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ORIGINAL ARTICLE

The role of therapy with aminoglycoside in the outcomes of kidney transplant recipients infected with polymyxin- and carbapenem-resistant *Enterobacteriaceae*

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Abstract

Kidney transplant recipients are at risk for infections due to carbapenem-resistant *Enterobacteriaceae* (CRE). Polymyxin-resistant CRE (PR-CRE) infections are especially difficult to treat. The aim of this study was to characterize PR-CRE infections among kidney transplant recipients and identify risk factors for treatment failure. This retrospective cohort study involved all kidney transplant recipients with PR-CRE infection between 2013 and 2017 at our center. Minimal inhibitory concentrations for polymyxin B were determined by broth microdilution. Carbapenem-resistant genes (bla_{KPC}, bla_{NDM}, and bla_{OXA-48}), aminoglycoside-resistance genes, and polymyxin-resistant gene *mcr*-1 were identified by polymerase chain reaction. All but one of the 47PR-CRE infections identified were due to *Klebsiella pneumoniae*. The most common type of infection (in 54.3%) was urinary tract infection (UTI). Monotherapy was used in 10 cases. Combined treatment regimens included double-carbapenem therapy in 19 cases, oral fosfomycin in 19, and amikacin in 13. Treatment failure occurred in 21 cases (45.7%). Clinical success was achieved 78.9% of patients who used aminoglycosides versus 37.0% of those who not used this drug ($p = 0.007$). Multivariate analysis showed diabetes mellitus to be a risk factor for treatment failure; amikacin use and UTI were found to be protective. Nine strains were RmtB producers. Although aminoglycosides constitute an important therapeutic option for PR-CRE infection, the emergence of aminoglycoside resistance could have a major impact on the management of CRE infection.

Keywords Polymyxin resistance · Kidney transplant · Fosfomycin · Double carbapenem · Mortality · Treatment

Introduction

Infections due to carbapenem-resistant *Enterobacteriaceae* (CRE) are becoming increasingly common worldwide, and solid organ transplant (SOT) recipients are described as group at risk for such infections. Among SOT recipients, the incidence of CRE infection ranges from 3 to 23% and the associated mortality ranges from 28 to 68%, higher than the 13 to 52% reported for the general population [1, 2].

The mortality associated with CRE infection among KT recipients could be as high as 42% and has been shown to be higher than that associated with carbapenem-susceptible *Enterobacteriaceae* infection [3–5].

There are few treatment options for CRE infections, and the options are even more limited in KT recipients, considering the potential nephrotoxicity of the few commonly

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available therapeutic drugs. Although the best therapeutic management has yet to be established, polymyxin is one of antimicrobial agents that has been most widely used in such infections [6]. Polymyxin resistance in CRE is primarily due to post-translational modification of the lipopolysaccharide molecules that form the outer layer of the outer membrane. In some countries, the incidence of infection with polymyxin-resistant CRE (PR-CRE) is high and is increasing. For example, it is estimated that approximately 15% and 20% of CRE strains are resistant to polymyxin in Italy and Greece, respectively, compared with approximately 27% in Brazil [7, 8].

There is a need for studies analyzing the treatment options for infection with PR-CRE in SOT recipients. Therefore, the aim of this study was to characterize infections with PR-CRE among KT recipients and to identify risk factors for failure of the treatment of such infections.

Materials and methods

Study design and patients

This was a retrospective cohort study involving all KT recipients who developed infection with PR-CRE between January 2013 and July 2017 at the University of São Paulo School of Medicine *Hospital das Clínicas*, in the city of São Paulo, Brazil. All patients diagnosed with PR-CRE infection were followed from the day of KT until graft loss, death, or the end of the study period (31 December 2017). We excluded patients who had received less than 48 h of targeted antibiotic treatment for PR-CRE, as well as those who had undergone combined transplantation, although those undergoing simultaneous kidney–pancreas transplantation were included.

All infections were identified through active surveillance in the KT ward and intensive care units, as well as from microbiology reports for patients in outpatient treatment. Surveillance culture for CRE was performed during all study period through perineum-rectal swab at admission, on weekly basis in KT ward and ICU until patient discharge, and at the day of KT. The criteria used in identifying and classifying healthcare-associated infections were those outlined by the US National Healthcare Safety Network [9].

Clinical success was defined as resolution or improvement of the signs and symptoms of infection with no evidence of infection recurrence within the first 30 days after the end of the initial treatment; all other scenarios were classified as treatment failure. We defined infection recurrence as new signs or symptoms of infection at the same site, with a positive culture for PR-CRE of the same phenotype and no evidence of a deep infection, more than 30 days after the end of the initial treatment. Combination therapy was defined as the use of at least two drugs in the targeted therapy, regardless of their in vitro sensitivity. In all combined therapy that used carbapenem, meropenem was administrated at high doses (6 g/day) and by extended infusion (3 to 4 h). Double carbapenem therapy (DCT) was performed with meropenem at high doses associated with 1 g of ertapenem administered daily 1 h prior to the first dose of meropenem. Oral fosfomycin was administered one dose daily from 3 to 14 days; intravenous fosfomycin is not available in Brazil. All patients who were treated with polymyxin used colistin. Tigecycline was used in high dose (loading dose 400 mg, followed by 200 mg daily). Aminoglycoside serum levels were not regularly monitored during the study period. A microbiological response was defined as eradication of baseline pathogens, as evidenced in subsequent cultures during treatment. Deaths in which patients showed signs and symptoms of PR-CRE-infection at the time of death were classified as PR-CRE-infection-related deaths, as were those occurring within 30 days after diagnosis of the infection if no other cause was identified.

Microbiological analysis

All CRE were initially identified and characterized (in terms of their susceptibility pattern) with an automated susceptibility testing system (VITEK or MALDI-TOF MS; bioMérieux, Marcy l'Étoile, France). The minimal inhibitory concentrations (MICs) of imipenem, meropenem, and tigecycline were determined by the stable-gradient method (Etest; AB Biodisk, Solna, Sweden); the MIC of polymyxin B was determined by broth microdilution, whereas that of fosfomycin was determined by agar dilution, with the addition of glucose-6-phosphate. The MICs were interpreted according to the Clinical and Laboratory Standards Institute breakpoints, except for those for polymyxin B and tigecycline, which were interpreted according to the guidelines established by the European Committee on Antimicrobial Susceptibility Testing [10, 11].

Carbapenem-resistant genes (bla_{KPC}, bla_{NDM}, and bla_{OXA-48}) and polymyxin-resistant genes (*mcr*-1) were identified by polymerase chain reaction [12]. Strains showing a high-level resistance to amikacin, gentamicin, and tobramycin in disk diffusion agar (with no inhibitory zone around the disks), with an MIC > 256 μg/mL, as determined by Etest strips (AB Biodisk), were also submitted to polymerase chain reaction for detection of 16S ribosomal RNA methyltransferase (16S-RMTase) genes, such as *arm*A and *rmt*A–*rmt*H [13, 14].

Pulsed-field gel electrophoresis

After extraction and digestion of whole bacterial DNA with XbaI restriction enzyme (50 units; New England Biolabs, London, UK), pulsed-field gel electrophoresis (PFGE) was performed according to the US Centers for Disease Control protocol, based on the methodology described by Gautom et al., with a variable angle system (CHEF-DR III; Bio-Rad Laboratories Inc., Hercules, CA, USA) [15]. The running parameters were

an initial switch time of 2.2 s, a final switch time of 54.2 s, a run time of 19 h, a temperature of 14 °C, and a gradient of 6 V/cm.

The images acquired were submitted to analysis with BioNumerics software, version 7.5 (Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was generated by the unweighted pair group method with arithmetic mean, based on the Dice coefficient (1.5% tolerance), and isolates that showed ≥ 80% similarity were defined as belonging to the same profile.

Statistical analysis

The outcome analyzed were clinical success of treatment for PR-CRE infection. We analyzed independent (continuous and categorical) variables related to KT—recipient age; recipient gender; donor type (living or deceased); type of transplantation (kidney or kidney–pancreas); re-transplantation; diabetes mellitus; end-stage renal disease; induction therapy; acute cellular rejection treatment in the last 3 months before PR-CRE infection; intensive care unit admission in the last 3 months before PR-CRE infection; dialysis in the last 3 months before PR-CRE infection; and time from transplantation to PR-CRE infection—variables related to infection—Sequential Organ Failure Assessment score at diagnosis of PR-CRE infection; glomerular filtration rate at diagnosis of PR-CRE infection; site of infection; positive blood culture; device-related infection; and another concomitant infection—and variables related to treatment—time from the first positive culture to the initiation of effective therapy; drug(s) used; and type of therapy (monotherapy or combination therapy).

In the statistical analysis, we used the chi-square test or Fisher's exact test, as indicated, for dichotomous variables, whereas we used the Mann-Whitney test for continuous variables. Variables showing a value of $p < 0.2$ in a univariate analysis were included in a multivariate analysis, which was performed by stepwise logistic regression. Variables that then reduced the −2 log likelihood or showed a value of $p < 0.05$ were retained in the model. All statistical analyses were performed with the R program (http://www.R-project.org/).

Results

Patients and infections

Among the 2333 KT recipients evaluated, we identified 47 patients with infection due to PR-CRE, which correspond to a cumulative incidence of 2.0%. One patient died within the first 48 h after the initiation of effective therapy, so we analyzed treatment of 46 PR-CRE infection episodes in 40 patients (one patient presented three episodes and two patients presented two episodes).

All PR-CRE infections were due to *Klebsiella pneumoniae*, except one, in which the causative agent was identified as *Serratia marcescens*. In 23 (57.5%) of the 40 patients, PR-CRE was identified in a surveillance culture obtained prior to the first infection episode, no patient was colonized previous to KT. Among patients colonized before infection, the median time from the first positive culture to infection was 5 days (range, 0–50 days). In addition, 23 (50.0%) cases had a polymyxin susceptible CRE infection previously identified.

As can be seen in Table 1, the most common type of infection was UTI, which accounted for 25 (54.3%) of the 46 infections, followed by 15 (32.6%) surgical site infection (SSI), three (6.5%) pneumonia, two (4.3%) catheter-related bloodstream infection, and one (2.2%) peritonitis. In the sample as a whole, the median age was 50 years (range, 15–72 years). Of the 46 PR-CRE infections evaluated, 29 (63.0%) occurred in female KT recipients, 41 (89.1%) occurred in patients who had received a kidney from a deceased donor, and six (13.0%) occurred in patients who had undergone simultaneous kidney–pancreas transplant. The most common cause of end-stage renal disease—identified in 16 (40.0%) of the patients—was diabetic nephropathy, followed by hypertensive nephrosclerosis, in eight (20.0%), uropathy, in six (15.0%), and glomerulonephritis in four (10.0%).

The median time from KT to infection was 94.5 days (range, 7–5241 days). Of the 46 PR-CRE infections, 20 (43.5%) were in patients who had had a positive blood culture, 11 (23.9%) were related to the use of an invasive device, and 21 (45.7%) were in patients in whom no intracavitary fluid collection was identified.

Treatment

The median duration of treatment was 17 days (range, 3–58 days). Monotherapy was used in 10 (21.7%) of the cases of PR-CRE infection: an aminoglycoside was used in five, fosfomycin was used in three, meropenem was used in one, and in one case, monotherapy with fosfomycin was preceded by monotherapy with amikacin. Combined therapy was used in 36 (78.3%) of the cases, including DCT in 19 and oral fosfomycin in 19 (52.7%). The proportion of treatment failure among patients treated with fosfomycin was similar using monotherapy (2 out of 4) or combined therapy (9 out of 19). Tigecycline was used as part of the combined therapy regimen in 72.2% (26/36) of the infections, including 11 UTI episodes, of those 10, tigecycline was the only available drug with in vitro activity. Aminoglycoside was used in combination with other drugs in 36.1% (13/36). In 12 cases (21.7%), colistin was used as part of the antibiotic therapy regimen (despite all strains had MICs > 16 μg/mL) and failed in all 12. Ceftazidime–avibactam was used as rescue therapy in four cases, three of those in combination with other drugs

Table 1 Description of the cases of infection due to polymyxin- and carbapenem-resistant *K. pneumoniae* among kidney transplant recipients

No. patient	Age	Type of transplant	Previous PR-CRE colonization	Previous PS-CRE isolated	Site of infection	Positive blood culture	Ureteral stent	AMK susceptibility	Antimicrobial therapy	Clinical success	30-day mortality
Urinary tract infections due to *K. pneumoniae*											
1	37	Pancreas-kidney	No	No	UTI	No	No	Yes	FOS	No	No
6	72	Kidney	Yes	Yes	UTI	No	No	Yes	AMK+TGC	Yes	No
6	72	Kidney	Yes	Yes	UTI	No	No	Yes	AMK	Yes	No
6	72	Kidney	Yes	Yes	UTI	No	No	Yes	FOS	Yes	No
10	67	Kidney	Yes	No	UTI	Yes	No	Yes	AMK+TGC	Yes	No
11	64	Kidney	No	Yes	UTI	No	Yes	Yes	AMK	Yes	No
12	63	Kidney	Yes	Yes	UTI	No	No	Yes	AMK+MERO+TGC	No	No
12	63	Kidney	Yes	Yes	UTI	Yes	No	Yes	GENT	Yes	No
14	34	Kidney	No	No	UTI	No	No	No	AMK+ERTA++MERO+FOS+TGC	Yes	No
14	34	Kidney	Yes	No	UTI	No	No	No	FOS+ERTA+MERO+TGC	Yes	No
16	55	Kidney	No	Yes	UTI	No	No	No	COL+FOS+MERO+TGC	No	Yes
17	49	Kidney	Yes	No	UTI	Yes	Yes	No	FOS+MERO+TGC	Yes	No
20	60	Kidney	Yes	Yes	UTI	No	No	No	FOS+ERTA+MERO	Yes	No
23	60	Kidney	Yes	No	UTI	Yes	Yes	No	COL+FOS+ERTA+MERO+TGC	No	Yes
24	69	Kidney	Yes	No	UTI	Yes	Yes	No	FOS+ERTA+MERO+TGC	No	No
25	69	Kidney	Yes	Yes	UTI	No	No	No	FOS+ERTA+MERO+TGC	Yes	No
26	37	Kidney	Yes	No	UTI	Yes	No	Yes	AMK+ERTA+MERO+FOS	Yes	No
28	50	Kidney	No	Yes	UTI	No	No	No	FOS	No	Yes
28	50	Kidney	No	Yes	UTI	No	No	No	COL+MERO	No	Yes
29	15	Kidney	Yes	Yes	UTI	No	Yes	Yes	AMK	Yes	No
33	58	Kidney	Yes	No	UTI	No	Yes	Yes	AMK+IMIP	Yes	No
36	40	Kidney	No	No	UTI	No	Yes	No	FOS+ERTA+MERO+TGC	Yes	No
37	34	Pancreas-kidney	No	Yes	UTI	No	No	No	COL+ERT+MERO+FOS	Yes	No
38	68	Kidney	Yes	No	UTI	No	No	Yes	AMK	Yes	No
40	46	Kidney	No	No	UTI	No	No	No	MERO	Yes	No
Other infections due to *K. pneumoniae*											
1	37	Pancreas-kidney	No	No	SSI	No	No	Yes	AMK+IMIP+TGC	No	No
2	59	Kidney	No	Yes	SSI	Yes	No	Yes	COL+IMIP+TGC	No	Yes
3	56	Kidney	No	No	SSI	Yes	No	Yes	COL+TGC	No	Yes
4	60	Pancreas-kidney	No	Yes	SSI	No	No	Yes	COL+IMIP	No	Yes
5	71	Kidney	No	Yes	SSI	Yes	No	Yes	AMK followed to FOS	Yes	No
7	57	Kidney	No	Yes	SSI	Yes	Yes	Yes	AMK+IMIP	Yes	No
9	50	Kidney	Yes	Yes	PNM	Yes	NA	Yes	AMK+COL+TGC	No	Yes
13	58	Kidney	Yes	No	SSI	Yes	Yes	No	FOS+MERO+TGC	No	No
15	50	Kidney	Yes	No	SSI	No	Yes	Yes	AMK+FOS+MERO	Yes	No
18	34	Kidney	No	No	SSI	Yes	No	No	FOS+ERTA+MERO+TGC	Yes	No
19	41	Kidney	Yes	Yes	CVC-BSI	Yes	NA	No	AMK+ERTA+MERO+TGC	Yes	No
21	27	Kidney	Yes	No	SSI	No	No	No	COL+FOS+ERTA+MERO+TGC	No	Yes
22	37	Pancreas-kidney	Yes	Yes	CVC-BSI	Yes	NA	No	FOS+ERTA+MERO+TGC	No	Yes
27	49	Kidney	No	Yes	PNM	Yes	NA	No	COL+ERTA+MERO+TGC	No	Yes
30	39	Pancreas-kidney	Yes	No	SSI	Yes	No	Yes	AMK+COL+ERTA+MERO	No	No
31	57	Kidney	No	No	SSI	No	No	No	FOS+ERTA+MERO+TGC	No	Yes
32	70	Kidney	No	Yes	PNM	Yes	NA	Yes	FOS+ERTA+MERO+TGC	No	Yes

Table 1 (continued)

No. patient	Age	Type of transplant	Previous PR-CRE colonization	Previous PS-CRE isolated	Site of infection	Positive blood culture	Ureteral stent	AMK susceptibility	Antimicrobial therapy	Clinical success	30-day mortality
34	47	Kidney	Yes	No	SSI	No	Yes	No	ERTA+MERO+TGC	No	Yes
35	41	Kidney	Yes	Yes	Peritonitis	No	NA	Yes	COL+MERO+TGC	No	Yes
39	44	Kidney	Yes	No	SSI	Yes	Yes	No	FOS+ERTA+MERO+TGC	Yes	Yes
Infections due to *S. marcescens*											
8	33	Kidney	No	No	SSI	Yes	No	Yes	GENT+MERO	Yes	No

PR, polymyxin resistance; *PS*, polymyxin sensitivity; *SSI*, surgical site infection; *PNM*, pneumonia; *UTI*, urinary tract infection; *CVC-BSI*, catheter-related bloodstream infection, *AMK*, amikacin; *COL*, colistin; *ERT*, ertapenem; *FOS*, fosfomycin; *GENT*, gentamicin; *IMIP*, imipenem; *MERO*, meropenem; *TGC*, tigecycline

(meropenem and tigecycline in two patients and fosfomycin in all three) and none of the three patients showed improvement and evolved to death. In one case, ceftazidime–avibactam was used as monotherapy after DCT failure and this patient had a favorable outcome.

Among patients who used aminoglycosides, 8 (40.0%) have other site of infection than UTI and the clinical success among those was achieved in 50.0% (4/8), among episodes in which aminoglycosides was not used and site of infection was not UTI, clinical success was achieved in 15.4% (2/13) (Table 1). In cases of UTI infections, patients who used aminoglycosides presented clinical success in 90.9% (10/11) and patients who did not use this drug had clinical success in only 57.1% (8/14) ($p = 0.09$).

Clinical success was identified in 25 (54.3%) of the infections, among those only one patient died during hospital stay, 42 days after PR-CRE infection diagnosis due to a hemorrhagic shock. PR-CRE infection-associated deaths occurred in 12 (26.1%) cases. Among the 28 KT recipients who were alive after 30 days of treatment, there was some degree of improvement in renal function in 40.0% of those who were treated with an aminoglycoside, compared with only 29.4% of those who were not ($p = 0.51$). Among these 28 patients, 6 (21.4%) lost the graft during the infection episode; four of them needed the graft removal for infection control, and one probably had the graft lost associated to amikacin nephrotoxicity.

In the multivariate analysis (Table 2), the only risk factor identified for treatment failure was diabetes mellitus ($p = 0.02$); the use of amikacin and having a UTI were found to be protective factors.

Microbiological analysis

In all but one patient, there was good concordance between the automatic (VITEK) and broth microdilution methods of microbiological analysis of polymyxin susceptibility. In that one patient, the MIC for polymyxin was ≥ 6 μg/mL and 4 μg/mL with the VITEK and broth microdilution methods, respectively. The MIC for polymyxin determined by microdilution ranged from 4 to > 64 μg/mL, and the MIC at which 50% of the isolates were inhibited was 32 μg/mL (Table 3).

Of the 46 strains isolated, 38 (82.6%) presented elevated MIC for carbapenem (MIC > 8 μg/mL); antimicrobial agent with best susceptibility was tigecycline 43 (93.5%), nine (19.6%) strains were susceptible to gentamicin, and 23 (50.0%) were susceptible to amikacin (Table 2). The proportion of strains resistant to all three aminoglycosides tested was 0% in 2014, 14.3% in 2015, 68.2% in 2016, and 55.6% in 2017 (Fig. 1).

Susceptibility to fosfomycin was tested in 14 strains isolated from patients who used fosfomycin, 11 (78.6%) of which were susceptible to fosfomycin at the beginning of treatment. Among those 11 cases, microbiological failure was observed

Table 2 Univariate and multivariate analyses of risk factors for treatment failure among 46 cases of polymyxin- and carbapenem-resistant *Klebsiella pneumoniae* infection in kidney transplant recipients

Variable	Clinical success ($n = 25$)	Treatment failure ($n = 21$)	Univariate analysis		Multivariate analysis	
			RR (95% CI)	*p* value	OR (95% CI)	*p* value
Recipient age (years), median (range)	50 (15–72)	50 (27–70)	–	0.85		
Recipient gender female, *n* (%)	16 (64.0)	13 (61.9)	0.96 (0.55–1.67)	0.88		
Simultaneous kidney–pancreas transplantation, *n* (%)	1 (4.0)	5 (23.8)	3.60 (0.59–21.93)	0.08		
Deceased donor, *n* (%)	22 (88.0)	19 (90.5)	1.11 (0.52–2.42)	> 0.99		
Cause of end-stage renal disease, *n* (%)						
Diabetic nephropathy	9 (36.0)	10 (47.6)				
Hypertensive nephrosclerosis	6 (24.0)	4 (19.0)				
Uropathy	4 (16.0)	3 (14.3)	–	0.72		
Undefined	2 (8.0)	3 (14.3)				
Glomerulonephritis	3 (12.0)	1 (4.8)				
Polycystic kidney disease	1 (4.0)	0				
Diabetes mellitus, *n* (%)	9 (36.0)	13 (61.9)	1.63 (0.92–2.90)	0.08	7.46 (1.35–41.25)	0.02
Re-transplantation, *n* (%)	5 (20.0)	3 (14.3)	0.84 (0.46–1.56)	0.71		
Induction therapy with ATG, *n* (%)	17 (68.0)	15 (71.4)	1.08 (0.62–1.88)	0.80		
ACR in the last 3 months before infection, *n* (%)	4 (16.0)	1 (4.8)	0.64 (0.38–1.09)	0.36		
ICU stay in the last 3 months before infection, *n* (%)	10 (40.0)	13 (61.9)	1.50 (0.86–2.61)	0.14		
Polymyxin use in the last 3 months before infection, *n* (%)	8 (32.0)	12 (57.1)	1.64 (0.89–2.99)	0.09		
Amikacin use in the last 3 months before infection, *n* (%)	9 (36.0)	6 (28.6)	0.86 (0.50–1.47)	0.59		
Carbapenem use in the last 3 months before infection, *n* (%)	13 (61.9)	16 (76.2)	1.58 (0.95–2.62)	0.09		
Dialysis in the last 3 months before infection, *n* (%)	13 (52.0)	13 (61.9)	1.20 (0.71–2.03)	0.50		
Ureteral stent at diagnosis of infection, *n* (%)	8 (32.0)	3 (14.3)	0.67 (0.41–1.10)	0.19		
SOFA score at infection, median (range)	3 (1–6)	5 (0–16)	–	0.04		
Time from transplantation to infection (days), median (range)	92 (7–5241)	132 (7–3790)	–	0.53		
Glomerular filtration rate (mL/min) at infection, median (range)	14.1 (5.7–51.2)	23.4 (2.8–81.8)	–	0.39		
Positive blood culture, *n* (%)	10 (40.0)	10 (47.6)	1.15 (0.67–2.00)	0.60		
Urinary tract infection, *n* (%)	18 (72.0)	7 (33.3)	0.46 (0.24–0.89)	0.009	0.10 (0.02–0.54)	0.007
Surgical site infection, *n* (%)	6 (24.0)	9 (42.9)	1.53 (0.78–3.03)	0.17		
Invasive device-related infection, *n* (%)	5 (20.0)	6 (28.6)	1.26 (0.62–2.55)	0.73		
Another concomitant HAI, *n* (%)	3 (12.0)	2 (9.5)	0.89 (0.41–1.93)	> 0.99		
Time from first positive culture to start of effective antibiotic therapy (days), median (range)	2 (0–12)	3 (0–21)	–	0.64		
Tigecycline included in antibiotic therapy regimen, *n* (%)	10 (40.0)	16 (76.2)	1.95 (1.13–17.33)	0.02		
Fosfomycin included in antibiotic therapy regimen, *n* (%)	13 (52.0)	10 (47.6)	0.92 (0.54–1.57)	0.77		
Amikacin included in antibiotic therapy regimen, *n* (%)	15 (60.0)	4 (19.0)	0.47 (0.27–0.81)	0.007	0.12 (0.02–0.64)	0.01
Polymyxin included in antibiotic therapy regimen, *n* (%)	0 (0.0)	12 (57.1)	–	< 0.001		
Double carbapenem as part of antibiotic therapy regimen, *n* (%)	10 (40.0)	9 (42.9)	1.06 (0.61–1.82)	0.85		
Combined therapy, *n* (%)	17 (68.0)	19 (90.5)	1.69 (1.07–2.69)	0.08		
Combined therapy including at least two drugs with in vitro susceptibility, *n* (%)	6 (24.0)	4 (19.0)	0.88 (0.49–1.59)	0.74		

ACR, acute cellular rejection; *ATG*, antithymocyte globulin; *HAI*, healthcare-associated infection; *ICU*, intensive care unit; *SOFA*, Sequential Organ Failure Assessment

Table 3 Susceptibility profile of 46 polymyxin-resistant *Klebsiella pneumoniae* strains isolated from infected kidney transplant recipients

Antibiotic	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range (μg/mL)
Ertapenem	> 8	> 8	4 to > 8
Imipenem	> 32	> 32	0.125 to > 32
Meropenem	> 32	> 32	0.5 to > 32
Polymyxin	24	32	4 to > 64
Tigecycline	0.5	1.05	0.125 to 4
Amikacin	16	> 64	< 2 to > 64
Gentamicin	> 16	> 16	< 1 to > 16

MIC_{50}, minimal inhibitory concentration (MIC) at which 50% of the isolates are inhibited; *MIC_{90}*, MIC at which 90% of the isolates are inhibited; *AT*, automated test (VITEK)

in six. Five strains were isolated after microbiological failure under treatment regimens including fosfomycin; in only one case, the isolated strain initially susceptible to fosfomycin became resistant after its use, whereas the strains remained susceptible to fosfomycin in the four remaining cases. Among the three patients infected with a PR-CRE that was resistant to fosfomycin at the beginning of treatment, one experienced clinical failure and died without additional positive cultures and clinical success was achieved in two. The overall rate of treatment success among the patients treated with fosfomycin was 52.2% (Fig. 2).

Mechanism of resistance and clonality

All but one of the strains were positive for bla_{KPC-2}, and all were negative for *mcr*-1, as well as being negative for the other carbapenemase genes tested. Strains isolated from nine of the episodes of infection presented high resistance to all aminoglycosides tested, all being confirmed as producers of RmtB enzyme, which was present in six different PFGE pulsotype strains. The clonality was analyzed through PFGE in 30 strains, 21 (70%) of which were found to belong to pulsotype A; the others were distributed among eight different pulsotypes (Fig. 3).

Discussion

Treatment of polymyxin- and carbapenem-resistant *Enterobacteriaceae* infections is challenging and there is currently no recommended standard antimicrobial regimen [16]. The present study described cases of PR-CRE infection in KT recipients, investigating the clinical/epidemiological course and microbiological features of those infections.

The incidence of PR-CRE infection varies widely among centers, reportedly accounting for 0–28% of all CRE infections in SOT recipients [1]. At our center, the proportion of PR-CRE infections is high. In the study, the PFGE analysis has been shown that part of this dissemination is through cross transmission but the presence of many pulsotypes probably indicates that these strains gain the hospital through several ways. As described before, many Brazilian institutions present a high level of polymyxin resistance among CRE strains [3, 17, 18].

In the present study, the majority of patients who developed PR-CRE infection were previously colonized by multidrug-resistant *K. pneumoniae*, and such colonization has been described as a major risk factor for CRE infection after SOT [18, 19].

In our sample, all strains were *mcr*-1-negative. Therefore, the probable mechanism of polymyxin resistance was post-translational modification of the lipopolysaccharide molecules that form the outer membrane. This modification occurred due

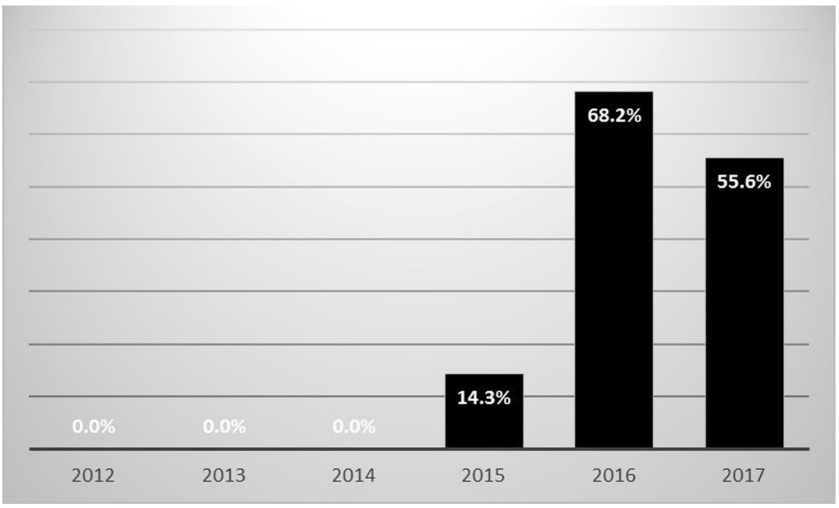

Fig. 1 Prevalence of aminoglycoside resistance during the study period (2012–2017)

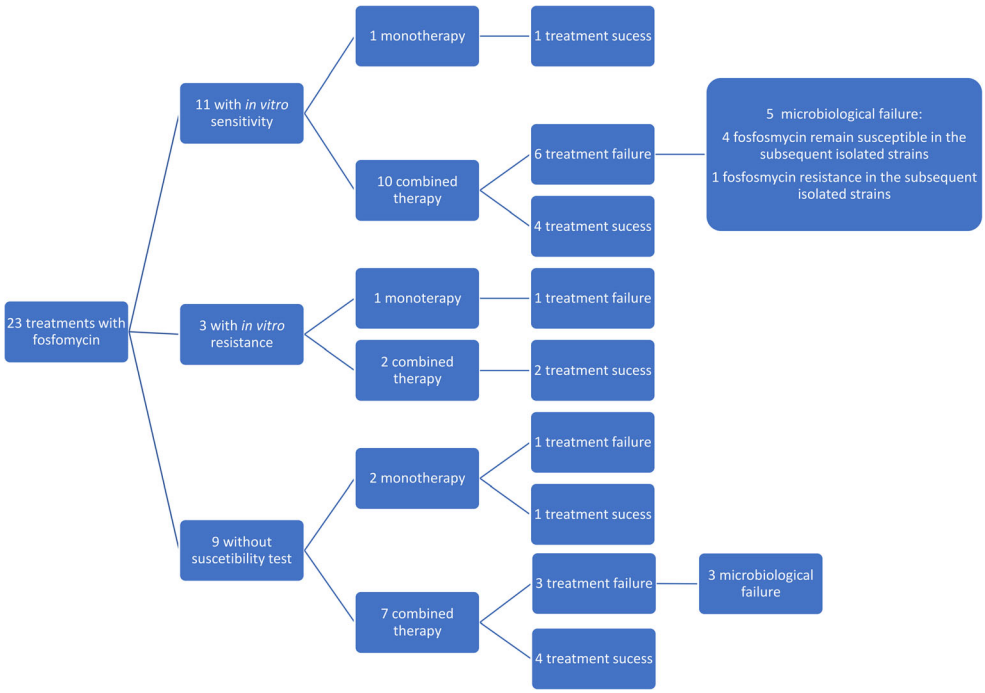

Fig. 2 Flowchart of 23 patients infected with polymyxin- and carbapenem-resistant *Enterobacteriaceae* and treated with fosfomycin

to dysregulation of the two-component regulatory systems PmrA/PmrB and PhoP/PhoQ, which control the expression of genes that modify lipopolysaccharide molecules and might be responsible for the majority of polymyxin resistance in CRE [7].

The use of carbapenem has been recommended in the treatment of CRE infections. Epidemiological and in vitro studies have demonstrated the benefit of using carbapenem, especially in combined therapy regimens for complicated and severe infections. However, the benefit of combined therapy with carbapenem has been observed most often in strains for which carbapenem has an MIC ≤ 8 μg/mL [2, 20].

In the present study, the strains isolated showed high levels of carbapenem resistance, which reduces the probability of carbapenem reaching its pharmacokinetic–pharmacodynamic target, even when a high dose and extended infusion are used. That could partially explain our finding of a higher proportion of treatment failure in patients receiving combined therapy with carbapenem.

In our sample, the rate of fosfomycin resistance was relatively high (21.4%). The rate of fosfomycin resistance has been shown to be higher among multidrug-resistant strains [21]. In a multicenter study that analyzed strains of *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*, fosfomycin resistance was identified in 60.8%, whereas other studies have reported fosfomycin resistance rates of 6.25–14.7% among CRE strains [16, 22, 23].

There have been reports of therapeutic success with antimicrobial regimens including fosfomycin even when in vitro resistance was detected [24]. That can be explained by the possible synergism between fosfomycin and other antimicrobial agents, such as carbapenem, colistin, and tigecycline [25]. In the present study, clinical success was achieved in two out of three patients who were infected with fosfomycin-resistant PR-CRE strains. Both of those patients were treated with DCT plus fosfomycin. The overall rate of treatment success among the patients treated with fosfomycin (as monotherapy or in combined therapy) was 52.2%. In the literature, the rate of microbiological cure for CRE infection achieved with oral fosfomycin ranges from 31 to 59% [26, 27].

One therapeutic strategy used for treating CRE infection in our study sample was DCT, which has been used when there are no other therapeutic options, such as when the strain in question is resistant to all other antimicrobial agents or when the antimicrobial agents to which it is susceptible have pharmacokinetic or toxicity profiles that preclude their use in some patients [24, 28]. Time-kill studies have shown that the bactericidal activity of meropenem increases when ertapenem is added, as well as a synergic effect when colistin is added to DCT, with an increase in anti-bacterial activity and inhibition of bacterial growth [29, 30]. One case-control study found that, in patients with CRE infection, mortality was 60.9% lower among those treated with DCT than among those treated with standard therapy [31]. In a case series, the reported rate of success of DCT is 39–78% [28, 32]. The 52.6% success rate observed in our sample is intermediate to those reported previously [29, 32]. Some cases of successful treatment with DCT have been reported among SOT recipients, even when DCT was used in isolation, although there might be a publication bias, because it is possible that only successful cases have been reported [24, 29, 30].

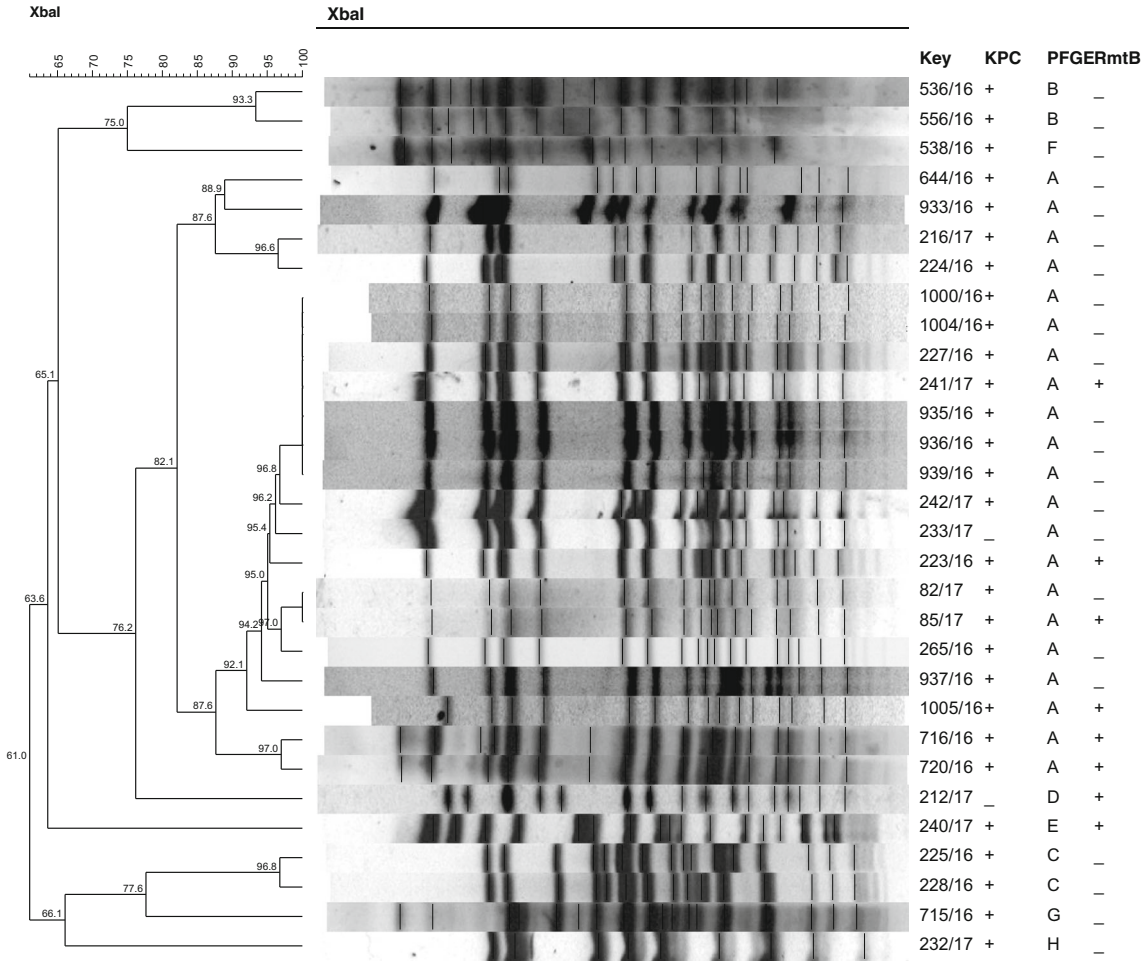

Fig. 3 Pulsed-field gel electrophoresis dendrogram of 30 strains of polymyxin- and carbapenem-resistant *Klebsiella pneumoniae* isolated from infected kidney transplant recipients

In our analysis, a UTI was found to be protective against treatment failure in PR-CRE infection. Other studies have shown that a UTI caused by CRE is associated with better outcomes when compared with other types of CRE infection [33]. Among KT recipients infected with CRE, mortality rates have been shown to be lower in those with UTI than in those with other types of infection. Although UTIs often affect the graft, the fewer systems involved and the high concentration of antibiotics in the urinary tract might be responsible for the better outcomes [3, 5].

Among the four patients treated with ceftazidime–avibactam in our sample, clinical success was achieved in only one. That combination was used as rescue therapy in all cases, which could account for the unfavorable result. The reports evaluating the use of ceftazidime–avibactam for treating CRE infection reported success rates of 46–92% [33, 34]. However, one large study of CRE infection that included SOT recipients reported microbiological failure of ceftazidime–avibactamin 27% [34]. The authors also found that 30% of the patients who failed developed resistance to ceftazidime–avibactam during treatment. The low rate of response to ceftazidime–avibactam was found in $bla_{\text{KPC-3}}$-producing strains. In our cohort, all KPC-producing strains were $bla_{\text{KPC-2}}$.

Aminoglycosides were the only antimicrobial agents associated with clinical success. CRE usually have remained highly susceptible to aminoglycosides, which have rapid bactericidal activity against CRE and a synergistic effect with other antimicrobial agents used in the treatment of CRE infections, such as carbapenem and polymyxin [35]. A cohort study of 33 patients with bacteremia due to CRE and treated with an aminoglycoside reported a clinical success rate of 54% [36].

Although there is concern about the use of aminoglycosides in KT recipients, because of their nephrotoxic potential, some studies have shown that they can be used safely in this population [3, 36]. At our cohort, we had six patients that lost the graft within 30 days of UTI by PR-CRE, and despite this is a high proportion, the majority of those lost the graft due to infection itself; in only one case, the probable cause of graft lost was amikacin nephrotoxicity.

The study of an outbreak involving 50 patients with CRE found that the use of gentamicin was independently associated with lower mortality, although only in the patients infected with strains for which gentamicin had an MIC ≤2 μg/mL [37].

In the present study, we observed a progressive increase in aminoglycoside resistance over time, more than 65% of the analyzed strains presenting resistance to at least one aminoglycoside tested and 9 strains presenting a high level of resistance to all aminoglycosides (MIC > 256 μg/mL). The investigation of the mechanism of resistance revealed that the former group of strains contained RmtB, a RMTase, which confers a high level of resistance to aminoglycosides. The *rmt*B gene encoding the RmtB enzyme is frequently harbored in plasmids, and resistance to other antibiotics are common [38]. Although the carbapenemase most often associated with RmtB is New Delhi metallo-beta-lactamase, KPC-2 has been identified in the same strains that contain RmtB [39]. Other studies have shown *rmt*B in different strains and species of carbapenem-resistant *Enterobacteriaceae* at the same hospital [40]. In our study, different *K. pneumonia* pulsotypes contained *rmt*B, showing the ability of plasmid spread of the resistance among strains.

This study has some limitations; first due to the lower frequency of this resistance profile, we could not include large number of cases. In addition, despite we included a great number of variables in treatment analysis, the retrospect design and the scarce therapeutic options entailed very heterogeneous treatment schemes and more severe patients probably were treated with large spectrum antibiotics. Therefore, prospective and multicenter studies are needed to confirm our finds.

Therefore, the treatment of PR-CRE infection among KT recipients is a challenge. The use of alternative drug combination regimens is imperative and might be efficacious. Aminoglycosides could represent an important therapeutic option in the treatment of CRE infection in KT recipients and the impact on renal function is much more due to the infection itself than to aminoglycoside nephrotoxicity. However, the emergence of aminoglycoside resistance through the presence of RMTase could have a major impact on the management of CRE infection in this specific population.

Compliance with ethical standards The study was approved by our Institutional Review Board and due to its retrospective study design, patient informed consent was waived.

Conflict of interest The authors declare that they have no competing interests.

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