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BRIEF REPORT

Epidemiologic and Microbiologic Characteristics of Hospitalized Patients Co-colonized With Multiple Species of Carbapenem-Resistant Enterobacteriaceae in the United States

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We describe the epidemiologic and microbiologic characteristics of patients co-colonized with different species of carbapenem-resistant *Enterobacteriaceae* (CRE) from 5 hospitals in 4 states. Twenty-eight of 313 patients (8.9%) were co-colonized with at least 2 different CRE species. Different species within the same patient showed identical mechanism resistance in 18/28 (64%) cases.

Keywords.: co-colonization; CRE.

The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) is an urgent public health threat due to the limited number of effective antibiotics to treat infections caused by these organisms. In 2017, >13 100 hospitalized patients had a CRE infection, and it is estimated that ~130 million dollars in health care costs arise from these infections [1]. Acquisition of CRE in the hospital setting can occur by patient-to-patient transmission or by transfer of resistance genes between different species within a patient's gastrointestinal tract or antibiotic selective pressure on the gut microbiota [1, 2]. It is estimated that thirty percent of clinical CRE isolates carry a mobile genetic element that can be transferred to other species, which can increase the likelihood that other species can become resistant to carbapenems [1]. Given this potential to acquire CRE both exogenously and endogenously, patients are at risk of colonization with >1 species of CRE. Furthermore, as CRE prevalence in the United States continues to increase, the risk of co-colonization with multiple CREs may also be increasing—with unknown implications for clinical management and infection control [1]. Despite this, there is a paucity of data on the epidemiology of patients co-colonized with >1 species of CRE. The aim of this study was to analyze the epidemiologic and microbiologic characteristics of patients co-colonized with at least 2 CRE species in 5 acute care hospitals in the United States.

METHODS

Patients were identified as part of a larger prospective multicenter cohort study to determine which health care personnel types and patient care interactions are risk factors for CRE transmission to gloves or gowns, a surrogate for transmission to other patients in the hospital setting [3]. Between January 2016 and June 2019, CRE-colonized or -infected patients admitted to 2 hospitals in Baltimore, Maryland, 1 in Torrance, California, 1 in New York, New York, and 1 in Pittsburgh, Pennsylvania, were enrolled in the study. CREs were defined as *Enterobacteriaceae* resistant to at least 1 of the carbapenem antibiotics. Included patients had a surveillance or clinical culture positive for CRE within 7 days of enrollment. Most patients were enrolled in the study within 24 hours of the positive culture. Policies for routine perirectal surveillance cultures upon intensive care unit (ICU) admission varied by hospital. We collected additional perirectal, stool, and skin samples at the time of enrollment. Patient demographic and clinical variables were obtained from electronic medical records. Methods for estimating rates of CRE transmission to health care personnel gown and gloves after patient interactions have been previously described in detail [3]. Briefly, following patient interactions but before removing personal protective equipment, the gown and gloves of each health care personnel were sampled using BBL dual-tipped CultureSwab (Becton Dickson, Sparks, MD, USA). The design of this work was approved by the Institutional Review Boards at the University of Maryland Baltimore (HP-00066759-16), Weill Cornell Medicine (1610017615), LABioMed (042087), and the University of Pittsburgh (PRO18020274). A waiver of documentation of consent was approved per 45 CFR 46.117(c)(1), and a waiver of consent was approved per 45 CFR 46.116(d).

Patient skin samples and stool samples collected at the time of enrollment were tested for CRE. Swabs were vortexed, and serial 1:10 dilutions using Butterfield's Buffer were performed. Each dilution was inoculated on CHROMagar *Klebsiella pneumoniae* carbapenemase (KPC; Northeast Labs,

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Winslow, ME, USA) in triplicate. Plates were incubated aerobically at 35°C ± 2°C for 24 hours. Based on the appearance and color of the colony, we identified gram-negative bacteria on the plate. All laboratory and clinically identified CRE-positive isolates underwent speciation and carbapenem susceptibility testing. We also performed modified carbapenem inactivation method (mCIM) testing on all CRE-positive isolates to identify production of carbapenemases, and we used EDTA-modified carbapenem inactivation (eCIM) methods to differentiate between serine- and metallo-carbapenemases [4]. All isolates then underwent molecular testing for carbanenemase gene identification. To differentiate between KPC and New Delhi metallo-β-lactamase (NDM), we used real-time multiplex quantitative polymerase chain reaction (qPCR) to detect the bla_{KPC} and bla_{NDM-1} in gram-negative bacteria based on Centers for Disease Control and Prevention (CDC) guidelines [5]. bla_{VIM} and bla_{IMP} gene detection was performed using multiplex PCR, and bla_{OXA-48} gene detection was performed using singleplex PCR. We conducted a descriptive epidemiologic analysis to examine the demographic and clinical characteristics of patients with at least 2 different species of CRE and calculated the transmission rates of patients co-colonized with CRE to health care personnel gown and gloves using SAS, version 9.4 (SAS Institute, Cary, NC, USA). We also conducted a nested case–control study to compare risk factors among the 28 co-colonized patients with a subset of 173 mono-colonized CRE patients from the larger cohort. The Institutional Review Board at each facility granted approval for waived consent of participants.

RESULTS

Of the total 313 patients with CRE enrolled in the study, 28 (8.9%) were found to be co-colonized with at least 2 different CRE species. Three different CREs were identified among 3 patients (1.0%), and 4 different CREs were identified in 1 patient (0.3%). Of the 28 co-colonized patients, 13 (46.4%) were from Maryland, 7 (25.0%) were from New York, 7 (25.0%) were from Pennsylvania, and 1 (3.6%) was from California. Of the 28 patients, 9 (32.1%) were identified by perirectal surveillance cultures. Positive clinical cultures were most frequently from urine specimens (n = 6; 21.4%). Two out of the 28 (7.1%) were known to be co-colonized from the clinical or surveillance cultures at the time of enrollment. We obtained a total of 62 CRE isolates from routine clinical or surveillance cultures performed as part of clinical and infection control care at the hospital, and these are identified as Organism 1 in Figure 1. CRE isolates from subsequent perirectal, stool, or skin cultures obtained for research purposes are identified as Organisms 2–4 in Figure 1.

Of the 28 patients who were co-colonized, 18 were male (64.3%) with a median age of 56 years. Forty-six percent of the patients were white (n = 13), and 53.6% were in the ICU at the time of enrollment (n = 15). The median number of invasive

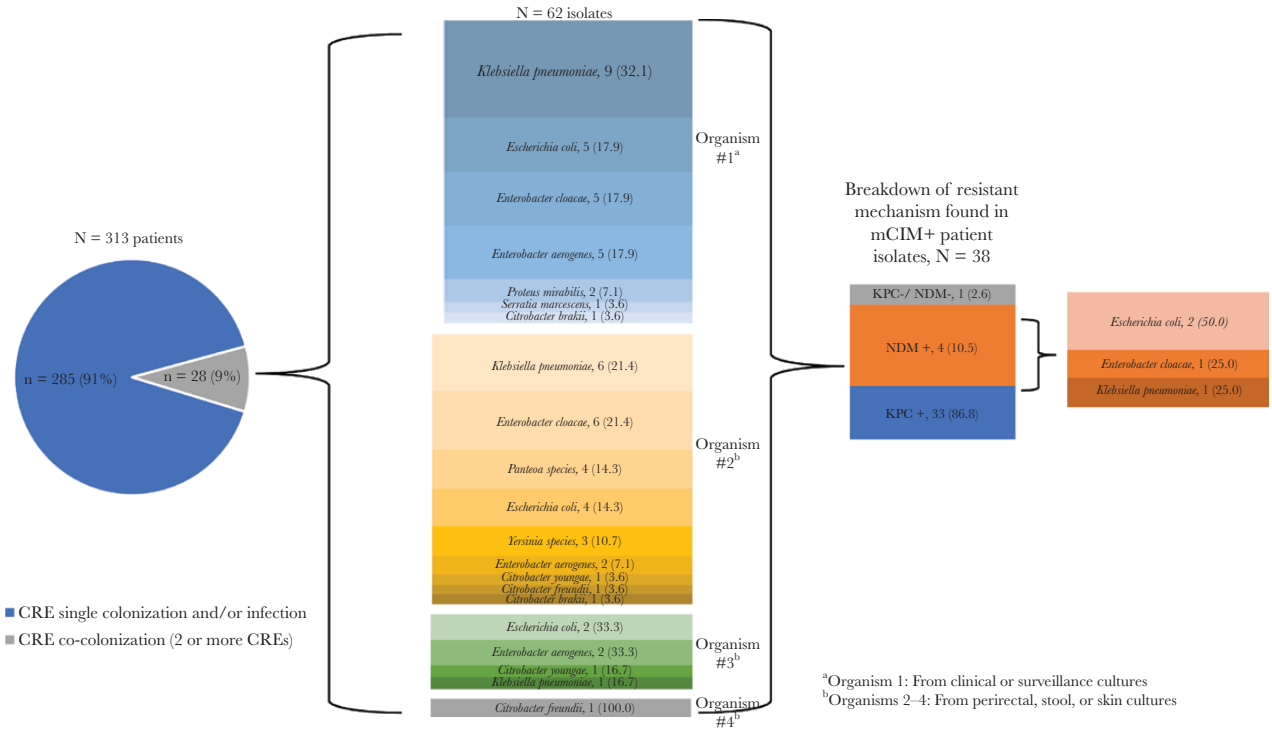

Figure 1. Description of isolates from 28 patients co-colonized with multiple species of CRE. Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; KPC, *Klebsiella pneumoniae* carbapenemase; mCIM, modified carbapenem inactivation method; NDM, New Delhi metallo-β-lactamase.

medical devices among this cohort was 4, and all patients had at least 1 device. Devices included an endotracheal tube, Foley catheter, nasogastric tube, rectal tube, surgical drain, or chest tube. Only 2 patients (7.1%) were known to have traveled outside of the United States in the year before admission. The median length of stay in the hospital (interquartile range) was 26.5 (16–60) days. The mean Elixhauser score for co-colonized patients (SD) was 2.6 (2.2). Upon hospital discharge, half of the co-colonized patients were transferred to another facility such as a long-term acute care (LTAC) hospital or nursing home (n = 14; 50.0%), 10 (35.7%) were discharged, and 4 (14.3%) died. The mean duration of antibiotic therapy from admission to positive culture (SD) was 23.4 (60.7) days, and the most frequently used antibiotic before positive culture was vancomycin (68%), followed by piperacillin/tazobactam (60%). Seven patients (25%) were receiving meropenem before positive culture.

When we compared the demographic and clinical characteristics of 173 patients with only 1 CRE to the 28 co-colonized patients (Table 1), we did not find statistically significant differences by age, sex, the number of medical devices, the number of antibiotics used before the positive CRE, length of hospital stay, or outcome. In multivariable analyses adjusted for race, the number of antibiotics used (odds ratio [OR], 1.1; 95% CI, 0.9–1.4), length of stay (OR, 1.0; 95% CI, 0.9–1.1), and whether the patient died (OR, 0.61; 95% CI, 0.2–2.2) were not associated with co-colonization.

We obtained a total of 62 isolates from 59 samples from the 28 co-colonized patients. Among the 62 isolates, the most common CRE species were *Klebsiella pneumoniae* (n = 18; 29.0%), *Escherichia coli* (n = 10; 16.1%), and *Enterobacter cloacae* (n = 9; 14.5%). Among the 62 isolates, 38 (61.35%) produced carbapenemase (mCIM-positive) and 8 (12.9%) produced MBL (eCIM-positive). Of the 38 isolates that were mCIM-positive, 33 (86.8%) were KPC+, 4 (10.5%) were NDM+, and 1 (2.6%) was negative for both KPC and NDM. Two *E. coli,* 1 *K. pneumoniae,* and 1 *E. cloacae* were determined to be NDM-producing CREs (Figure 1). We tested for VIM, IMP, and OXA-carbapenemase; however, none of the patient isolates exhibited these resistant mechanisms. Different species within the same patient showed identical mechanism resistance in 18/28 (64%) cases. Among co-colonized patients, transmission to health care personnel's gloves occurred in 21 out of 261 observations (8.0%), to gowns in 12 of 261 observations (4.6%), and transmission to either glove or gown occurred 28 times (10.7% of the time).

DISCUSSION

In a large multistate cohort of 313 patients colonized with CRE, we found that 9% were co-colonized with at least 2 different species. It is noteworthy that only 2 (7%) of these patients were identified as co-colonized by the initial culture (routine clinical or surveillance methods), suggesting that co-colonization may occur more frequently than previously estimated. Our findings also suggest that it is common for different CRE species to have the same resistance mechanisms within a single patient. It is possible that CRE co-colonization is occurring frequently because there is ongoing cross-species transmission of carbapenemases as suggested by other studies [10].

To our knowledge, this study is the first to characterize CRE co-colonization among geographically diverse acute care hospitals and to compare clinical and demographic characteristics among co-colonized and mono-colonized patients; however, a limitation of this study is that we did not assess previous stays

Table 1. Demographic and Clinical Characterstics of Patients Co-colonized and Mono-colonized With CRE (n = 201)

Characterstics	Co-colonized (n = 28)	Mono-colonized (n = 173)	*P* Value[a]
Age, mean (SD)	56.8 (16.4)	60.1 (16.6)	.29
Male	18 (64.3)	105 (60.3)	.69
Race			*P* < .001
Black/African American	8 (46.4)	84 (49.1)	
White	13 (28.6)	77 (45.0)	
Other	7 (25.0)	10 (5.9)	
Missing	0 (0.0)	3 (1.7)	
No. of devices, median (IQR)	4 (1–5)	3 (2–5)	.67
No. of antibiotics used before positive CRE, median (IQR)	4.5 (3–6)	5 (3–7)	.19
Length of stay, mean (SD), d	55.2 (71.5)	47.6 (60.8)	.5
Outcome			.76
Died	4 (14.3)	31 (17.8)	
Discharged	10 (35.7)	45 (25.9)	
Transfered to other facility	14 (50.0)	97 (55.8)	
Other	0 (0.0)	1 (100.0)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; IQR, interquartile range.

[a]Calculated using the chi-square test, Fisher exact test, Wilcoxon rank sum test, or Student *t* test.

in long-term care facilities or nursing homes. Other studies have described CRE co-colonization among patients in a single health care facility [6–9]. In a study conducted in 1 long-term care facility, Snyder and colleagues reported that 11 residents (21%) were colonized with at least 2 different species of multidrug-resistant gram-negative organisms [6]. A second study in a long-term care setting also found that co-colonization with multidrug-resistant gram-negative bacteria of different species was frequent and that 61% of 33 patients were colonized with at least 2 different species, of whom 15% were colonized with 3 or 4 different species [7]. Similarly, a study by Marchaim et al. at a hospital in Detroit examined co-colonization with any resistant gram-negative bacteria and found that 40% of 86 patients were co-colonized with multidrug-resistant gram-negative bacteria [8]. The demographic profiles of the patients who were co-colonized in this study were similar to ours, in that these patients were older and admitted in the ICU. We were unable to identify any demographic or clinical risk factors for co-colonization, implying that further microbiologic research is necessary to better understand CRE co-colonization. Transmission rates to health care personnel gown and gloves among co-colonized patients were very similar to those among the patients who were known to be colonized with only 1 CRE, suggesting that additional infection control precautions for co-colonized patients may not be necessary [3].

CONCLUSIONS

Co-colonization with multiple carbapenem-resistant *Enterobacteriaceae* occurs frequently in the acute care setting. Understanding the epidemiology of CRE co-colonization is important to inform interventions to limit the spread of these high-consequence organisms and can help guide the optimal empiric antibiotic selection for these patients.

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