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Multicenter Study of the Risk Factors for Colonization or Infection with Carbapenem-Resistant *Enterobacteriaceae* in Children

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ABSTRACT Carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly identified in children in the United States, but data on the epidemiology of CRE in this population are limited. The objectives of this study were to characterize the risk factors for colonization or infection with CRE and describe the microbiologic characteristics of pediatric CRE isolates. We performed a multicenter matched case-control study from January 2011 to October 2015 at three tertiary care pediatric centers. Case patients were hospitalized children with CRE isolated from clinical cultures and were matched in a 2:1 ratio to control patients with carbapenem-susceptible *Enterobacteriaceae* (CSE). Risk factors for colonization or infection with CRE were then evaluated using a multivariable conditional logistic regression. Additionally, we comprehensively reported the antimicrobial susceptibility pattern for CRE isolates. Sixty-three case patients were identified and matched to 126 control patients. On multivariable analysis, antipseudomonal antibiotic exposure within the previous 3 months (odds ratio [OR], 5.20; 95% confidence interval [CI], 1.71 to 15.9; $P = 0.004$), prior surgery (OR, 6.30; 95% CI, 1.83 to 21.6; $P = 0.003$), and mechanical ventilation (OR, 12.4; 95% CI, 1.26 to 122; $P = 0.031$) were identified as risk factors for colonization or infection with CRE. Pediatric CRE isolates demonstrated relatively low rates of resistance to amikacin (5%) and ciprofloxacin (25%). Our findings support an important role for antibiotic stewardship interventions limiting the unnecessary use of antipseudomonal antibiotics as a strategy to prevent widespread emergence of CRE in children. Future studies should further characterize molecular determinants of antibiotic resistance among pediatric CRE isolates.

KEYWORDS *Klebsiella pneumoniae* carbapenemase, pediatrics, multidrug-resistant organism, Gram-negative resistance

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Infections due to multidrug-resistant *Enterobacteriaceae* are increasing in frequency and represent a major public health threat (1). While carbapenems have historically been the most effective agents for the treatment of these infections, carbapenem-resistant *Enterobacteriaceae* (CRE) have increasingly been identified as causes of health care-associated infections in adults (2–5). The rapid worldwide spread of CRE is largely attributable to the dissemination of carbapenemase enzymes, which represent highly transmissible plasmid-mediated resistance determinants that render the carbapenems, and most other classes of antibiotics, ineffective (6). In the United States, 80% of CRE

isolates from adult patients produce *Klebsiella pneumoniae* carbapenemases (KPCs), whereas metallo-β-lactamases and OXA-48-like carbapenemases are more common in other regions of the world (5). Several studies have identified risk factors for colonization or infection with CRE in adults, which include a prior exposure to broad-spectrum antibiotics, long-term acute care hospital (LTACH) residence, indwelling medical devices, and an immunocompromised status (7–15).

While there is a comparative lack of data on CRE in children, available surveillance information suggests an increasing prevalence of carbapenem resistance in this population. Specifically, a study of >300,000 pediatric *Enterobacteriaceae* isolates collected between 1999 and 2012 demonstrated that in 2011 to 2012, 0.47% of isolates overall and 4.5% of isolates from intensive care unit (ICU) patients were resistant to the carbapenems. In contrast, no carbapenem-resistant isolates were identified between 1999 and 2000 (16). The degree to which this increase in carbapenem resistance can be attributed to the dissemination of carbapenemase enzymes versus an increased prevalence of other resistance mechanisms, namely, extended-spectrum β-lactamase (ESBL) production or AmpC cephalosporinase production coupled with porin mutations, has not yet been systematically studied. Similarly, there is a paucity of clinical data on risk factors for CRE colonization or infection in children, with existing studies limited largely by a lack of control groups and heterogeneous patient populations and health systems. However, available data suggest that underlying medical conditions, especially malignancy, ICU admission, and prior antibiotic exposure, are common among children with CRE colonization or infection (17–26). However, few of these studies were performed in the United States, thereby limiting the generalizability to this population (17–19).

Given the increasing prevalence of CRE and the growing population of children with complex medical conditions at high risk for colonization and infection with multidrug-resistant organisms, an improved understanding of the risk factors for CRE in this population is urgently needed. In particular, this knowledge would aid in identifying potentially modifiable risk factors as well as inform optimal infection prevention and antibiotic stewardship strategies. Therefore, the aims of our study were to (i) evaluate the risk factors for CRE colonization or infection among pediatric patients hospitalized at three tertiary care institutions in the Untied States and (ii) describe the microbiologic characteristics (e.g., resistance profiles) of these CRE isolates.

RESULTS

Baseline characteristics of the study population. During the nearly 5-year study period, we identified 63 patients with index clinical cultures positive for CRE who were matched to 126 control patients with clinical cultures positive for CSE. Twenty-six patients (41%) had CRE infections, and the remaining patients were colonized. Among the entire study cohort of 189 patients, the median age was 24 months (interquartile range, 8 to 120 months) and 105 (56%) were male (Table 1). The sources of cultures were urine (40%), respiratory (35%), wound (13%), blood (10%), and peritoneal fluid (3%). Thirteen patients were excluded because 2 matched controls could not be identified. Though the excluded patients were more often from The Johns Hopkins Hospital and <1 year of age, unmatched patients did not differ from the matched patients on clinical characteristics.

Risk factors for colonization or infection with CRE. In a bivariable analysis, several variables were associated with colonization or infection with CRE. Compared to matched control patients with CSE, patients with CRE were more likely to have been admitted to the ICU (odds ratio [OR], 16.4; 95% confidence interval [CI], 3.83 to 69.9; $P < 0.001$), have had a recent surgical procedure (OR, 7.68; 95% CI, 2.94 to 20.1; $P < 0.001$), require mechanical ventilation (OR, 28.7; 95% CI, 3.81 to 216; $P = 0.001$), have prior exposure to antipseudomonal antibiotics (OR, 8.29; 95% CI, 3.19 to 21.6; $P < 0.001$), and have an invasive device, including a central venous catheter (OR, 3.50; 95% CI, 1.80 to 6.84; $P < 0.001$) or a urinary catheter (OR, 4.80; 95% CI, 1.69 to 13.6; $P = 0.003$) (Table 2).

On a subsequent multivariable analysis, antipseudomonal antibiotic exposure (OR, 5.20; 95% CI, 1.71 to 15.9; $P = 0.004$), recent surgery (OR, 6.30; 95% CI, 1.83 to 21.6; $P =$

TABLE 1 Baseline matching characteristics of children colonized or infected with carbapenem-resistant *Enterobacteriaceae* and carbapenem-susceptible *Enterobacteriaceae*

Variable	No. (%) of patients or median (IQR)[a]	
	CRE (n = 63)	CSE (n = 126)
Hospital location		
Philadelphia	16 (25)	32 (25)
Boston	10 (16)	20 (16)
Baltimore	37 (59)	74 (59)
Age (mo)	24 (6–32)	30 (8–108)
Year of culture		
2011	15 (24)	30 (24)
2012	12 (19)	24 (19)
2013	14 (22)	28 (22)
2014	14 (22)	28 (22)
2015	8 (13)	16 (13)
Infection	26 (41)	52 (41)
Culture source		
Urine	25 (40)	50 (40)
Respiratory	22 (35)	44 (35)
Wound	8 (13)	16 (13)
Blood	6 (10)	12 (10)
Peritoneal fluid	2 (3)	4 (3)

[a]IQR, interquartile range; CRE, carbapenem-resistant *Enterobacteriaceae*; CSE, carbapenem-susceptible *Enterobacteriaceae*.

0.003), and mechanical ventilation (OR, 12.4; 95% CI, 1.26 to 122; $P = 0.031$) remained significant risk factors for CRE colonization or infection. Given its identification as a confounder, as well as its *a priori* defined clinical significance, ICU admission (for at least 48 h at the time of positive culture) (OR, 4.32; 95% CI, 0.83 to 22.7; $P = 0.083$) was also included in the final multivariable model.

Microbiologic characteristics of CRE isolates. Of 63 unique CRE isolates, the most commonly identified species were *Enterobacter* species (57%), followed by *Klebsiella* species (25%). In contrast, of 126 CSE isolates, the most commonly identified species was *Escherichia coli* (43%). Among the 63 isolates demonstrating carbapenem resistance, carbapenemase testing was performed on 45 (71%), and a carbapenemase was detected in approximately half (21 isolates [47%]). Nine of these isolates underwent only phenotypic testing for carbapenemase production, and 12 were tested for specific carbapenemase genes, with 9 KPC-, 2 NDM-, and 1 VIM-producing isolate identified. Carbapenemase production was identified in 5 *Klebsiella* species isolates (50% of those tested), 14 *Enterobacter* species isolates (54% of those tested), and 2 *Citrobacter freundii* isolates (100% of those tested). The results of antibiotic susceptibility testing for all CRE isolates are shown in Table 3. Notably, 32 isolates met the CDC CRE definition based on ertapenem resistance alone with retained susceptibility to either meropenem or imipenem; 23 of these isolates underwent carbapenemase testing, and carbapenemase production was detected in 8, including 5 with confirmed KPCs. Additionally, among the 63 isolates, 46 (73%) were susceptible to ciprofloxacin and 59 (92%) were susceptible to amikacin. Among the 10 isolates tested for susceptibility to colistin, 8 (80%) were susceptible. Finally, when restricted to the 21 known carbapenemase-producing isolates, 62% of isolates demonstrated susceptibility to ciprofloxacin and 95% demonstrated susceptibility to amikacin. Of the 6 carbapenemase-producing isolates tested for colistin susceptibility, 4 (67%) were susceptible (Table 3).

DISCUSSION

We performed a multicenter observational study evaluating risk factors for CRE colonization or infection in children over a nearly 5-year study period. We identified prior antipseudomonal antibiotic exposure, mechanical ventilation, and recent surgery

TABLE 2 Bivariable analysis of risk factors for colonization or infection with carbapenem-resistant *Enterobacteriaceae* in children

Variable	No. (%) of patients or median (IQR)[a]		OR (95% CI)[b]	*P* value
	Cases	Controls		
Clinical characteristics[c]				
Male sex	43 (68)	62 (49)	2.60 (1.26–5.36)	0.010
Nonwhite race	40 (63)	77 (61)	1.11 (0.58–2.12)	0.744
LTCF resident	3 (5)	5 (4)	1.20 (0.29–5.02)	0.803
Healthcare abroad[d]	9 (14)	8 (6)	2.67 (0.93–7.69)	0.069
UAE	3	4		
India/Pakistan	2	0		
Kuwait	3	1		
Saudi Arabia	1	3		
Prior admission[e]	34 (54)	60 (47)	1.32 (0.70–2.49)	0.389
Prior surgery[f]	43 (68)	44 (35)	7.68 (2.94–20.1)	<0.001
OSH transfer[g]	17 (27)	11 (9)	4.09 (1.67–10.0)	0.002
ICU admission[h]	34 (54)	30 (24)	16.4 (3.83–69.9)	<0.001
LOS before culture (days)	16 (1–39)	1 (0–10)	1.01 (1.00–1.02)	0.038
Healthcare associated[i]	46 (73)	46 (37)	8.45 (3.25–22.0)	<0.001
Comorbid conditions				
Immunocompromised[j]	17 (27)	20 (16)	2.14 (0.96–4.73)	0.062
Prematurity[k]	12 (19)	11 (9)	3.24 (1.10–9.55)	0.033
Renal insufficiency/failure	6 (10)	4 (3)	3.00 (0.85–10.6)	0.089
Urogenital tract abnormality	9 (14)	12 (10)	1.71 (0.63–4.66)	0.294
Medical devices				
Central venous catheter	38 (60)	40 (32)	3.50 (1.80–6.84)	<0.001
Tracheostomy	10 (16)	26 (21)	0.65 (0.25–1.66)	0.368
Endotracheal tube	24 (38)	16 (13)	28.7 (3.81–216)	0.001
Foley catheter	12 (19)	5 (4)	4.80 (1.69–13.6)	0.003
Prior antibiotic exposure[l]				
Fluoroquinolone	6 (10)	7 (6)	1.92 (0.57–6.52)	0.294
Antipseudomonal[m]	44 (70)	42 (33)	8.29 (3.19–21.6)	<0.001
Carbapenem	20 (32)	5 (4)	12.67 (3.75–42.8)	<0.001

[a]IQR, interquartile range.
[b]OR, odds ratio; CI, confidence interval.
[c]LTCF, long-term-care facility; OSH, outside hospital; ICU, intensive care unit; LOS, length of stay.
[d]Medical care provided in an international location within 12 months of the positive culture. UAE, United Arab Emirates.
[e]Admission to an acute care hospital in the 3 months preceding the positive culture.
[f]Any surgical procedure other than percutaneous enteric tube placement or central line placement in the 3 months preceding the positive culture.
[g]Transfer to the current institution after >48-h stay at another acute care hospital.
[h]ICU admission for at least 48 h prior to the positive culture.
[i]Culture obtained after >48 h in the hospital.
[j]Includes patients with malignancies receiving chemotherapy in the preceding 3 months, hematopoietic stem cell or solid organ transplants, primary immunodeficiencies, and patients receiving immunosuppressants (e.g., methotrexate, azathioprine, and corticosteroids [equivalent of prednisone ≥20 mg daily] for at least 2 weeks) for nonmalignant disease.
[k]Patients <2 years old who were born at <32 weeks gestational age.
[l]All antibiotics received for at least 48 h in the preceding 3 months.
[m]Cefepime, ceftazidime, piperacillin-tazobactam, ticarcillin-clavulanate, imipenem, meropenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and levofloxacin.

as significant risk factors. To our knowledge, this is the largest comparative study evaluating risk factors for CRE colonization or infection in children in the United States, and the results are further strengthened by the multicenter design and comprehensive capture of potential risk factors through a standardized medical record review. We additionally provided a comprehensive description of the antibiotic susceptibility profiles of the identified isolates, which was most notable for the relatively high rates of susceptibility to ciprofloxacin and amikacin.

Our study showed that receipt of antipseudomonal antibiotics in the previous 3 months was a significant risk factor for colonization or infection with CRE in children,

TABLE 3 Antibiotic susceptibility results for pediatric carbapenem-resistant *Enterobacteriaceae* isolates[a]

Antibiotic	All isolates ($n = 63$)				Carbapenemase-producing isolates ($n = 21$)			
	No. of isolates tested	No. (%)			No. of isolates tested	No. (%)		
		Susceptible	Intermediate	Resistant		Susceptible	Intermediate	Resistant
Gentamicin	63	42 (67)	2 (3)	19 (30)	21	9 (43)	0	12 (57)
Tobramycin	54	30 (56)	3 (6)	21 (39)	18	5 (28)	2 (11)	11 (61)
Amikacin	63	58 (92)	2 (3)	3 (5)	21	20 (95)	0	1 (5)
Ciprofloxacin	63	46 (73)	1 (2)	16 (25)	21	13 (62)	0	8 (38)
Ertapenem	41	2 (5)	0	39 (95)	15	1 (7)	0	14 (93)
Meropenem	58	34 (59)	1 (2)	23 (40)	20	8 (40)	0	12 (60)
Imipenem	37	8 (13)	3 (5)	26 (41)	14	2 (14)	2 (14)	10 (71)
Tigecycline[b]	13	7 (54)	0	6 (46)	9	4 (44)	0	5 (56)
Colistin[c]	10	8 (80)	0	2 (20)	6	4 (67)	0	2 (33)

[a]Susceptibilities are based on 2017 CLSI breakpoints unless otherwise noted.
[b]Tigecycline susceptibility was defined as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines as an MIC of ≤ 1 μg/ml and resistance was defined as an MIC of >2 μg/ml.
[c]Colistin susceptibility was defined as per the EUCAST guidelines as an MIC of ≤ 2 μg/ml and resistance was defined as an MIC if >2 μg/ml.

a finding consistent with adult observational studies and available pediatric case series (7–9, 13, 15, 20, 22, 26). This association is likely due in part to alterations in commensal gastrointestinal flora in the presence of broad-spectrum antibiotics resulting in selection for antibiotic-resistant organisms and underscores the importance of antibiotic stewardship interventions focused on limiting unnecessary exposure to antipseudomonal antibiotics. Further supporting the important role of antibiotic stewardship interventions in preventing the emergence of CRE in children, approximately half of the isolates tested for carbapenemase production in our study appeared to not be carbapenemase producing, suggesting that perhaps the selective pressure imposed by antibiotic exposure (rather than dissemination of carbapenemases) resulted in carbapenem resistance, though future studies in which genotypic carbapenemase testing is systematically performed are needed to confirm this conclusion.

Also consistent with adult and available pediatric literature, mechanical ventilation was significantly associated with subsequent CRE colonization or infection, and the association with ICU admission approached statistical significance (10, 11, 14, 15, 22, 26). This association is likely multifactorial, including a high risk of colonization with CRE in the presence of indwelling devices (e.g., endotracheal tubes), as well as in the presence of severe underlying illnesses resulting in the need for ICU admission and mechanical ventilation. Related, recent prior surgery was also identified as a risk factor for CRE, an association we suspect is most likely reflective of underlying medical complexity and illness severity. Given these identified risk factors and recent data showing a disproportionate increase in CRE prevalence in the pediatric ICU population (now approaching 5% nationwide), as well as the high utilization of broad-spectrum antibiotics in these patients, antibiotic stewardship efforts targeting the pediatric ICU population may be particularly impactful in preventing the emergence of CRE in children (16, 27). Further, we have identified a subpopulation of children in whom enhanced infection prevention and control measures, such as targeted active surveillance, as well as empirical use of CRE-active antimicrobials, may be of benefit in certain scenarios, though further studies are needed to evaluate the utility of these strategies in children, given the overall low prevalence of CRE in the pediatric population.

The results of our study also provide a comprehensive description of the microbiology of pediatric CRE isolates, which are notable for several findings. First, in contrast to the higher rates of fluoroquinolone resistance described in the adult literature, 73% of isolates in our study were susceptible to ciprofloxacin (28, 29). This finding was consistent when the analysis was restricted to carbapenemase-producing isolates, with a ciprofloxacin susceptibility rate of 62%. The lower rate of fluoroquinolone resistance demonstrated in our study is likely due in part to the infrequent use of ciprofloxacin in our cohort, which would be expected to lead to less selective pressure on gastrointestinal flora and promote the development of resistance to this antibiotic. Furthermore,

amikacin resistance was infrequent. While clinical data evaluating the effectiveness of ciprofloxacin and amikacin for the treatment of infections due to CRE are limited, these antibiotics represent potentially effective and relatively safe treatment strategies for children with CRE infections, particularly for those with mild infections (e.g., cystitis) or as combination therapy for those with more moderate to severe infections, especially given the dearth of novel antimicrobials with activity against CRE in development for use in children. Second, *Enterobacter* species accounted for the majority of CRE isolates in our study both in the overall cohort (57%) and in the carbapenemase-producing isolates (67%). Notably, this is substantially different than the reported adult data in which *K. pneumoniae* is the most common CRE and warrants further study.

Finally, mechanisms of carbapenem resistance among pediatric isolates were diverse, with approximately half of isolates tested for carbapenemase production found to harbor carbapenemase genes. While the identification of carbapenemase production itself has important infection prevention and epidemiologic implications, specifically, the identification of VIM and NDM production in three patients who received medical care abroad in the United Arab Emirates, Kuwait, and India suggests that patients receiving medical care abroad may be an important route by which carbapenemase-producing CRE is introduced into pediatric institutions. This finding is of particular concern given a growing trend in tertiary care pediatric institutions to provide care to medically complex patients from regions of the world where CRE is endemic and warrants further study, including the study of a potential role for targeted active surveillance in this population.

Our study has several potential limitations. We combined infected and colonized patients into one group, which precluded the evaluation of risk factors specifically for infection. However, given that gastrointestinal colonization with antibiotic-resistant organisms most likely precedes infection, the risk factors are likely to be similar. We additionally defined CRE phenotypically as an organism demonstrating resistance to at least one carbapenem antibiotic and therefore could not assess risk factors specifically for carbapenemase-producing CRE, which may be distinct from those for non-carbapenemase-producing CRE. Relatedly, carbapenemase testing was variably performed at the respective institutions during the study period, which may have impacted the estimates of carbapenemase prevalence and type. Additionally, given the retrospective nature of the study, it is possible that there was an incomplete capture of clinical and demographic variables, including prior antibiotic exposures (especially outpatient prescriptions) and the receipt of health care abroad, if these were not documented in the electronic health record. However, we sought to minimize this as much as possible by limiting our cohort to inpatients and by having physicians trained in infectious diseases perform comprehensive medical record reviews. Finally, the study was performed in three East Coast tertiary care pediatric hospitals; therefore, the findings may not be generalizable to smaller institutions or other geographic regions. However, as most children colonized or infected with CRE in the United States are medically complex and likely to be cared for at large academic medical centers, the risk factors we identified are anticipated to be similar at other institutions caring for children with CRE. Furthermore, while one institution contributed a greater number of cases than the other two, there was no concern for a CRE outbreak during the study period, and so we expect the risk factors identified across the three institutions to be generalizable to other nonoutbreak settings.

In conclusion, the results of our study demonstrate that prior antipseudomonal antibiotic exposure, recent surgery, and mechanical ventilation were significant risk factors for colonization or infection with CRE in children. These findings underscore the importance of antimicrobial stewardship interventions aimed at limiting the unnecessary use of antipseudomonal antibiotics, especially among high-risk patients, including those hospitalized in the pediatric ICU. Future studies should further characterize potentially modifiable risk factors for CRE, describe the molecular epidemiology of CRE in this population, and evaluate the role of fluoroquinolones and aminoglycosides in the treatment of CRE infections in children.

MATERIALS AND METHODS

Study setting. The source population for the study included patients who were hospitalized at Boston Children's Hospital (Boston, MA; 395 beds), The Children's Hospital of Philadelphia (Philadelphia, PA; 527 beds), or The Johns Hopkins Hospital (Baltimore, MD; 205 beds) between 1 January 1 2011 and 15 October 2015. All study hospitals are tertiary care pediatric centers and serve as regional referral centers. This study period was selected to ensure a uniform interpretation of carbapenem resistance, as the Clinical and Laboratory Standards Institute (CLSI) implemented new MIC breakpoints for carbapenem resistance in 2010 (30). This study was approved by the institutional review board at each institution, with a waiver of informed consent.

Study design and population. We performed a retrospective matched case-control study to identify risk factors for CRE colonization or infection. All patients less than 21 years old with clinical cultures positive for any *Enterobacteriaceae* species who were inpatients or admitted the day of their positive cultures were eligible for inclusion. Outpatients and patients who had cultures performed in the emergency department and not admitted were excluded. Patients with clinical cultures positive for CRE were included as case patients, with each case patient eligible for inclusion only once on the date of their first positive culture during the study period. CRE was defined according to the 2015 Centers for Disease Control and Prevention (CDC) definition as an isolate resistant to imipenem, meropenem, doripenem, or ertapenem (31). Carbapenem resistance was defined per the 2017 CLSI guidelines throughout the study period as an imipenem, doripenem, or meropenem MIC of $\geq$4 μg/ml or an ertapenem MIC of $\geq$2 μg/ml (30). Control patients were patients with carbapenem-susceptible *Enterobacteriaceae* (CSE) isolated from clinical cultures. Carbapenem susceptibility was defined per the 2017 CLSI guidelines throughout the study period as an imipenem, doripenem, or meropenem MIC of $\leq$1 μg/ml or ertapenem MIC of $\leq$0.5 μg/ml (30). Control patients were matched in a 2:1 ratio to case patients on the following criteria: (i) hospitalization at same institution, (ii) age strata (infant, $<$1 year; child, 1 to 12 years; adolescent, $\geq$13 years), (iii) the year of positive culture, (iv) a clinical source of positive culture, and (v) infection versus colonization status. If a patient had multiple positive cultures from different sources within 7 days of the index culture, the most clinically significant source of infection was used for matching. All blood and peritoneal cultures were considered representative of infection; urine, respiratory, and wound cultures were classified as infection versus colonization based on modified National Healthcare Safety Network (NHSN) surveillance definitions by physicians trained in infectious diseases at each site (32). If more than two eligible control patients were identified for a case patient, the two control patients with the cultures closest in date to that of the case patient were selected. If fewer than two eligible control patients were identified for a case patient, the case patient was excluded from the analysis.

Data collection. Clinical and demographic data from the electronic health record were abstracted at each institution using a standardized data collection form. Demographic data included age, sex, race, the receipt of health care abroad within 12 months of the positive culture, long-term-care facility residence, transfer from an outside hospital, and acute care admission within the 3 months preceding the positive culture. Clinical data included ICU admission, the length of hospital stay prior to the positive culture, significant comorbid medical conditions, the presence of an invasive device (including endotracheal tubes) that had been in place for $>$48 h prior to the positive culture, prior surgery, and prior antibiotic exposures. Several comorbid medical conditions were combined into categories for analysis, including (i) immunocompromising conditions (malignancy with the receipt of chemotherapy within 6 months, solid organ or bone marrow transplantation, primary immunodeficiency, or receipt of immunosuppressive medications within 30 days of the positive culture), (ii) renal disease (dialysis dependence within 30 days of the positive culture or chronic kidney disease), and (iii) urogenital tract abnormality (neurogenic bladder or other bladder or collecting system diagnosis). Patients were classified as having had surgery if they underwent any surgical procedure other than the placement of a central venous line or percutaneous enteric tube within 3 months of the positive culture. An exposure to a given antibiotic was defined categorically as any exposure of $>$48 h in the 3 months prior to the positive culture, including both inpatient and outpatient prescriptions, and was abstracted from prescription data or physician notes within the hospital electronic health record. Individual antibiotics were combined for purposes of analysis into the following categories: (i) antipseudomonal antibiotics (cefepime, ceftazidime, piperacillin-tazobactam, ticarcillin-clavulanate, ciprofloxacin, levofloxacin, gentamicin, tobramycin, amikacin, imipenem, and meropenem), (ii) fluoroquinolones (ciprofloxacin or levofloxacin), and (iii) carbapenems (ertapenem, imipenem, and meropenem).

Susceptibility testing and molecular testing of *Enterobacteriaceae* isolates. Microbiologic data were collected, including organism species, source of the positive culture, antibiotic susceptibilities (including specific MIC values), and the results of any carbapenemase testing performed. The antibiotic susceptibilities were performed at Children's Hospital of Philadelphia (CHOP) and Boston Children's Hospital using the Vitek 2 (bioMérieux, Marcy l'Etoile, France) and at The Johns Hopkins Hospital using the BD Phoenix automated system (BD Diagnostics, Sparks, MD). At CHOP, carbapenemase testing was not routinely performed during the study period, but one isolate was tested on an exploratory basis for the presence of carbapenemases using an in-house-developed PCR assay. At Boston Children's Hospital, the modified Hodge test (MHT) for carbapenemase production was used throughout the study period. At The Johns Hopkins Hospital, the MHT was performed from January 2011 until March 2015, and subsequently, the Carba NP CLSI method was performed as described elsewhere (33). As part of an ongoing study at The Johns Hopkins Hospital, a portion of carbapenemase-producing isolates underwent β-lactamase gene identification using the DNA microarray-based assay Check-MDR CT103XL kit (Check-Points, Wageningen, Netherlands) (34). This assay tests for the presence of β-lactamase genes, including

those encoding ESBLs and AmpC cephalosporinases, as well as for the major genes encoding carbapenemases, including KPC, NDM, VIM, IMP, and OXA.

Statistical analysis. Categorical variables were summarized by frequencies and continuous variables were summarized by medians and interquartile ranges. The bivariable analysis was conducted using conditional logistic regression to determine the unadjusted association between each hypothesized risk factor and CRE colonization or infection. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to determine the strengths of these associations. Variables involved in confounding or interacting were identified using conditional logistic regression, adding one potential confounder at a time to assess its impact on the covariate of interest. A multivariable model using manual forward selection was then developed, starting with our primary hypothesized risk factor, antipseudomonal antibiotic exposure, and any risk factor identified as a confounder or effect modifier. All risk factors with a P value of <0.10 in the bivariable analysis were considered for inclusion in the final model. For all calculations, a 2-tailed P value of < 0.05 was considered statistically significant. All analyses were performed using Stata, version 14 (StataCorp, College Station, TX).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01440-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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