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EPIDEMIOLOGY AND SURVEILLANCE

Antibiotic Prophylaxis Is Associated with Subsequent Resistant Infections in Children with an Initial Extended-Spectrum-Cephalosporin-Resistant *Enterobacteriaceae* Infection

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ABSTRACT The objective of this study was to assess the association between previous antibiotic use, particularly long-term prophylaxis, and the occurrence of subsequent resistant infections in children with index infections due to extended-spectrum-cephalosporin-resistant *Enterobacteriaceae*. We also investigated the concordance of the index and subsequent isolates. Extended-spectrum-cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp. isolated from normally sterile sites of patients aged <22 years were collected along with associated clinical data from four freestanding pediatric centers. Subsequent isolates were categorized as concordant if the species, resistance determinants, and *fumC-fimH* (*E. coli*) or *tonB* (*Klebsiella pneumoniae*) type were identical to those of the index isolate. In total, 323 patients had 396 resistant isolates; 45 (14%) patients had ≥1 subsequent resistant infection, totaling 73 subsequent resistant isolates. The median time between the index and first subsequent infections was 123 (interquartile range, 43 to 225) days. In multivariable Cox proportional hazards analyses, patients were 2.07 times as likely to have a subsequent resistant infection (95% confidence interval, 1.11 to 3.87) if they received prophylaxis in the 30 days prior to the index infection. In 26 (58%) patients, all subsequent isolates were concordant with their index isolate, and 7 (16%) additional patients had at least 1 concordant subsequent isolate. In 12 of 17 (71%) patients with *E. coli* sequence type 131 (ST131)-associated type 40-30, all subsequent isolates were concordant. Subsequent extended-spectrum-cephalosporin-resistant infections are relatively frequent and are most commonly due to bacterial strains concordant with the index isolate. Further study is needed to assess the role prophylaxis plays in these resistant infections.

KEYWORDS antibiotic resistance, pediatric infectious disease

Infections caused by extended-spectrum-cephalosporin-resistant (ESC-R) *Escherichia coli* and *Klebsiella* species, including extended-spectrum beta-lactamase (ESBL)- and AmpC-producing organisms, are an emerging problem in children. These infections are

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increasing in frequency and are associated with prolonged hospitalization and mortality (1–6). Additionally, antimicrobial resistance among these organisms is often mediated by mobile genetic elements, resulting in multiple avenues for resistance transfer and infection transmission.

Subsequent resistant infections, due either to the same organism as the initial infection or to a new resistant organism, are a concerning clinical outcome associated with ESC-R *Enterobacteriaceae* infection. Reported rates of subsequent ESC-R infections range between 16% and 45% (7–11), but few studies have evaluated potential risk factors for subsequent ESC-R infections (9, 12). In our prior single-center study, we identified the receipt of ≥14 days of antibiotics in the previous 91 days as an independent predictor for subsequent ESC-R urinary tract infection (UTI) in children. The vast majority (91%) of the patients who received ≥14 days of antibiotics in the previous 91 days received long-term prophylaxis and were likely driving this association; however, this study was limited by a small sample size (9). Long-term prophylactic antibiotics are often prescribed for children with certain UTI risk factors and/or frequent UTIs (13, 14), and this prolonged exposure may increase the risk of multiple ESC-R infections (1, 9).

Few studies of ESC-R infections have compared the molecular characteristics between index and subsequent isolates in adults or children (9, 11). In our previous study, we found that the majority of patients had at least one subsequent isolate with the same molecular characteristics as the index isolate (9).

The objective of this study was to assess the association between previous antibiotic use, including a separate examination of long-term prophylaxis, and the occurrence of subsequent ESC-R infections among a cohort of patients with index ESC-R *E. coli* and *Klebsiella* sp. infections identified at four U.S. tertiary care pediatric centers. Furthermore, we compared the species, bacterial sequence types (*fumC-fimH* type for *E. coli* or *tonB* type for *Klebsiella pneumoniae*), and resistance determinants to evaluate the concordance between index and subsequent isolates.

RESULTS

A total of 323 patients with 396 ESC-R *E. coli* (83%) or *Klebsiella* sp. (17%) isolates were identified; 45 (14%) patients had ≥1 subsequent ESC-R *E. coli* and *Klebsiella* sp. infection, totaling 73 subsequent ESC-R *E. coli* and *Klebsiella* spp. isolates. The median number of subsequent infections for patients was 1 (interquartile range [IQR], 1 to 2 infections); the median number of days between the index and the first subsequent infections was 123 (IQR, 43 to 225 days).

Demographics and clinical characteristics. The median age of patients with subsequent infections was 5.1 (IQR, 1.7 to 10.2) years and was 4.9 (IQR, 1.1 to 11.7) years for patients without a subsequent infection. Urine was the source of 348 (88%) isolates, and 344 (99%) of these met the criteria for likely UTI. Of the 344 that met the criteria for likely UTI, 244 (71%) met microbiological criteria and the patients had symptoms of UTI, 96 (28%) met the microbiological criteria and symptom data were not available, and 4 (1%) met the criteria based on symptoms alone (cultures did not meet standard microbiological criteria). Of the 248 patients with symptoms reported, 144 (58%) had fevers, 66 (27%) had abdominal or flank pain, 66 (27%) had dysuria, frequent or urgent urination, or changes in continence patterns, 50 (20%) had changes in the color or odor of urine, 16 (6%) had hematuria, and 27 (11%) had vomiting.

Patients with subsequent ESC-R infections were more likely than those with only one isolate to have been hospitalized in the year prior to the index infections and have underlying medical conditions (Table 1). Furthermore, patients with subsequent ESC-R infections were more likely than those without to have received any antibiotics in the 30 days prior to the index (62% versus 46%, respectively) and to have received prophylactic antibiotics in the 30 days prior to the index (47% versus 22%, respectively) (Table 2). Of the 81 patients who received prophylactic antibiotics, 72 (90%) were on the prophylactic regimen for the entire 30-day period and the remaining 9 (10%) patients had been receiving the prophylactic regimen for several months and discon-

TABLE 1 Demographic and clinical characteristics of children with and without subsequent infections[a]

Characteristic	No. (%) unless otherwise noted		
	Subsequent infection?		
	Yes (n = 45)	No (n = 278)	P value
Median age (years)	5.1	4.9	0.98
IQR[b]	1.7–10.2	1.1–11.7	
Female sex	32 (71)	202 (73)	0.83
Hispanic ethnicity	7 (16)	39 (15)	0.90
Race			0.50
Caucasian	29 (64)	154 (59)	
African American	4 (9)	46 (18)	
Asian	10 (22)	49 (19)	
Native American	0 (0)	6 (2)	
Pacific Islander	2 (4)	6 (2)	
More than one race	0 (0)	1 (0)	
Site of index infection			0.37
Urine	38 (85)	246 (89)	
Blood	6 (13)	20 (7)	
Other	1[c] (2)	12[d] (4)	
Onset[e]			<0.01
Community associated	1 (2)	91 (33)	
Healthcare associated	39 (87)	136 (49)	
Hospital associated	5 (11)	51 (18)	
Previously hospitalized in last year	28 (62)	123 (44)	0.03
Median hospitalizations in last year	1	2	0.56
IQR	1–5	1–4	
Medical condition category			<0.01
Urological	32 (71)	87 (31)	
Other[f]	10 (22)	84 (30)	
Immunocompromised[g]	9 (20)	54 (20)	0.53
In-dwelling devices[h]	23 (51)	103 (37)	0.10
Central venous catheter	10 (22)	49 (18)	
Foley catheter	4 (9)	15 (5)	
Nasogastric tube	9 (20)	49 (18)	
Other	11 (24)	42 (41)	

[a]Table does not account for missing data.
[b]IQR, interquartile range.
[c]Peritoneal fluid (n = 1).
[d]Peritoneal fluid (n = 5), bone (n = 3), surgical wound (n = 3), and cerebrospinal fluid (n = 1).
[e]Hospital-associated onset defined as a culture obtained >48 h after hospitalization from a patient without signs or symptoms of infection on hospital admission or ≤48 h after hospital discharge from a patient without signs and symptoms of infection on admission. Community-associated onset defined as a culture obtained in an outpatient setting or ≤48 h after hospital admission from an otherwise healthy patient without hospitalization in the previous year. Health care-associated onset defined as a culture obtained in an outpatient setting or ≤48 h after hospitalization from a patient who had been hospitalized in the last year and/or had a chronic medical condition requiring frequent contact with health care facilities or prolonged/recurrent antibiotic courses.
[f]Includes neuromuscular, cardiovascular, respiratory, gastrointestinal, hematology/oncology, metabolic, and other congenital/chromosomal and rheumatologic conditions.
[g]Includes malignancy, immunosuppression (chemotherapy, glucocorticoids ≥2 mg/kg for ≥2 weeks, tumor necrosis factor [TNF] inhibitors, calcineurin inhibitors, mycophenolate mofetil [MMF], antineoplastic agents), and any transplant.
[h]Total is >100% as patients could have more than one device.

tinued the regimen during the 30-day period. In the Cox proportional hazards analyses, after adjusting for potential confounders, receipt of any antibiotics in the 30 days prior to their index infection was not statistically significantly associated with having a subsequent resistant infection (Table 2). However, when examining prophylaxis only,

TABLE 2 Antibiotic exposure in pediatric patients with and without subsequent infections

Exposure	No. (%)		Hazard ratio (95% CI)	
	Subsequent infection?			
	Yes (n = 45)	No (n = 278)	Univariate	Multivariate[a]
Any antibiotic in last 30 days[b]	28 (62)	128 (46)	1.84 (1.01–3.36)	1.16 (0.61–2.21)
Prophylaxis in last 30 days	21 (47)	60 (22)	2.80 (1.56–5.03)	2.07 (1.11–3.87)

[a]Adjusted for any medical condition, hospitalized in year prior to index infection, indwelling device, and immunosuppression.
[b]Includes treatment and prophylaxis in the 30 days prior to the index infection.

patients were 2.07 times more likely (95% confidence interval [CI], 1.11 to 3.87) to have subsequent ESC-R infections if they received prophylaxis in the 30 days prior to the index ESC-R infections, even after adjusting for potential confounders.

Patients with index infections due to ESC-R *K. pneumoniae*, as opposed to *E. coli*, were overrepresented in the group with subsequent infections (P = 0.03) (Table 3). When comparing strains of *E. coli*, *fumC-fimH* type 40-30 (also known as sequence type 131 [ST131]-*H*30 or clade C) was significantly more common in patients with subsequent ESC-R infections (P = 0.01) (Table 3).

Comparison of microbiological characteristics of index and subsequent isolates. In 26 (58%) of 45 patients with subsequent infections due to ESC-R organisms, all subsequent isolates were concordant with their index isolate (Table 4; see also Table S2 in the supplemental material). An additional 7 patients (16%) had at least 1 subsequent isolate that was concordant with the index isolate. The remaining 12 (26%) patients had subsequent isolates that were only discordant with the index isolates. In total, 48 (66%)

TABLE 3 Microbiological profiles of index isolates of children with and without subsequent infections

Characteristic	No. (%)		P value
	Subsequent infection?		
	Yes (n = 45)	No (n = 278)	
Species			0.03
E. coli	33 (73)	240 (86)	
K. pneumoniae	12 (27)	38 (14)	
E. coli fumC-fimH sequence type[a]			0.005
Alleles 40-30	17 (52)	66 (28)	
Others	16 (48)	174 (72)	
K. pneumoniae tonB sequence type[b]			0.24
Allele 39	2 (17)	2 (5)	
Others	10 (83)	36 (95)	
Resistance phenotype			0.84
ESBL	32 (71)	179 (64)	
AmpC	11 (25)	83 (30)	
ESBL and AmpC	1 (2)	5 (2)	
Carbapenem resistant	1 (2)	8 (3)	
Undetermined	0	3 (1)	
Resistance determinants[c]			
CTX-M-15	20 (44)	103 (37)	0.34
CTX-M others	9 (20)	61 (22)	0.77
CMY-2	9 (20)	71 (26)	0.42
DHA	1 (2)	4 (1)	0.53
FOX	1 (2)	3 (1)	0.45
SHV	4 (9)	12 (4)	0.25
None detected	5 (11)	32 (12)	>0.99

[a]Denominator is all *E. coli* isolates.
[b]Denominator is all *K. pneumoniae* isolates.
[c]Total is >100% as more than one determinant can be detected.

TABLE 4 Distribution of resistance determinants by species and sequence type among index and subsequent isolate series

Index isolate sequence type	Determinant	No. of patients	Subsequent isolate series							
			Concordant isolates		Mixed discordant and concordant isolates				Discordant isolates	
						No. of isolates				
			No. of patients	No. of isolates	No. of patients	Total	Concordant	Discordant	No. of patients	No. of isolates
E. coli fumC-fimH										
40-30	CTX-M-15	11	10	18	1	3	2	1	0	0
40-30	CTX-M-27	4	2	3	2	7	5	2	0	0
Others		18	8	8	2	4	2	2	8	12
Total *E. coli*		33	20	29	5	14	9	5	8	12
K. pneumoniae tonB										
*tonB*39	CMY-2	1	1	1	0	0	0	0	0	0
*tonB*39	FOX-5	1	1	1	0	0	0	0	0	0
Others		10	4	6	2	5	2	3	4	5
Total *K. pneumoniae*		12	6	8	2	5	2	3	4	5
Overall total		45	26	37	7	19	11	8	12	17

of subsequent isolates were concordant with the index isolates and the remaining 25 isolates (34%) were discordant.

The discordances between index and subsequent isolates included instances of different resistance determinants with the same *fumC-fimH* or *tonB* type ($n = 11$ isolates), different *fumC-fimH* or *tonB* types with shared resistance determinants ($n = 5$ isolates), different species with a shared resistance determinant ($n = 6$ isolates), and both different resistance determinants and different *fumC-fimH* or *tonB* types ($n = 3$ isolates) (Table S2).

Of the 17 patients with index infections caused by *E. coli fumC-fimH* type 40-30, in 12 (71%), all subsequent isolates were concordant with their index isolate (Table 4; see also Table S1). An additional 3 (18%) patients had at least 1 subsequent isolate that was concordant with the index isolate. The remaining 2 (12%) patients had isolates that were only discordant to the index isolates. In total, 28 (80%) of the 35 *fumC-fimH* type 40-30 subsequent isolates were concordant with the index isolates and the remaining 7 (20%) were discordant (Table S2).

Among the *fumC-fimH* type 40-30 isolates, all instances of discordance between subsequent and index isolates were instances of different resistance determinants with the same *fumC-fimH* type (40-30) (Table S2).

DISCUSSION

In this study, we observed that 14% of patients with an initial ESC-R infection experienced a subsequent ESC-R infection during our observation period. Most patients (58%) had only 1 subsequent infection, and the median time between the index and the first subsequent infections was 123 days. We found that previous antibiotic use and prophylaxis use were associated with subsequent infections in univariate analyses, but only prophylaxis remained an independent risk factor for subsequent infections after adjusting for potential confounders. Subsequent infections were most commonly (66%) due to bacterial strains of the same *fumC-fimH* or *tonB* type and resistance determinants as index ESC-R isolates. *E. coli* ST131-associated *fumC-fimH* type 40-30, also known as ST131-*H*30 or clade C, had an even higher ratio of concordance (80%) between index and subsequent isolates.

Other studies have identified antibiotic use as an independent risk factor for subsequent ESC-R *Enterobacteriaceae* infections, but variations in study population and design make direct comparisons to these studies challenging. A previous study from our research group found that receipt of ≥14 days of antibiotics within the 3 months

prior to subsequent infections was an independent risk factor for subsequent resistant infections (9). Another recent study conducted in adults with bacteremia caused by ESBL *E. coli* and *Klebsiella* spp. found that definitive treatment of the index infections with flomoxef (an oxacephem or fourth-generation cephalosporin) was an independent risk factor for recurrence (11). In this study, we were able to examine only antibiotic use prior to the index infection, and while overall antibiotic use in the 30 days prior to index infections was not an independent risk factor for subsequent resistant infections, patients who received prophylaxis prior to the index infections were twice as likely to have subsequent ESC-R infections than patients who did not receive prophylaxis, even after adjusting for underlying medical conditions and other potential confounders. There are several possible explanations for this observation. First, given the prolonged nature of the prophylactic regimens included in this analysis, patients who received prophylaxis in the 30 days prior to the index infections likely continued to receive it after the index infections, leading to ongoing selective pressure on the intestinal microbiome and resulting in a greater susceptibility to resistant infections. Alternatively, this observation might be a result of residual confounding due to underlying urologic conditions, as children with urological abnormalities often experience recurrent UTIs and thus receive prophylaxis. Unfortunately, we did not have a sufficient number of subjects to be able to differentiate between urologic and other underlying medical conditions in our multivariable model. The type of antimicrobial used for prophylaxis is also likely important; one previous study found that among patients with vesicoureteral reflux, children who received cephalosporin prophylaxis were more likely to have breakthrough ESBL urinary tract infections than those who received prophylaxis with co-trimoxazole (16). Again, our sample size precluded an examination of specific prophylactic agents.

Consistent with other studies, 66% of subsequent isolates were concordant with the index isolates (9, 11, 12). This finding suggests a persistent reservoir of resistant bacteria, likely in the gastrointestinal tract or the genitourinary tract, which was not eliminated during the treatment of the preceding infection. In our previous study, we found that among the patients with stool and subsequent infection isolates available for comparison, nearly half had stool and subsequent infection isolates that were both concordant with the index isolates (9). Unfortunately, stool isolates were not included in this study. Nevertheless, to reduce the risk of subsequent infections, persistent reservoirs may need to be addressed therapeutically; fecal transplantation may be such an intervention for intestinal reservoirs, while surgical interventions or maneuvers to promote thorough bladder drainage may be required for urinary reservoirs.

While the majority of subsequent isolates were concordant with the index isolates, we observed instances where subsequent isolates differed from the index isolates by *fumC-fimH* or *tonB* types but shared resistance determinants, suggesting possible transfer of plasmids between organisms of the same or different species within a patient, presumably due to ongoing selective pressure due to antibiotic exposure. We also observed instances where subsequent isolates differed from the index isolates both by *fumC-fimH* or *tonB* types and by resistance determinants, suggesting the vulnerability of the patient to the acquisition of new resistant organisms, presumably due to ongoing dysbiosis with or without selective pressure.

We observed that a higher proportion of patients with ESC-R *K. pneumoniae* index infections experienced subsequent infections than those with ESC-R *E. coli* index infections, similar to previous findings (9, 17). This observation may be due to differences in host factors, as patients with ESC-R *K. pneumoniae* are more likely than those with ESC-R *E. coli* to have hospital-acquired infections or previous health care exposure and/or chronic medical conditions (18, 19). Finally, we observed an especially high rate of concordance (71%) among patients with index infections with the ST131-associated *E. coli* type 40-30 strain. This clone, which has spread rapidly to become the dominant cause of multidrug-resistant extraintestinal *E. coli* infections in many populations (18, 20), has been shown to persist clinically irrespective of host factors and appropriate antibiotic treatment (21).

This study has several limitations. First, it was designed only to capture subsequent infections that were detected at the study hospitals; therefore, our infection data may be incomplete. However, our observed rate of subsequent infections (14%) is similar to other pediatric studies that have reported rates of 17 to 18%. Similarly, we were limited by our incomplete capture of antibiotic exposure; we did not capture antibiotic use that occurred outside the study hospitals or that occurred after the index infections. This may have resulted in an underestimate of true exposure. It is also possible that our definition for UTI captured patients who had colonizations with ESC-R organisms rather than true infections; however, given that most patients met microbiological criteria and had signs and symptoms of UTI, we believe that the misclassification of colonization as a UTI was likely infrequent. Further, the study was not of sufficient power to adjust for specific medical conditions, which may have resulted in residual confounding. Finally, some variability in resistance genotype profiles (specifically, patients with 1 isolate each that was negative for a specific resistance determinant but was otherwise concordant) may have been due to plasmid loss during the freeze/thaw cycles of archival storage. This study also had several strengths, including its multicenter multiyear design, relatively large sample size, and combination of detailed clinical and molecular data.

Antibiotic prophylaxis may contribute to the risk of subsequent ESC-R infections in children, and in most cases, the molecular profiles of index and subsequent isolates are concordant. These findings highlight the need to study antimicrobial stewardship interventions in children requiring prophylaxis and assess emerging therapies, such as fecal microbiota transplantation, to identify effective strategies for preventing recurrent ESC-R infections.

MATERIALS AND METHODS

Setting and institutional review. This prospective surveillance study involved four freestanding pediatric hospitals in the United States. 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 candidate ESC-R *E. coli*, *K. pneumoniae*, and *K. oxytoca* isolates recovered from urine or other normally sterile sites during routine clinical care of hospitalized and outpatient children <22 years of age. Candidate ESC-R isolates included those nonsusceptible (resistant or intermediate) to aztreonam, ceftazidime, ceftriaxone, cefotaxime, or cefepime. Each hospital used its standard clinical microbiological methods for species identification and susceptibility testing. Isolates were archived at −70°C and shipped to the coordinating center quarterly.

Coordinating center methods for confirmation and characterization of study isolates. (i) Overview. Upon arrival from participating laboratories, candidate resistant isolates were further evaluated at the coordinating center using standardized methods to confirm species and antibiotic susceptibility and to characterize resistance phenotype 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 in 2010 (22) and carbapenem breakpoints recommended in 2011 (23) were applied to all candidate resistant isolates to confirm resistance.

(iv) Phenotypic characterization. The class A ESBL phenotype and class C AmpC phenotype were identified as previously described (5, 9, 18). Carbapenem resistance was confirmed in those isolates identified as nonsusceptible to meropenem using disk diffusion as previously described (18). Control strains included CLSI-recommended type strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603, two laboratory-characterized strains, an *E. coli* strain containing bla_{CMY-2}, and a *K. pneumoniae* strain containing bla_{KPC}.

(v) Resistance genotyping. Study isolates were tested by PCR using primer sets for genes encoding common extended-spectrum cephalosporinases, including class A CTX-M, extended-spectrum TEM and SHV, and class C CMY, DHA, and FOX as previously described (5, 9, 18, 24–27). Isolates exhibiting phenotypic resistance to carbapenems were tested with primers for *bla* genes encoding class A (KPC), class B (IMP, VIM, and NDM), and class D (OXA-48/181-like) carbapenemases as previously described (9, 18, 28). All amplicons were sequenced, and assembly and alignment of nucleotide sequences was performed to identify all *bla* determinants to the variant level (e.g., CTX-M-15 versus CTX-M-14), as previously described (5, 9, 18).

(vi) Sequence-based strain typing. To characterize clonal relatedness of ESC-R strains, PCR and sequencing were carried out using previously described primers for *fumC* and *fimH* for *E. coli* or for *tonB* for *K. pneumoniae* (5, 9, 18, 29, 30).

Definitions. "Index isolate" signifies the first ESC-R *E. coli* or *Klebsiella* sp. isolated from a patient during the study period. "Subsequent isolate" signifies an ESC-R *E. coli* or *Klebsiella* sp. isolate obtained ≥28 days after any prior isolate during the study period. Our goal in choosing this 28-day window was to select a time point when patients would have completed treatment for the prior infections and after which any infections occurring would likely be new infections rather than a continuation of previous infections. Subsequent isolates were classified as either concordant or discordant. "Concordant subsequent isolate" signifies an isolate where the species, *fumC-fimH* type for *E. coli* or *tonB* type for *K. pneumoniae*, and molecular resistance determinants were the same as those for the index isolate. "Discordant subsequent isolate" signifies an isolate in which any of these characteristics differed from that of the index isolate.

Clinical data. Demographic and clinical data were collected from the medical records using a standardized case report form. Data on complex chronic conditions expected to last at least 12 months and require frequent or specialty medical care were collected and categorized using the strategy developed by Feudtner et al. using International Classification of Diseases, 9th revision (ICD-9) diagnosis codes (31). Additionally, we added a "urological" category that included neurogenic bladder and vesicoureteral reflux. For patients who contributed urine isolates, symptom and culture data (collection method, CFU per ml, etc.) were collected. These data were used by the coordinating site to characterize patients as likely having a UTI. Given the heterogeneity of our patient population, which included infants, patients with neurogenic bladders, and immunocompromised patients with neutropenia, it would be difficult to develop a highly sensitive and specific definition for UTI. Therefore, patients met criteria for likely UTI if the culture met standard microbiology lab criteria for susceptibility testing (≥10,000 CFU/ml in a clean catch specimen or indwelling catheter or ≥1,000 CFU/ml in a straight catheter specimen) (32) 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 frequent/urgent urination).

Antibiotic exposure. Given the nature of the study, only antibiotic exposures in the year prior to the index infections were collected. These exposures included all systemic (i.e., oral or intravenous) inpatient and outpatient antibiotic treatments and prophylaxes. Outpatient treatment and prophylaxis data were collected from pharmacy records or clinical chart notes and recorded using the case report form. Inpatient antibiotic treatment data were obtained from the Pediatric Health Information System (PHIS) database as previously described (5). Antibiotics were categorized as treatment or prophylaxis as indicated in the medical record. We defined "any antibiotic exposure" as receipt of any antibiotic (whether treatment or prophylaxis). We defined "prophylactic antibiotic exposure" as receipt of a long-term prophylactic regimen of daily oral antibiotics; short courses, such as 2 to 7 day courses, for surgical site infection prophylaxis were excluded. Prophylactic antibiotics that were administered 2 to 3 days per week (e.g., trimethoprim-sulfamethoxazole for *Pneumocystis* prophylaxis) were considered as administered daily as long as the regimen continued. A breakdown of antibiotic exposures (any antibiotic and prophylaxis) by each class and/or individual antibiotic is provided in Table S1 in the supplemental material.

Statistical analyses. We first described and compared demographic and clinical variables between patients with one ESC-R infection and patients with subsequent ESC-R infections. The chi-square or Fisher exact test was used to compare categorical variables and a Mann-Whitney U test was used for continuous variables. Subcategories of underlying medical conditions, immunosuppressants, and indwelling devices were initially described but were later collapsed for the multivariable model due to small sample size. An "immunosuppression" variable was created that included diagnosis of cancer, receipt of immunosuppressants within the year prior to the index infection, or receipt of hematopoietic cell or solid organ transplantation.

Exposures to "any" and "prophylactic" antibiotics in the 30 days prior to the index infection were tabulated for patients with and those without subsequent infections. The impact of antibiotic exposure was then further explored using two Cox proportional hazards models: one with any antibiotic use in the 30 days prior to the index infection (whether prophylaxis or treatment indicated) as the predictor of interest and one limited to prophylaxis use in the 30 days prior to the index infection as the predictor of interest. Potential confounders were chosen *a priori* using a conceptual framework (see Fig. S1) and included immunosuppression, underlying medical condition, indwelling device, and previous hospitalization.

We also described and compared microbiological and molecular characteristics of index isolates between patients with one ESC-R infection and patients with subsequent ESC-R infections. Descriptive statistics for resistance phenotypes, molecular resistance determinants, and *fumC-fimH* type (*E. coli*) or *tonB* type (*K. pneumoniae*) were tabulated and summarized. The associations between dichotomous variables were evaluated using chi-square or Fisher exact tests.

Finally, we compared the microbiological and molecular characteristics of subsequent ESC-R isolates with those of index isolates. The duration between the index and first subsequent infections was also described. All statistical analyses were performed using Stata (version 12.1; StataCorp, College Station, TX).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02656-16.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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