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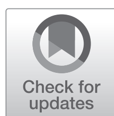

Acquisition of Carbapenemase-Producing *Enterobacteriaceae* in Solid Organ Transplantation Recipients

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ABSTRACT

Background. Carbapenemase-producing *Enterobacteriaceae* (CPE) can lead to life-threatening outcomes with rapid spread of the carbapenemase gene in solid organ transplantation (SOT) recipients because of limitations of available antibiotics. We examined the characteristics and importance of CPE acquisition in SOT recipients with large numbers of CPE isolates.

Methods. Between November 2015 and October 2016, 584 CPE isolates were found in 37 recipients and verified by carbapenemase gene multiplex polymerase chain reaction (PCR). One hundred recipients with at least 2 negative results in carbapenemase PCR for stool surveillance and no CPE isolates in clinical samples were retrospectively included.

Results. Most CPE isolates were *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (546, 93.5%). The most frequent transplantation organ was lung (43.3%), and the most common sample with CPE isolates other than stool was respiratory tract (22.6%). The median time between SOT and first CPE acquisition was 7 days. All-cause mortality was significantly higher in recipients with CPE than in those without CPE (24.3% vs 10.0%; $P = .03$). In multivariate regression analysis, stool colonization of vancomycin-resistant *Enterococci* and/or *Clostridium difficile* during 30 days before SOT (odds ratio [OR], 3.28; 95% CI, 1.24–8.68; $P = .02$), lung transplantation (OR, 4.50; 95% CI, 1.19–17.03; $P = .03$), and intensive care unit stay $\geq$2 weeks (OR, 6.21; 95% CI, 1.72–22.45; $P = .005$) were associated with acquisition of CPE.

Conclusions. Early posttransplantation CPE acquisition may affect the clinical outcome of SOT recipients. Careful screening for CPE during the early posttransplantation period would be meaningful in recipients with risk factors.

INVASIVE infection with multidrug-resistant gram-negative bacteria (MDR-GNB) after solid organ transplantation (SOT) is a consistent and growing global problem that is associated with life-threatening detrimental outcomes including graft dysfunction and high

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mortality [1,2]. Among MDR-GNB pathogens, colonization or infection with carbapenem-resistant *Enterobacteriaceae* (CRE), most commonly carbapenem-resistant *Klebsiella pneumoniae* (KPN), in SOT recipients is recently emerging, mainly in the general population of high CRE-endemic regions [3–8]. Notably, carbapenemase-producing *Enterobacteriaceae* (CPE) might be more virulent than CRE that do not produce the carbapenemase and may be clinically and epidemiologically important because the carbapenemase gene could easily spread to immunosuppressed SOT recipients who are especially vulnerable to disseminated infections through recipient-to-recipient, donor-to-recipient, or hospital environment and/or health care worker-to-recipient transmission of highly mobile genetic elements [8–13]. In this respect, physicians have to comply with several strict infection prevention and control measures to control the CPE outbreak [10,14]. Another ominous implication for the spread of CPE is the lack of limited possibilities for the selection of appropriately verified antimicrobial agents against CPE [15–17].

After the isolation of CPE-producing *K. pneumoniae* carbapenemase (KPC)-2 in kidney transplant recipients was first reported in New York in 2006 [18], the threat of CPE acquisition as infection or colonization, especially of KPC-producing *Enterobacteriaceae*, in SOT recipients has continuously been increasing since 2011 [19–23]. However, these reports were commonly case series with a small number of SOT recipients with CPE infection (maximum 21) and limited numbers of CPE isolates [19–24]. Therefore, we might not have sufficient knowledge about risk factors and the clinical impact of CPE acquisition on outcome in SOT recipients. Because our hospitals have experienced a great increase in CPE cases, we were able to collect a variety of clinical data for hundreds of CPE isolates in SOT recipients. This analysis aimed to evaluate the clinical characteristics and effects of CPE acquisition in SOT recipients through examination of a large number of CPE isolates.

METHODS

Study Design and Patient Collection

We retrieved all data from antibiotic susceptibility tests for *Enterobacteriaceae* and polymerase chain reaction (PCR) for carbapenemase genes from electronic medical records for 309 recipients aged at least 20 years who received SOT between November 2015 and October 2016 at Yonsei University Health System, Severance and Gangnam Severance Hospital, university-affiliated tertiary care centers with a total of 3500 beds in Seoul, South Korea. We confined the *Enterobacteriaceae* family to the genera of *Citrobacter*, *Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Raoultella,* and *Serratia spp,* which are common human pathogens [12]. We excluded 12 recipients who received retransplantation and/or had already taken immunosuppressive drugs before inductive immunosuppressive therapy for SOT. Finally, after additional exclusion of 3 recipients with carbapenemase-nonproducing CRE, 37 recipients who ever had CPE in any clinical sample, including stool or rectal swab, during hospital stay for SOT were selected as the group with CPE. We included all 100 SOT recipients without CRE and CPE isolates, defined as 2 or more negative results in a PCR test for carbapenemase genes for stool surveillance and no CPE isolates in clinical samples during hospital stay for SOT, as a control group to identify the factors associated with CPE acquisition in SOT recipients and to evaluate the effects of CPE on clinical outcomes. During the study period, active surveillance for CPE in stool or rectal swab using a carbapenemase gene PCR test to detect stool CPE carriers and contact precaution control measures were implemented for patients who had CRE and/or CPE clinical isolates, were admitted to the intensive care unit (ICU) before/after SOT, or had suspected history of contact with other patients infected or colonized by CPE [10,14]. This study was approved by the local Ethics Committee of the Institutional Review Board with waiver of informed consent.

Isolation of Carbapenemase-Producing *Enterobacteriaceae* and Verification of Carbapenemase Type

We used the MALDI Biotyper system (Bruker Co, Ltd, Billerica, Mass, United States) to identify species of *Enterobacteriaceae* from positive cultures in clinical sample. The antimicrobial susceptibility tests for *Enterobacteriaceae* were performed according to the breakpoints and standard guidelines from the Clinical and Laboratory Standards Institute (CLSI) in M100-S25 [25]. If the *Enterobacteriaceae* isolate was resistant to meropenem and ertapenem, we performed the modified Hodge test (MHT) for first-isolated CRE in each recipient in conformity with CLSI standards and designated it as CPE when an isolate with positive MHT had any carbapenemase gene in the multiplex PCR test [25,26]. When each recipient had repeatedly isolated CRE for least 3 days in an identical sample, CPE was directly confirmed through the carbapenemase gene PCR test without MHT. The carbapenemase gene PCR test was performed using a PANA RealTyper CRE Kit according to the manufacturer's instructions (PANAGENE Inc, Daejeon, Korea) [27]. This multiplex real-time PCR kit can detect 9 kinds of carbapenemase gene including class A carbapenemase of KPC and Guiana extended-spectrum, class B metallo-β-lactamases (MBL) carbapenemase of imipenemase-type carbapenemase, New Delhi MBL (NDM) and Verona integrin-encoded MBL (VIM), and class D OXA carbapenemases (OXA-23, OXA-48, OXA-58, and *ISAba1*-OXA-51) using peptide nucleic acid fluorescent probe and melting curve analysis [28–31]. Briefly, 4 probe dyes, FAM, HEX, ROX, and Cy5, were used, and the real-time PCR was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc, Calif, United States) with the following cycling conditions: 2 minutes at 50°C and 15 minutes at 95°C, followed by 45 cycles of 15 seconds at 95°C, 45 seconds at 58°C, and 15 seconds at 72°C. Afterward, additional reaction for 5 minutes at 95°C, 5 minutes at 35°C, and 5 seconds at 35°C to 80°C was performed for melting curve analysis. The unique melting temperature value for each of the 4 peptide nucleic acid fluorescent probes can identify the presence of each carbapenemase gene [27,30].

Collection of Clinical Information

Together with the usual information for SOT, we collected data for pretransplantation sensitization to HLAs. These include the complement-dependent cytotoxicity-lymphocyte cross-match test for T cells (time long and antihuman globulin phages) and B cells (warm phages) as well as the Luminex panel-reactive antibody (PRA) screen and identification of multiple class I and II HLAs by bead-based immunoassay using LABScreen Mixed assay and

Table 1. Characteristics of 37 Solid Organ Transplantation Recipients With Carbapenemase-Producing *Enterobacteriaceae* and 584 Carbapenemase-Producing *Enterobacteriaceae* Isolates

Characteristics	
Time between SOT and first isolation of CPE, median (range), d	7 (−19 to 230)*
Time between date of admission to hospital for SOT and first isolation of CPE, median (range), d	29 (2 to 236)
Features of CPE isolation in 37 recipients, No. (%)	
Clinical samples excluding stool/rectal swab (no stool carrier)†	2 (5.4)
Clinical samples and stool/rectal swab	24 (64.9)
Stool/rectal swab excluding other clinical samples‡	11 (29.7)
Results by CPE acquisition in 37 recipients, No. (%)	
Apparent infection§	11 (29.7)
Bacteremia	6 (16.2)
Pneumonia‖ with bacteremia	2 (5.4)
Pneumonia‖ without bacteremia	5 (13.5)
Colonization	26 (70.3)
Species (n = 584), No. (%)	
Klebsiella pneumonia	546 (93.5)
Escherichia coli	17 (3.0)
Enterobacter aerogenes	11 (1.9)
Raoultella ornithinolytica	3 (0.5)
Klebsiella oxytoca	2 (0.3)
Citrobacter amalonaticus	2 (0.3)
Citrobacter freundii	2 (0.3)
Enterobacter cloacae	1 (0.2)
Type of carbapenemase (n = 584), No. (%)	
KPC	580 (99.3)
NDM	4 (0.7)
ESBL production among CPE isolates cultured in samples except stool/rectal swab (n = 216), No. (%)	
Yes	4 (1.9)
No	212 (98.1)
Isolated sites (n = 584), No. (%)	
Blood	19 (3.3)
Respiratory tract	132 (22.6)
Upper¶	96 (16.4)
Lower**	36 (6.2)
Urine	25 (4.3)
Peritoneal fluid from indwelling catheter	3 (0.5)
Pleural fluid from indwelling catheter	9 (1.5)
Bile from indwelling catheter	3 (0.5)
Wound	21 (3.6)
Cervix	4 (0.7)
Stool/rectal swab	368 (63.0)
Changing pattern of MIC for amikacin in 37 recipients, No. (%)	
Not changed	12 (32.4)
Increased	9 (24.3)
All resistant	1 (2.7)
Not evaluated††	15 (40.5)

Abbreviations: CPE, carbapenemase-producing *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumonia* carbapenemase; MIC, minimal inhibitory concentration; NDM, New Delhi metallo-beta-lactamase; SOT, solid organ transplantation.

*A negative value means that CPE was isolated before transplantation.

†No carbapenemase gene was detected in PCR in ≥2 samples of stool or rectal swab.

‡CPE was not isolated in tests of all cultures of clinical samples (excluding stool or rectal swab) performed to diagnose infection or monitor colonization according to the physician's decision.

§Apparent infection was strictly confined to only bacteremia and pneumonia.

‖Pneumonia was strictly defined as CPE isolated from a sample from the lower respiratory tract with other clinical/laboratorial/radiological evidence.

¶Includes samples from oral cavity, throat, sputum, and nasopharyngeal or oropharyngeal suction as well as suction from endobronchial tube.

**Includes samples from bronchial washing and bronchoalveolar lavage through bronchoscopy.

††Cases in which CPE was isolated once and/or in only stool/rectal swab.

LABScreen PRA Class I/II assay, respectively (One Lambda, Inc, Canoga Park, Calif, United States) [32–34]. The percentage score of the PRA identification test was calculated by the following equation: (no. of positive bead reaction/total no. of beads) $\times$ 100 [34]. When considered clinically necessary by the physician, further evaluation including culture, PCR of *van A* and/or *van B*, and enzyme-linked immunoassay of toxin A and/or B for vancomycin-resistant *Enterococci* (VRE) or *Clostridium difficile* in stool was performed for diagnosis of *C difficile* infection or for infection control measurement of VRE.

Definitions

We used the phenotype-based definition based on antimicrobial susceptibility patterns to identify CRE [26]. We considered an isolate as CRE if it was resistant to meropenem with minimal inhibitory concentration (MIC) ≥4 μg/mL and to ertapenem with MIC ≥2 μg/mL according to the breakpoints of CLSI M100-S25 [25,26]. CPE was defined as an isolate among CRE with any carbapenemase gene in PCR [26]. We considered the patient a *C difficile* stool carrier if *C difficile* was isolated in loose stool irrespective of production of toxin A or B. The VRE isolates were verified by PCR test for *vanA* or *vanB*. We strictly confined apparent infection by CPE to bacteremia and/or pneumonia to avoid any subjective decisions. CPE bacteremia was defined as CPE isolation in at least 1 pair of samples from peripheral blood of a patient with symptoms and signs of systemic infection [13]. Pneumonia was strictly defined as CPE isolation in the lower respiratory tract, including bronchial washing or bronchoalveolar lavage, together with lung parenchymal infiltration and signs of systemic infection [35]. Acute rejection was defined as a transplantation organ–specific classification [36–38]. Cytomegalovirus reactivation was defined as asymptomatic viremia regardless of viral titer, tissue-invasive disease, or cytomegalovirus syndrome [39]. We defined the follow-up endpoint as the discharge date from hospital stay for SOT for recipients who were alive or as the date of death for recipients who died.

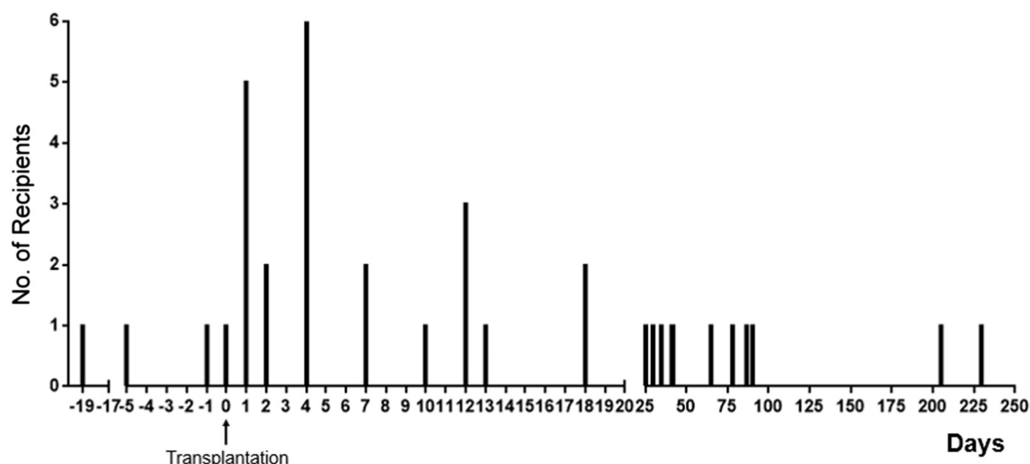

Fig 1. Time between solid organ transplantation surgery and first isolation of carbapenemase-producing *Enterobacteriaceae* (CPE). A negative value means that CPE was isolated before transplantation.

Statistical Analysis

Data were expressed as number (percent) or median (range or interquartile range [IQR]). To compare continuous variables between 2 groups, we used the independent *t* test and Mann-Whitney test for normal and non-normal distributions, respectively. Categorical variables were compared between 2 groups by χ^2 test or Fisher exact test. We performed multivariate logistic regression analysis to identify independent clinical factors associated with CPE acquisition in SOT recipients with inclusion of all nominal variables that showed statistical significance with P value $\leq$.05 in univariate analysis. Survival analysis was performed using a log-rank test. All P values were 2-tailed, and a P value $\leq$.05 was considered statistically significant. We used SPSS V23 (IBM Corp, Armonk, NY, United States) and GraphPad Prism V6 (GraphPad Software, Inc, La Jolla, Calif, United States) software for statistical analyses and graphs.

RESULTS

Characteristics of Carbapenemase-Producing *Enterobacteriaceae* Isolates

The great majority of total CPE isolates were KPC-producing and extended-spectrum beta-lactamase (ESBL)-nonproducing KPN (546 of 584, 93.5%). Four isolates producing NDM were *Citrobacter amalonaticus*, *Raoultella ornithinolytica*, *Klebsiella oxytoca*, and *Enterobacter cloacae*. CPE was most commonly isolated in samples from respiratory tract (132 of 584, 22.6%) irrespective of the transplantation organ when we did not consider CPE isolated in stool or rectal swab (Table 1). The CPE isolation site did not show a distinct association with transplantation organ. The percentage of respiratory tract isolates among total CPE isolation sites in recipients of lung (n = 16) and liver (n = 14) transplants was 76.8% (116 of 151) and 88.5% (46 of 52), respectively. Of the total 216 CPE isolates in samples except stool or rectal swab, 151 (70.0%) were identified in lung transplant recipients. Two different CPE species, KPC-producing KPN and KPC-producing *Escherichia coli*, were isolated concurrently in the same stool and/or rectal swab sample of 4 SOT recipients. The median time between transplantation and first isolation of CPE was 7 days, and the longest interval was 230 days. Three recipients showed the first CPE isolate 1, 5, and 19 days before transplantation (Table 1, Fig 1).

Only 20 (9.3%) among 216 CPE isolates were susceptible to tigecycline. The resistance rates for gentamicin (87.5%), levofloxacin (95.8%), and trimethoprim and/or sulfamethoxazole (93.5%) were very high. However, susceptibility to amikacin was maintained in the majority of isolates (87.5%) (Table 2). Nevertheless, 9 of 37 (24.3%) recipients had increased MIC values for amikacin in repeatedly isolated CPE (Table 1).

Characteristics of SOT Recipients With Carbapenemase-Producing *Enterobacteriaceae* Isolates

Transplant recipients who did not have CPE isolates in stool were in the minority (5.4%). CPE was isolated in both clinical samples and stool and/or rectal swab in 24 of 37 (64.9%) recipients, and 11 (29.7%) recipients had CPE isolates in only stool or rectal swab, but not other clinical samples (Table 1). Bacteremia by CPE occurred in 6 (16.2%) recipients (5 with lung transplant and 1 with liver transplant). Fifty percent of CPE-bacteremia recipients had died at 71, 110, and 176 days after transplantation and at 32, 53, and 164 days after the first CPE bacteremia.

Comparison of Characteristics Between SOT Recipients With and Without Carbapenemase-Producing *Enterobacteriaceae* Isolates

The frequency of recipients who received lung transplantation was significantly higher in the group with CPE compared with SOT recipients without CPE acquisition (43.3% vs 10.0%; P = .001). Age, type of donor, type of induction immunosuppressive drugs, and degree of

Table 2. Antimicrobial Susceptibilities of 216 CPE Isolates Cultured in Samples Other Than Stool/Rectal Swab

Susceptibility and MIC, No. (%)	MEM		ETP		AMK		GEN		LVX		SXT		TGC	
Susceptible*	0		0		189 (87.5)		26 (12.0)		8 (3.7)		14 (6.5)		20 (9.3)	
Intermediate*	0		0		0		1 (0.5)		1 (0.5)		0		15 (6.9)	
Resistant*	216 (100)		216 (100)		27 (12.5)		189 (87.5)		207 (95.8)		202 (93.5)		181 (83.8)	
	MIC†		MIC†		MIC†		MIC†		MIC†		MIC†		MIC†	
	≤2	0 (0.0)	≤2	0 (0.0)	≤2	125 (57.9)	≤1	24 (11.1)	≤0.12	8 (3.7)	≤20	12 (5.6)	≤0.5	11 (5.1)
	4	1 (0.5)	4	2 (0.9)	4	23 (10.6)	2	2 (0.9)	≤0.5	1 (0.5)	40	2 (0.9)	1	4 (1.9)
	8	4 (1.8)	≥8	214 (99.1)	8	30 (13.9)	8	1 (0.5)	≥8	207 (95.8)	160	5 (2.3)	2	5 (2.3)
	≥16	211 (97.7)			16	11 (5.1)	≥16	189 (87.5)			≥320	197 (91.2)	4	15 (6.9)
					≥64	27 (12.5)							≥8	181 (83.8)

Abbreviations: AMK, amikacin; CPE, carbapenemase-producing *Enterobacteriaceae*; ETP, ertapenem; GEN, gentamicin; LVX, levofloxacin; MEM, meropenem; MIC, minimal inhibitory concentration; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

*The breakpoints of MIC to discriminate the susceptibility followed the guideline of Clinical and Laboratory Standards Institute M100-S25.

†μg/mL.

pretransplantation sensitization did not show significant differences between the 2 groups (Table 3).

We compared prior isolation of another bacteria, antibiotic use, and duration of hospital stay as well as ICU stay during the period between 30 days before transplantation and first CPE acquisition in the group with CPE or the endpoint of follow-up in the group without CPE. Prior isolation of carbapenem-sensitive *Enterobacteriaceae*, irrespective of whether it produced ESBL, was more frequent in the group with CPE (45.9% vs 22.0%; $P = .006$). The number of recipients who received carbapenem treatment was significantly larger in the group with CPE (54.1% vs 30.0%; $P = .009$), and the duration of carbapenem use was significantly longer in the group with CPE (median [IQR], 2 [0–8] vs 0 [0–4] days; $P = .02$). The total hospital days before transplantation were similar between 2 groups, but duration of ICU stay was significantly longer in the group with CPE (median [IQR], 13 [3–29] vs 3 [2–7] days; $P < .001$) (Table 3). All-cause mortality was significantly higher in recipients with CPE acquisition (24.3% vs. 10.0%; $P = .025$) (Fig 2).

Independent Factors Associated With CPE Acquisition

We used the median value of 2 weeks in the group with CPE as the cutoff duration for longer ICU stay for logistic analysis. The multivariate regression model revealed that VRE or *C difficile* colonization in stool (OR, 3.3; 95% CI, 1.2–8.7; $P = .02$), longer ICU stay ≥ 2 weeks (OR, 6.2; 95% CI, 1.7–22.5; $P = .005$) during the 30 days before SOT, and lung transplantation (OR, 4.5; 95% CI, 1.2–17.0; $P = .03$) were independently associated with CPE acquisition (Table 4).

DISCUSSION

Compared with previous reports, this study included the largest number of CPE isolates, nearly all KPC-producing ESBL-nonproducing KPN, identified in SOT recipients. We collected and analyzed clinical and microbiological data for 584 CPE isolates from various sites including stool and/or rectal swab. Our study showed that CPE acquisition was associated with high all-cause mortality after SOT, as reported for other MDR gram-positive and -negative bacteria [1,2,13,40]. CPE acquisition occurred at the immediate early period after transplantation, with a median time of 1 week. However, more profound induction of immunosuppression according to pretransplantation sensitization, which could affect early immune status, did not have a significant association with CPE acquisition. These findings are similar to the universal hallmark of health care–associated MDR bacterial infections occurring within 1 month after SOT [1,2]. Pretransplantation CPE acquisition might occur more frequently if the length of admission before transplantation is longer.

The most vulnerable population for CPE acquisition in our study was lung transplant recipients with a longer ICU stay before transplantation. A history of ICU admission and

Table 3. Comparison of Clinical Characteristics of Solid Organ Transplantation Recipients With or Without Acquisition of Carbapenemase-Producing *Enterobacteriaceae*

Characteristics	CPE Positive (N = 37)	CPE Negative (N = 100)	*P* Value
Age, mean (SD), y	56.0 (10.2)	55.0 (10.8)	.65*
Sex, male, No. (%)	22 (59.5)	71 (71.0)	.22†
Transplantation organ, No. (%)			.001‡
Kidney	4 (10.8)	17 (17.0)	
Liver	14 (37.8)	54 (54.0)	
Lung	16 (43.3)	10 (10.0)	
Heart	3 (8.1)	14 (14.0)	
Pancreas	0 (0)	5 (5.0)	
Type of donor, No. (%)			.12†
Living	11 (29.7)	26 (26.0)	
Deceased	26 (70.3)	55 (55.0)	
Induction immunosuppressive drug, No. (%)			
Antithymocyte globulin	1 (2.7)	8 (8.0)	.44‡
Basiliximab	3 (8.1)	22 (22.0)	.08†
Pretransplantation sensitization			
HLA mismatch, No. (%)			
T lymphocyte	1 (2.7)	8 (8.0)	.44‡
B lymphocyte	2 (5.4)	7 (7.0)	>.99‡
Panel-reactive antibody			
Screen, anti-HLA antibody, positive, No. (%)			
Class I	11 (29.7)	37 (37.0)	.43†
Class II	8 (21.6)	22 (22.0)	>.99†
Identification, median (IQR), %			
Class I	2 (0–31)	3 (0–29)	.82§
Class II	2 (0–40)	0 (0–29)	.20§
Prior isolation of carbapenem-sensitive *Enterobacteriaceae**, EBSL-nonproducing or ESBL-producing, No. (%)	17 (45.9)	22 (22.0)	.006†
Only ESBL-producing	8 (21.6)	11 (11.0)	.11†
Only ESBL-nonproducing	4 (10.8)	9 (9.0)	.75‡
Use of carbapenems‖, No. (%)	20 (54.1)	30 (30.0)	.009†
Total duration of carbapenem use‖, median (IQR), d	2 (0–8)	0 (0–4)	.02§
Hospital stay before SOT¶, median (IQR), d	10 (4–26)	8 (2–14)	.14§
Total duration of ICU stay‖, median (IQR), d	13 (3–29)	3 (2–7)	<.001‡
VRE or *C difficile* isolation in stool‖, yes, No. (%)	16 (43.2)	16 (16.0)	.001†
Acute rejection, yes, No. (%)	2 (5.4)	17 (17.0)	.10†
CMV reactivation, yes, No. (%)	25 (67.6)	34 (34.0)	.001†

Abbreviations: CMV, cytomegalovirus; CPE, carbapenemase-producing *Enterobacteriaceae*; ESBL, extended-spectrum beta-lactamase; ICU, intensive care unit; IQR, interquartile range; SOT, solid organ transplantation; VRE, vancomycin-resistant enterococci.

*Independent sample *t* test.

†Pearson χ^2 test.

‡Fisher exact test.

§Mann-Whitney test.

‖For the period between 30 days before transplantation and endpoint of follow-up in recipients without CPE and between 30 days transplantation and first CPE acquisition in recipients with CPE.

¶From time of admission for SOT.

late discharge from ICU are general risk factors for various MDR pathogens, even in nontransplant patients [1,17,35]. In our center, lung transplant recipients usually received longer ICU care before and after transplantation compared with kidney and liver transplant recipients because of mechanical ventilation or percutaneous cardiopulmonary support treatment. Nevertheless, lung transplantation itself was an independent factor of CPE acquisition.

An interesting finding was that previous stool colonization of VRE and/or *C difficile* was independently associated with CPE acquisition. This suggests that alteration of gastrointestinal flora caused by various factors including prolonged ICU stay or broad-spectrum antibiotic treatment might intensify the effect of colonization with other easily transmissible and highly environment-resistant enteric bacteria on the probability of CPE acquisition. Further microbiological or molecular studies of gut microbiota in patients with CPE and MDR-GNB will be helpful to understand this feature. It might have important clinical meaning for controlling the further spread of CPE, a new detrimental MDR pathogen, into the SOT recipient population that is especially vulnerable to invasive CPE infection

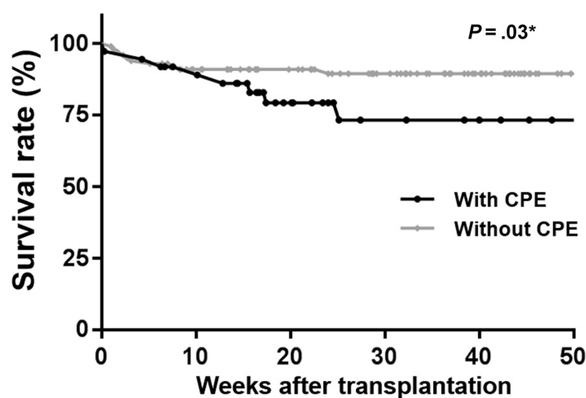

Fig 2. Kaplan–Meier survival curve for solid organ transplantation recipients with or without acquisition of carbapenemase-producing *Enterobacteriaceae*. *Log-rank test. Abbreviations: CPE, carbapenemase-producing *Enterobacteriaceae*.

Table 4. Multivariate Logistic Regression Analysis to Identify Independent Clinical Factors Associated With Carbapenemase-Producing *Enterobacteriaceae* Acquisition in Solid Organ Transplantation Recipients

Variables	OR	95% CI	*P* Value
Transplantation organ			
Lung	4.50	1.19–17.03	.03
Liver	2.16	0.67–7.00	.20
Prior isolation of carbapenem-sensitive *Enterobacteriaceae,* ESBL-nonproducing or ESBL-producing*, yes	1.82	0.64–5.14	.26
VRE or *C difficile* colonization in stool*, yes	3.28	1.24–8.68	.02
Previous use of carbapenems*, yes	1.50	0.47–4.84	.495
Longer ICU stay*, >2 wk	6.21	1.72–22.45	.005

Abbreviations: CI, confidence interval; ESBL, extended-spectrum β-lactamase; ICU, intensive care unit; OR, odds ratio; VRE, vancomycin-resistant enterococci.
*At 30 days before transplantation.

because of various medical or surgical conditions. Use of old antimicrobial agents such as polymyxin E to treat invasive CPE infection could bring additional morbidity and mortality caused by drug toxicity, especially dysfunction of renal allografts due to polymyxin E-induced nephrotoxicity. CPE might have nearly identical epidemiologic and clinical features to VRE, namely prior stool colonization in most cases and development of life-threatening invasive infection as well as a long time for eradication from stool.

Even though we strictly applied several infection control measures including the most important one of active surveillance through stool and/or rectal swab examination in patients with high risk, those admitted to ICU, and those with contact isolation, effective elimination of CPE was extremely difficult. Repeated admission to the transplantation unit or ward can increase the risk of CPE spread in the relatively homogenous SOT recipient population. We also experienced CPE isolation as stool colonization in most cases in a general ward with restricted admission for SOT recipients.

Our data showing the coexistence of KPC-producing KPN and KPC-producing *E coli* in stool raise concern about interspecies spread of carbapenemase genes between KPN and *E coli* in a single recipient through mobile genetic cassettes. These 2 bacteria are common pathogens of health care–associated infection together with glucose nonferment GNB of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* of majors for MDR-GNB. We found that KPN and *E coli* could coexist in the colon at a relatively high frequency, especially in SOT recipients with life-long immunosuppressive treatment, unlike with glucose non-fermenting GNB.

The major limitation of the study was that susceptibility tests for polymyxin E were not routinely performed; therefore, we could not obtain the rate of resistance to polymyxin E, which is currently the most potent antibiotic against CPE. In addition, our recipients included a small number of heart and lung dual transplantation recipients and did not include intestinal transplantation recipients, populations that are susceptible to health care–associated MDR-GNB infection [1]. We were also unable to continuously perform strict surveillance with a regular monitoring schedule in all recipients although almost all recipients with risk factors received PCR tests for carbapenemase genes with stool samples at a 1-week interval. These data did not include microbiological molecular analyses including subtype of KPC/NDM, pulsed gel electrophoresis and/or multilocus sequence typing, and detailed analyses of the structure of carbapenemase genes; therefore, we cannot provide any information on whether these CPE isolates originated from single or multiple clones. However, this study has the following unique strengths: (1) implementation of the strictest definitions for CRE and apparent infection and (2) the first detailed analysis of large data for all CPE isolates including stool colonization in a homogeneous SOT recipient population.

CONCLUSIONS

Our analyses revealed that the acquisition of KPC-producing KPN in SOT recipients occurred in the early posttransplantation period and was associated with lung transplantation, prior VRE or *C difficile* stool colonization, and longer ICU stay. The implementation of active control measures should be applied to SOT recipients, especially with these risk factors, to decrease the mortality caused by CPE acquisition itself and the morbidities related to invasive CPE infection.

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