isolates with an ESBL phenotype; no determinant was detected in the remaining 9%. An AmpC determinant was detected in 88% of the isolates with an AmpC phenotype; no determinant was detected in the remaining 12%.

Demographic and clinical factors. Overall, the median age of the subjects was 5.2 years (range, 0.1 to 21.9; interquartile range, 1.4, 12.2). Subjects with ESBL isolates were younger than the controls, while those with AmpC isolates were older than the controls (Table 1). In addition, compared to the controls, patients with ESBL- or AmpC-producing isolates were more likely to be male and to have underlying medical conditions, indwelling devices, and previous hospitalizations in the past year ($P \leq 0.01$ for all comparisons) (Table 1). Both AmpC- and ESBL-producing isolates were more likely than controls to be resistant to TMP-SMX, ciprofloxacin, and gentamicin (Table 1).

Antibiotic exposure as a risk factor for an ESBL- or AmpC-producing isolate. Compared to controls, a larger proportion of patients with ESBL- or AmpC-producing isolates were exposed to antibiotics in the 30 and 90 days prior to the culture date of the study isolate (Table 2). A similar pattern was seen when examining the subcategories of broad-spectrum beta-lactams and TMP-SMX exposure (Table 2).

After controlling for potential confounding factors, the relative risk of having an ESBL-producing isolate rather than a susceptible isolate was 2.19 times higher (95% confidence interval [CI], 1.49 to 3.25) among those with antibiotic exposure in the 30 days prior to infection than in those with no antibiotic exposure (Table 2). Similar results were found when antibiotic exposure in the 90 days prior to the index date was examined. Similar results were also found when examining exposure to certain specific antibiotic categories in the 30 days prior to the index date, including noncarbapenem broad-spectrum beta-lactams and TMP-SMX. In contrast, exposure to other antibiotic subgroups was not associated with an increased adjusted relative risk of an ESBL-producing isolate (Table 2). In the dose-response analysis of antibiotic exposure in the 90 days prior to index infection, we found that compared to patients with 1 to 30 days of antibiotic exposure, patients with no exposure to antibiotics had a lower adjusted relative risk of having an ESBL-producing isolate (adjusted relative risk ratio [aRRR] of 0.53; 95% CI, 0.35 to 0.80); however, patients with 31 to 90 days of antibiotic exposure did not have a higher adjusted relative risk of having an ESBL-producing isolate compared to patients with 1 to 30 days of exposure to antibiotics (aRRR of 1.05; 95% CI, 0.65 to 1.70). Therefore, a dose-response relationship between antibiotic exposure and ESBL-producing isolates was not supported by these data.

In contrast to the ESBL findings, after controlling for potential confounding factors, the relative risk of having an AmpC-producing isolate compared to a susceptible isolate was not higher with antibiotic exposure in the 30 or 90 days prior to the index date (Table 2). However, when examining subgroups of antibiotics, the adjusted relative risk of having an AmpC-producing isolate was higher for patients with exposure to third-generation cephalosporins in the 30 days and 90 days prior to the index date. Additionally, the adjusted relative risk of having an AmpC-producing isolate was higher with exposure to broad-spectrum beta-lactams in the 90 days prior to the index date. Exposure to other antibiotic subgroups was not associated with an increased adjusted relative risk of an AmpC-producing isolate (Table 2). In the dose-response analysis, patients with no exposure to antibiotics did not have a significantly lower relative risk of having an AmpC-producing isolate and patients with 31 to 90 days of exposure to antibiotics did not have a significantly higher adjusted relative risk of having an AmpC-producing isolate compared to patients with 1 to 30 days of antibiotic exposure (aRRR of 1.06 [95% CI, 0.58 to 1.92] and aRRR of 1.24 [95% CI, 0.63 to 2.47], respectively). Therefore, a dose-response relationship between the use of any antibiotic and having an AmpC-producing isolate was not supported by these data.

Hospitalization in resistant cases versus controls. The odds of hospitalization for infection were 1.64 times higher (95% CI, 1.09 to 2.47; $P = 0.02$) in patients with ESBL-producing isolates than in controls with susceptible isolates, even after controlling for potential confounders. The odds of hospitalization were not higher for patients with AmpC-producing isolates than for controls with susceptible isolates (adjusted odds ratio of 0.72; 95% CI, 0.37 to 1.41; $P = 0.33$).

TABLE 2 Descriptive statistics and adjusted odds ratios for antibiotic exposure in patients with ESBL-producing, AmpC-producing, and susceptible infections in previous 30 days, 90 days, and by antibiotic category

Time of exposure, drug category[a]	No. (%)			Adjusted relative risk ratio (95% CI)[b]	
	ESBL $n = 210$	AmpC $n = 94$	Controls $n = 900$	ESBL vs control	AmpC vs control
30 days before culture					
Any antibiotic	100 (48)	32 (34)	200 (22)	2.19 (1.48–3.23)	1.12 (0.65–1.91)
Broad-spectrum beta-lactams	33 (16)	11 (12)	52 (6)	1.98 (1.15–3.40)	1.88 (0.86–4.11)
Third-generation cephalosporins	14 (7)	8 (9)	19 (2)	2.32 (1.09–4.93)	4.47 (1.75–11.41)
Carbapenems	3 (1)	1 (1)	3 (0)	2.35 (0.45–12.37)	2.06 (0.18–23.25)
Cefepime and/or BL/BLIs	24 (11)	4 (4)	34 (4)	2.01 (1.07–3.74)	0.88 (0.28–2.77)
Anaerobic agents	19 (9)	3 (3)	43 (5)	1.20 (0.65–2.20)	0.40 (0.12–1.37)
Aminoglycosides	10 (4)	1 (1)	22 (2)	0.89 (0.37–2.12)	0.37 (0.05–2.98)
Fluoroquinolones	7 (3)	3 (3)	12 (1)	1.62 (0.58–4.50)	1.71 (0.42–6.89)
TMP-SMX	44 (21)	19 (20)	74 (8)	1.81 (1.11–2.96)	1.69 (0.88–3.23)
90 days before culture					
Any antibiotic	120 (57)	45 (48)	289 (32)	1.91 (1.31–2.79)	1.03 (0.62–1.73)
Broad-spectrum beta-lactams	49 (23)	25 (27)	109 (12)	1.31 (0.84–2.05)	1.91 (1.07–3.41)
Third-generation cephalosporins	21 (10)	20 (21)	62 (7)	0.92 (0.53–1.60)	2.68 (1.45–4.94)
Carbapenems	6 (3)	4 (4)	14 (2)	1.04 (0.37–2.90)	1.85 (0.53–6.48)
Cefepime and/or BL/BLIs	37 (18)	11 (12)	62 (7)	1.80 (1.07–3.02)	1.30 (0.60–2.81)
Anaerobic agents	30 (14)	12 (13)	80 (9)	1.08 (0.64–1.78)	0.96 (0.47–1.98)
Aminoglycosides	13 (6)	6 (6)	38 (4)	0.61 (0.30–1.28)	1.06 (0.38–2.94)
Fluoroquinolones	14 (7)	5 (5)	22 (2)	1.76 (0.83–3.74)	1.30 (0.44–3.82)
TMP-SMX	51 (24)	25 (27)	101 (11)	1.53 (0.97–2.41)	1.62 (0.90–2.92)

[a] BL/BLIs, beta-lactams/beta-lactamase inhibitors; TMP-SMX, trimethoprim-sulfamethoxazole.
[b] Multinomial logistic regression was performed controlling for age, sex, previous hospitalization in the last year, presence of an indwelling device, underlying medical conditions, and immunosuppression as defined in Table 1.

DISCUSSION

We assessed the importance of prior antibiotic exposure as a risk factor for ESBL- or AmpC-producing versus susceptible *E. coli* and *K. pneumoniae* infections in children using prospectively collected data from a 4-year, multicenter study. We found significant associations between previous antibiotic exposure and infections with ESBL- or AmpC-producing isolates even after adjusting for potential confounding factors; however, the nature and the strength of the associations varied by resistance phenotype. We also found that the odds of hospitalization were higher in patients with infections due to ESBL-producing organisms, but not AmpC-producing organisms, than in controls.

The body of work supporting an association between previous antibiotic use and infection with ESBL-producing *E. coli* and *K. pneumoniae* in children is not as extensive as that in adults, but it is growing (32–39). Several of the available pediatric studies have focused on prophylaxis to prevent urinary tract infections (36–39) and/or did not adjust for important potential confounders (34, 38). We are aware of only one published study that focused on exposure to extended-spectrum cephalosporins (32). The current study is the first to examine the separate relationships between the risk of infection due to ESBL-producing organisms (compared to infection due to susceptible organisms) in children and previous exposure to different categories of antibiotics, including third-generation cephalosporins, broad-spectrum beta-lactams, fluoroquinolones, aminoglycosides, TMP-SMX, and anaerobic antibiotics. We found that the association between any antibiotic use and having an ESBL-producing isolate seemed to be driven by the use of third-generation cephalosporins and other broad-spectrum beta-lactams, as might be expected due to selection pressure and overall impact on the microbiota. The use of TMP-SMX was also significantly associated with an increased relative risk of having an ESBL-producing isolate, perhaps as a reflection of coselection, as ESBL-producing organisms frequently display coresistance to TMP-SMX. ESBL-producing organisms are also frequently coresistant to fluoroquinolones, and yet, fluoroquinolone use did not demonstrate an increased relative risk of ESBL-producing infection; however, fluoroquinolone use in pediatrics is less common and we were likely underpowered to identify such an association. These results reinforce the importance of antimicrobial stewardship efforts targeting the use of third-generation cephalosporins and other broad-spectrum beta-lactams. Our findings may also provide a basis for stewardship efforts to focus on the use of TMP-SMX in an effort to prevent ESBL-producing *Enterobacteriaceae* infections.

To our knowledge, this study is also the first to examine the epidemiology of infections due to AmpC-producing *E. coli* and *K. pneumoniae* in pediatrics. Studies that have examined previous antibiotic use as a risk factor for the development of infections due to AmpC-producing organisms in adult populations have had mixed results: some have demonstrated an association (12, 13, 16), while others have not (11, 14, 15). Interestingly, we found that the epidemiology of AmpC-producing organisms is different from that of ESBL-producing organisms; any previous antibiotic use was not a significant risk factor for having an AmpC-producing isolate compared to a susceptible isolate, while exposure to

third-generation cephalosporins in particular was significantly associated with AmpC-producing isolates. The reason for this differential association is unknown; one hypothesis is that there is less coselection of AmpC-producing isolates than of ESBL-producing isolates when examining "any antibiotic" exposure due to the relatively lower frequency of coresistance to other antimicrobials in AmpC-producing isolates (15).

Several studies have examined differences in lengths of hospitalization between pediatric patients with ESBL-producing and non-ESBL-producing infections, with mixed results (32–34, 36, 38). To our knowledge, no studies have examined differences in risks of hospitalization or lengths of stay for either adult or pediatric patients with AmpC-producing infections compared to susceptible infections. We found that the odds of hospitalization were larger for patients with infections due to ESBL-producing organisms than for patients with infections due to susceptible organisms. We did not find the same relationship for infections due to AmpC-producing organisms. Higher rates of coresistance to non-beta-lactam antibiotics, such as ciprofloxacin and TMP-SMX, in the ESBL-producing organisms could potentially explain this finding by leading to more discordant empirical antimicrobial therapy or lack of commonly used oral choices for definitive treatment in the patients with infections due to ESBL-producing organisms. Another possible explanation is that ESBL-producing organisms are more virulent and cause more severe symptoms than AmpC-producing and susceptible organisms, as a large proportion of these infections are caused by *E. coli* sequence type 131 (ST131), which is known to be highly virulent (40). Finally, it is possible that the provider's knowledge of ESBL status drove the decision to hospitalize, and in parallel, a lack of awareness about AmpC-producing organisms (since they were not routinely flagged by all the clinical microbiology laboratories) influenced management decisions. Together, these findings suggest that future research assessing the epidemiology of infections due to third-generation-cephalosporin-resistant organisms should differentiate between ESBL- and AmpC-producing variants when possible.

This study has several limitations. There are possibly unmeasured confounding variables for which we could not adjust and which may have biased our results. Additionally, it may have been ideal to include a second control group without infection to gain a better understanding of the impact that antibiotic exposure may have had on the development of resistant infections relative to the uninfected state. While the lack of an uninfected control group has also been shown to lead to an overestimation of the risk of antibiotic exposure for resistance (41, 42), an additional control group was beyond the scope of this study. Because the majority of our isolates were *E. coli* obtained from urine specimens, our results may not be generalizable to other specimen types or organisms. Also, our dose-response analyses were limited by the lack of daily data for outpatient antibiotic exposure, which may have led to either over- or underestimating true exposure, as well as by the lack of variability in exposure duration in our data, which left us unable to assess finer cut points of exposure. Finally, because this study was performed in four tertiary-care pediatric hospitals, our findings may not be generalizable to all pediatric settings. The strengths of this study include the multicenter involvement, its matched case-control design, its large (for pediatric research) sample size, and the ability to distinguish between ESBL- and AmpC-producing infections.

Antibiotic exposure appears to be an important factor in the development of resistant infections in children, but the strength of this association (and whether it is attributable to specific types of antimicrobials) varies by the mechanism of antibiotic resistance. These results reinforce the need to study and implement antimicrobial stewardship strategies in children, including those children with underlying health conditions.

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REFERENCES

1. **Kollef MH, Golan Y, Micek ST, Shorr AF, Restrepo MI.** 2011. Appraising contemporary strategies to combat multidrug resistant gram-negative bacterial infections—proceedings and data from the Gram-Negative Resistance Summit. Clin Infect Dis **53**(Suppl 2):S33–S55. http://dx.doi.org/10.1093/cid/cir475.
2. **Centers for Disease Control and Prevention.** 2014. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
3. **Levy SB, Marshall B.** 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med **10:**S122–S129. http://dx.doi.org/10.1038/nm1145.
4. **Doernberg SB, Winston LG.** 2012. Risk factors for acquisition of extended-spectrum beta-lactamase-producing Escherichia coli in an urban county hospital. Am J Infect Control **40:**123–127. http://dx.doi.org/10.1016/j.ajic.2011.04.001.
5. **Muro S, Garza-Gonzalez E, Camacho-Ortiz A, Gonzalez GM, Llaca-Diaz JM, Bosques F, Rositas F.** 2012. Risk factors associated with extended-spectrum beta-lactamase-producing Enterobacteriaceae nosocomial bloodstream infections in a tertiary care hospital: a clinical and molecular analysis. Chemotherapy **58:**217–224. http://dx.doi.org/10.1159/000339483.
6. **Rodriguez-Bano J, Picon E, Gijon P, Hernandez JR, Ruiz M, Pena C, Almela M, Almirante B, Grill F, Colomina J, Gimenez M, Oliver A, Horcajada JP, Navarro G, Coloma A, Pascual A, Spanish Network for Research in Infectious Diseases.** 2010. Community-onset bacteremia due to extended-spectrum beta-lactamase-producing Escherichia coli: risk factors and prognosis. Clin Infect Dis **50:**40–48. http://dx.doi.org/10.1086/649537.
7. **Gudiol C, Calatayud L, Garcia-Vidal C, Lora-Tamayo J, Cisnal M, Duarte R, Arnan M, Marin M, Carratala J, Gudiol F.** 2010. Bacteraemia due to extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-EC) in cancer patients: clinical features, risk factors, molecular epidemiology and outcome. J Antimicrob Chemother **65:**333–341. http://dx.doi.org/10.1093/jac/dkp411.
8. **Kuster SP, Hasse B, Huebner V, Bansal V, Zbinden R, Ruef C, Ledergerber B, Weber R.** 2010. Risks factors for infections with extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae at a tertiary care university hospital in Switzerland. Infection **38:**33–40. http://dx.doi.org/10.1007/s15010-009-9207-z.
9. **Ben-Ami R, Rodriguez-Bano J, Arslan H, Pitout JD, Quentin C, Calbo ES, Azap OK, Arpin C, Pascual A, Livermore DM, Garau J, Carmeli Y.** 2009. A multinational survey of risk factors for infection with extended-spectrum

beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis **49:**682–690. http://dx.doi.org/10.1086/604713.

10. **Azap OK, Arslan H, Serefhanoglu K, Colakoglu S, Erdogan H, Timurkaynak F, Senger SS.** 2010. Risk factors for extended-spectrum beta-lactamase positivity in uropathogenic Escherichia coli isolated from community-acquired urinary tract infections. Clin Microbiol Infect **16:**147–151. http://dx.doi.org/10.1111/j.1469-0691.2009.02941.x.
11. **Pai H, Kang C, Byeon JH, Lee KD, Park WB, Kim HB, Kim EC, Oh MD, Choe KW.** 2004. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother **48:**3720–3728. http://dx.doi.org/10.1128/AAC.48.10.3720-3728.2004.
12. **Pascual V, Ortiz G, Simo M, Alonso N, Garcia MC, Xercavins M, Rivera A, Morera MA, Miro E, Espejo E, Navarro F, Gurgui M, Perez J, Rodriguez-Carballeira M, Garau J, Calbo E.** 2015. Epidemiology and risk factors for infections due to AmpC beta-lactamase-producing Escherichia coli. J Antimicrob Chemother **70:**899–904. http://dx.doi.org/10.1093/jac/dku468.
13. **Lee CH, Lee YT, Kung CH, Ku WW, Kuo SC, Chen TL, Fung CP.** 2015. Risk factors of community-onset urinary tract infections caused by plasmid-mediated AmpC beta-lactamase-producing Enterobacteriaceae. J Microbiol Immunol Infect **48:**269–275. http://dx.doi.org/10.1016/j.jmii.2013.08.010.
14. **Reuland EA, Halaby T, Hays JP, de Jongh DM, Snetselaar HD, van Keulen M, Elders PJ, Savelkoul PH, Vandenbroucke-Grauls CM, Al Naiemi N.** 2015. Plasmid-mediated AmpC: prevalence in community-acquired isolates in Amsterdam, the Netherlands, and risk factors for carriage. PLoS One **10:**e0113033. http://dx.doi.org/10.1371/journal.pone.0113033.
15. **Matsumura Y, Nagao M, Iguchi M, Yagi T, Komori T, Fujita N, Yamamoto M, Matsushima A, Takakura S, Ichiyama S.** 2013. Molecular and clinical characterization of plasmid-mediated AmpC beta-lactamase-producing Escherichia coli bacteraemia: a comparison with extended-spectrum beta-lactamase-producing and non-resistant E. coli bacteraemia. Clin Microbiol Infect **19:**161–168. http://dx.doi.org/10.1111/j.1469-0691.2012.03762.x.
16. **Park YS, Yoo S, Seo MR, Kim JY, Cho YK, Pai H.** 2009. Risk factors and clinical features of infections caused by plasmid-mediated AmpC beta-lactamase-producing Enterobacteriaceae. Int J Antimicrob Agents **34:**38–43. http://dx.doi.org/10.1016/j.ijantimicag.2009.01.009.
17. **Weissman SJ, Adler A, Qin X, Zerr DM.** 2013. Emergence of extended-spectrum beta-lactam resistance among Escherichia coli at a US academic children's hospital is clonal at the sequence type level for CTX-M-15, but not for CMY-2. Int J Antimicrob Agents **41:**414–420. http://dx.doi.org/10.1016/j.ijantimicag.2013.01.006.
18. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. M100-S20. CLSI, Wayne, PA.
19. **Qin X, Zerr DM, Weissman SJ, Englund JA, Denno DM, Klein EJ, Tarr PI, Kwong J, Stapp JR, Tulloch LG, Galanakis E.** 2008. Prevalence and mechanisms of broad-spectrum beta-lactam resistance in Enterobacteriaceae: a children's hospital experience. Antimicrob Agents Chemother **52:**3909–3914. http://dx.doi.org/10.1128/AAC.00622-08.
20. **Zerr DM, Qin X, Oron AP, Adler AL, Wolter DJ, Berry JE, Hoffman L, Weissman SJ.** 2014. Pediatric infection and intestinal carriage due to extended-spectrum-cephalosporin-resistant Enterobacteriaceae. Antimicrob Agents Chemother **58:**3997–4004. http://dx.doi.org/10.1128/AAC.02558-14.
21. **Ingram PR, Inglis TJ, Vanzetti TR, Henderson BA, Harnett GB, Murray RJ.** 2011. Comparison of methods for AmpC beta-lactamase detection in Enterobacteriaceae. J Med Microbiol **60:**715–721. http://dx.doi.org/10.1099/jmm.0.029140-0.
22. **Japoni-Nejad A, Ghaznavi-Rad E, van Belkum A.** 2014. Characterization of plasmid-mediated AmpC and carbapenemases among Iranain nosocomial isolates of Klebsiella pneumoniae using phenotyping and genotyping methods. Osong Public Health Res Perspect **5:**333–338. http://dx.doi.org/10.1016/j.phrp.2014.09.003.
23. **Barroso H, Freitas-Vieira A, Lito LM, Cristino JM, Salgado MJ, Neto HF, Sousa JC, Soveral G, Moura T, Duarte A.** 2000. Survey of Klebsiella pneumoniae producing extended-spectrum beta-lactamases at a Portuguese hospital: TEM-10 as the endemic enzyme. J Antimicrob Chemother **45:**611–616. http://dx.doi.org/10.1093/jac/45.5.611.
24. **Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD, Davies RH, Liebana E.** 2005. *bla*$_{CTX-M}$ genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob Agents Chemother **49:**1319–1322. http://dx.doi.org/10.1128/AAC.49.4.1319-1322.2005.
25. **Perez-Perez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol **40:**2153–2162. http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002.
26. **Yan JJ, Wu SM, Tsai SH, Wu JJ, Su IJ.** 2000. Prevalence of SHV-12 among clinical isolates of Klebsiella pneumoniae producing extended-spectrum beta-lactamases and identification of a novel AmpC enzyme (CMY-8) in Southern Taiwan. Antimicrob Agents Chemother **44:**1438–1442. http://dx.doi.org/10.1128/AAC.44.6.1438-1442.2000.
27. **Feudtner C, Hays RM, Haynes G, Geyer JR, Neff JM, Koepsell TD.** 2001. Deaths attributed to pediatric complex chronic conditions: national trends and implications for supportive care services. Pediatrics **107:**E99. http://dx.doi.org/10.1542/peds.107.6.e99.
28. **Garcia LS.** 2010. Clinical microbiology procedures handbook, 3rd ed. American Society for Microbiology, Washington, DC.
29. **Chow AW, Benninger MS, Brook I, Brozek JL, Goldstein EJ, Hicks LA, Pankey GA, Seleznick M, Volturo G, Wald ER, File TM, Jr, Infectious Diseases Society of America.** 2012. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. Clin Infect Dis **54:**e72–e112. http://dx.doi.org/10.1093/cid/cis370.
30. **Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, Joffe MD, Miller DT, Rosenfeld RM, Sevilla XD, Schwartz RH, Thomas PA, Tunkel DE.** 2013. The diagnosis and management of acute otitis media. Pediatrics **131:**e964–e999. http://dx.doi.org/10.1542/peds.2012-3488.
31. **Maclure M, Greenland S.** 1992. Tests for trend and dose response: misinterpretations and alternatives. Am J Epidemiol **135:**96–104.
32. **Zaoutis TE, Goyal M, Chu JH, Coffin S, Bell LM, Nachamkin I, McGowan KL, Bilker WB, Lautenbach E.** 2005. Risk factors for and outcomes of bloodstream infection caused by extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella species in children. Pediatrics **115:**942–949. http://dx.doi.org/10.1542/peds.2004-1289.
33. **Megged O.** 2014. Extended-spectrum beta-lactamase-producing bacteria causing community-acquired urinary tract infections in children. Pediatr Nephrol **29:**1583–1587. http://dx.doi.org/10.1007/s00467-014-2810-y.
34. **Fan NC, Chen HH, Chen CL, Ou LS, Lin TY, Tsai MH, Chiu CH.** 2014. Rise of community-onset urinary tract infection caused by extended-spectrum beta-lactamase-producing Escherichia coli in children. J Microbiol Immunol Infect **47:**399–405. http://dx.doi.org/10.1016/j.jmii.2013.05.006.
35. **Hanna-Wakim RH, Ghanem ST, El Helou MW, Khafaja SA, Shaker RA, Hassan SA, Saad RK, Hedari CP, Khinkarly RW, Hajar FM, Bakhash M, El Karah D, Akel IS, Rajab MA, Khoury M, Dbaibo GS.** 2015. Epidemiology and characteristics of urinary tract infections in children and adolescents. Front Cell Infect Microbiol **5:**45. http://dx.doi.org/10.3389/fcimb.2015.00045.
36. **Dayan N, Dabbah H, Weissman I, Aga I, Even L, Glikman D.** 2013. Urinary tract infections caused by community-acquired extended-spectrum beta-lactamase-producing and nonproducing bacteria: a comparative study. J Pediatr **163:**1417–1421. http://dx.doi.org/10.1016/j.jpeds.2013.06.078.
37. **Kizilca O, Siraneci R, Yilmaz A, Hatipoglu N, Ozturk E, Kiyak A, Ozkok D.** 2012. Risk factors for community-acquired urinary tract infection caused by ESBL-producing bacteria in children. Pediatr Int **54:**858–862. http://dx.doi.org/10.1111/j.1442-200X.2012.03709.x.
38. **Bitsori M, Maraki S, Kalmanti M, Galanakis E.** 2009. Resistance against broad-spectrum beta-lactams among uropathogens in children. Pediatr Nephrol **24:**2381–2386. http://dx.doi.org/10.1007/s00467-009-1255-1.
39. **Jhaveri R, Bronstein D, Sollod J, Kitchen C, Krogstad P.** 2008. Outcome of infections with extended spectrum beta-lactamase producing organisms in children. J Pediatr Infect Dis **3:**229–233.
40. **Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M.** 2010. Escherichia coli sequence type ST131 as the major cause of serious multidrug-resistant E. coli infections in the United States. Clin Infect Dis **51:**286–294. http://dx.doi.org/10.1086/653932.
41. **Harris AD, Samore MH, Lipsitch M, Kaye KS, Perencevich E, Carmeli Y.** 2002. Control-group selection importance in studies of antimicrobial resistance: examples applied to Pseudomonas aeruginosa, Enterococci, and Escherichia coli. Clin Infect Dis **34:**1558–1563. http://dx.doi.org/10.1086/340533.
42. **Kaye KS, Harris AD, Samore M, Carmeli Y.** 2005. The case-case-control study design: addressing the limitations of risk factor studies for antimicrobial resistance. Infect Control Hosp Epidemiol **26:**346–351. http://dx.doi.org/10.1086/502550.