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Short Communication

Induction and nosocomial dissemination of carbapenem and polymyxin-resistant *Klebsiella pneumoniae*

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ABSTRACT

Introduction: Polymyxins are antimicrobial agents capable of controlling carbapenemase-producing *Klebsiella pneumoniae* infection. **Methods:** We report a cluster of four patients colonized or infected by polymyxin-resistant and *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae.* **Results:** Three patients were hospitalized in adjacent wards, and two were admitted to the intensive care unit. The index case maintained prolonged intestinal colonization by KPC-producing *K. pneumoniae*. Three patients received polymyxin B before the isolation of polymyxin-resistant *K. pneumoniae*. **Conclusions:** Colonization by KPC-producing *K. pneumoniae* and previous use of polymyxin B may be causally related to the development of polymyxin-resistant microorganisms.

Keywords: *Klebsiella pneumoniae.* KPC. Polymyxin B. Colistin.

Klebsiella pneumoniae has adapted to the extensive and intensive use of antibacterial drugs in hospitals. Over the last 30 years, *K. pneumoniae* went from having partial resistance to ampicillin and narrow-spectrum cephalosporins to current pandemic resistance to broad-spectrum cephalosporins due to extended-spectrum beta-lactamase production as well as multidrug resistance to penicillins, cephalosporins, and monobactams[1]. Likewise, carbapenemase-producing strains such as *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase producers, which are not susceptible to imipenem or other carbapenem drugs, have rapidly emerged and spread worldwide[2] [3]. Colistin and polymyxin B are among the few remaining drugs able to combat these multidrug-resistant strains and having satisfactory efficacy in the treatment of patients infected with KPC-producing *K. pneumoniae*[4]. However, KPC producers also resistant to polymyxins have recently been detected[5].

Here, we report the cases of four patients involved in a cluster of polymyxin-resistant and KPC-producing *K. pneumoniae* in order to clarify the factors associated with the induction and dissemination of this extensively drug- resistant strain.

Infection by polymyxin-resistant KPC-producing *K. pneumoniae* occurred in patients admitted to the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil – a public university hospital that provides tertiary medical care – in November 2011. The hospital units where transmission and/or isolation of the extensively drug-resistant clone occurred are located on the 6th floor (hematology ward and bone marrow transplant unit), 5th floor (geriatric ward), and 2nd floor (intensive care unit [ICU]). Clinical and epidemiological data were obtained retrospectively from the patient's medical records.

Bacterial identification and initial susceptibility testing were performed by using the VITEK 2 automated microbial identification system (BioMérieux, Mercy L'Etoile, France). E-test® strips (BioMérieux) were used to determine the minimum inhibitory concentrations (MICs) of colistin and polymyxin B for KPC-producing *K. pneumoniae* isolates in duplicate. The MICs for colistin were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[6]. The breakpoints proposed for *Acinetobacter baumannii*[7] were adopted for the MICs of polymyxin B.

Polymyxin-resistant and KPC-producing *K. pneumoniae* were isolated from the four patients from December 8, 2011 to January 27, 2012. All patients had serious underlying diseases, and three

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were receiving immunosuppressive drugs **(Table 1)**. All four patients had been hospitalized previously. Two were readmitted to the bone marrow transplant unit, one to the hematology ward, and one to the geriatric ward; the latter two were also hospitalized for some time in the ICU **(Figure 1)**. Their clinical data are presented below.

Case 1

Case 1 was of a 39-year-old woman with acute myeloid leukemia who was admitted to the hematology ward for chemotherapy. She developed pulmonary aspergillosis and bloodstream infection with KPC-producing *K. pneumoniae* as a consequence of neutropenia and had been treated with voriconazole, polymyxin B (25 days), and tigecycline (14 days). Although KPC-producing *K. pneumoniae* was further isolated within 10 days of the initiation of treatment with polymyxin B, both infections were controlled. The patient was readmitted and received to bone marrow transplantation. She developed another bloodstream infection with KPC-producing *K. pneumoniae* and was treated again with polymyxin B (25 days) plus tigecycline (21 days). The two polymyxin B treatments were separated by 50 days. The patient developed severe mucositis after bone marrow transplantation, and polymyxin-resistant and KPC-producing *K. pneumoniae* was isolated from an oropharyngeal ulcer 14 days after restarting polymyxin B. However, she died from a new bloodstream infection with multi-susceptible *K. pneumoniae*. Throughout both admissions, polymyxin-susceptible and KPC-producing *K. pneumoniae* was isolated from 10 rectal swabs, revealing persistent colonization for more than 100 days.

Case 2

Case 2 was of a 74-year-old woman who had been suffering from Sheehan syndrome for 30 years and developed acute myeloid leukemia. While receiving chemotherapy, she suffered several complications and consequently stayed in the ICU for 20 days. She developed febrile neutropenia and received courses of antibacterial drugs including polymyxin B. On the 42nd day of hospitalization, a rectal swab culture revealed KPC-producing *K. pneumoniae*. Polymyxin-resistant and KPC-producing *K. pneumoniae* was subsequently isolated from two blood cultures on the 49th day and a urine culture on the 53rd day. The patient was being treated with meropenem, followed by the addition of amikacin. However, she developed septic shock and died.

Case 3

Case 3 was of a 36-year-old man who experienced acute myeloid leukemia relapse after bone marrow transplantation and was readmitted to the bone marrow transplantation ward for chemotherapy. He developed febrile neutropenia, tonsillitis, pulmonary infiltrate, and cellulitis at the venous catheter implantation site. Antibacterial agents and voriconazole were administered continuously. On the 49th day of hospitalization, polymyxin-resistant and KPC-producing *K. pneumoniae* was isolated from two consecutive blood cultures. The patient was treated empirically with polymyxin B and tigecycline for two days, but suffered septic shock and died.

Case 4

Case 4 was of a 64-year old woman who had chronic arterial disease, diabetes mellitus, and chronic renal failure. She was

TABLE 1 - Demographic, clinical, and hospital epidemiological data of patients infected with polymyxin B-resistant and KPC-producing *Klebsiella pneumoniae.*

Case	Age (y)	Sex	Underlying disease	Ward/unit	Hospitalization duration (days)[a]	Previous antimicrobials		Antibiotic therapy[c]	Clinical outcome
						days[b]	drugs		
1	39	F	Acute myeloid leukemia	Hematology, bone marrow transplant unit	32	32	T/S-MEM-MTZ-PB-TGC-AMK-CIP-VOR	_	Death (related to multisusceptible *K. pneumoniae*)
2	74	F	Acute myeloid leukemia	Hematology, intensive care unit	49	42	CPM-VAN-MEM-AMK-CIP-PB- AMPH	MEM AMK	Death
3	36	M	Acute myeloid leukemia	Bone marrow transplant unit	49	49	CPM-LVX-VAN-MEM-LNZ-VOR	PB TGC	Death
4	65	F	Chronic arterial disease, diabetes, renal failure	Geriatrics, intensive care unit	60	67	CIP-CLI-P/T-VAN-PB-GEN	TGC	Survival

KPC: *Klebsiella pneumoniae* carbapenemase; **F:** female; **M:** male; **T/S:** trimethoprim/sulfamethoxazole; **MEM:** meropenem; **MTZ:** metronidazole; **PB:** polymyxin B; **TGC:** tigecycline; **AMK:** amikacin; **CIP:** ciprofloxacin; **VOR:** voriconazole; **CPM:** cefepime; **VAN:** vancomycin; **AMPH:** amphotericin B; **LVX:** levofloxacin; **LNZ:** linezolide; **CLI:** clindamycin; **P/T:** piperacillin/tazobactam; **GEN:** gentamicin. [a]Time of hospitalization. [b]Antimicrobial administration before the isolation of polymyxin-resistant *K. pneumoniae*. [c]Antibiotics administered after the isolation of this bacterium.

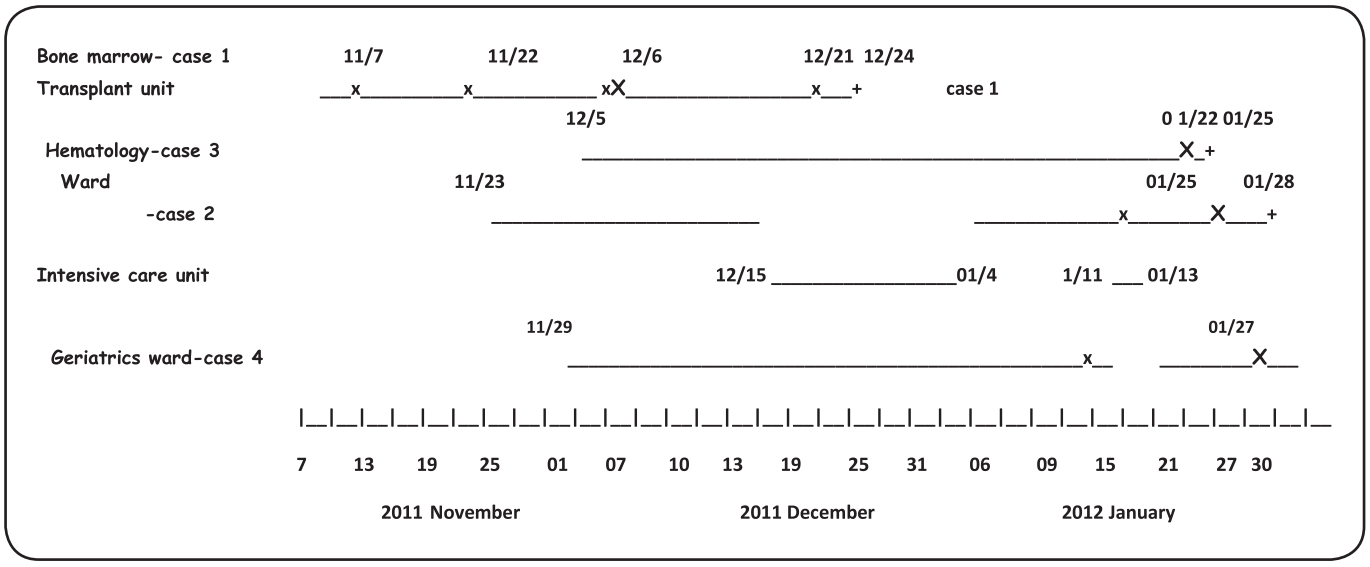

FIGURE 1 - A cluster of carbapenem- and polymyxin-resistant *Klebsiella pneumoniae* cases in four patients: hospital ward, transference to intensive care unit, and time of isolation of carbapenemase- producing *K. pneumoniae* susceptible (x) or resistant to polymyxins (X), and death (+).

admitted to the geriatric ward and subsequently transferred to the ICU seven days after case 2 left the unit. She underwent surgery for revascularization of the right leg. However, a surgical site infection occurred, from which *A. baumannii* and KPC-producing *K. pneumoniae* were isolated. Empirical antibiotics therapy was replaced with polymyxin B and gentamicin. After 19 days of polymyxin B treatment, she developed fever and cellulitis at the venous catheter insertion site. The catheter was removed, and polymyxin-resistant and KPC-producing *K. pneumoniae* was isolated from its tip. The patient was treated with tigecycline, and her fever stopped.

Polymyxin B 20,000 IU/Kg/day was administered for cases 1, 2, and 4. The microbiological data of the patients are shown in **Table 2**. In addition to resistance to polymyxin B and colistin, three KPC-producing *K. pneumoniae* isolates were also resistant or had intermediate susceptibility to tigecycline. However, all isolates were susceptible to amikacin.

The present report describes the clinical and hospital factors associated with the emergence and transmission of polymyxin-resistant *K. pneumoniae*. Three previously studied isolates (cases 2-4) carried the bla_{KPC2} and *gnrS1* genes and belong to sequence type (ST)-11, an internationally occurring high-risk clone[8]; these bacterial isolates simultaneously carry genes encoding virulent phenotypes and related to multidrug resistance. Different multidrug-resistant *K. pneumoniae* clones associated with patient colonization or infection, such as extended-spectrum and CTX-M beta-lactamase producers have been detected in the hospital where the cluster occurred[9]. The isolation of carbapenem-resistant *K. pneumoniae* from blood cultures and other samples has increased in recent years; these isolates were characterized as KPC-2 producers as well as ST-258, ST-11, and ST-48 clones[10]. This epidemiological change is similar to those that have occurred in hospitals in other regions and countries, i.e., increases in cases of infection attributed to the ST-11 and other clones of KPC-producing *K. pneumoniae*[11].

The dissemination of Gram-negative bacilli resistant to imipenem and other carbapenem drugs led to increased use of polymyxins for infected patients in hospitals. However, previous use of colistin is the only independent factor for the isolation of Gram-negative bacilli resistant to this antibiotic[12]. In three of the four cases reported herein, polymyxin B was administered immediately for over 14, 19, and 25 days, respectively, before the isolation of polymyxin-resistant and KPC-producing *K. pneumoniae*. The highest MIC was observed in an isolate from case 1, who received polymyxin for a longer period (25 + 14 days). The emergence of polymyxin B-resistant isolates has been observed during treatment with this drug for KPC-producing *K. pneumoniae* infection or colonization probably due to the selective pressure of polymyxin B on the heterogeneous bacterial population[13]. An *in vitro* study of multidrug-resistant colistin-susceptible *K. pneumoniae* revealed colistin had a rapid bactericidal effect albeit a low post-antibiotic effect; bacterial regrowth was attributed to the heteroresistance phenomena, which was detected in 15 of 16 isolates[14]. In case 1, the persistence of bacteremia during the first KPC-producing *K. pneumoniae* infection for up to 10 days of polymyxin B administration as well as rectal colonization for more than 100 days despite the therapeutic course with this antibiotic are suggestive of heteroresistance.

Other factors may be involved in the development of polymyxin resistance. In the present cluster patients had severe organic alterations and was invaded with catheters and drains. Three patients received immunosuppressants, and all four received broad-spectrum antibiotic therapy for more than 30 days, which favored infection with Gram-negative bacilli. Advanced age, a history of surgery, and the administration of

TABLE 2 - MICs of antimicrobials against polymyxin-resistant and carbapenemase-producing *Klebsiella pneumoniae* and other bacteria isolated during patient hospitalization.

Case	Clinical sample	MIC (µg/mL)					Other bacteria isolated (clinical sample)
		PB[a]	CL[a]	IPM[b]	AMK[b]	TGC[b]	
1	Tonsil, ulcer	48	64	≥16	4	≥8	Polymyxin-susceptible and KPC-producing *K. pneumoniae* (blood, urine, rectal swab)
		(R)	(R)	(R)	(S)	(R)	Multisusceptible *K. pneumoniae* (blood)
2	Blood, urine	12	16	≥16	≤2	≥8	Polymyxin-susceptible and KPC-producing *K. pneumoniae* (rectal swab)
		(R)	(R)	(R)	(S)	(R)	Oxacillin-resistant *Staphylococcus epidermidis* (blood)
3	Blood	12	16	≥16	≤2	2	Cephalosporin-resistant *Enterobacter cloacae* (rectal swab)
		(R)	(R)	(R)	(S)	(I)	Oxacillin-resistant *Staphylococcus epidermidis* (blood)
4	Venous catheter tip	24	32	≥16	≤2	1	Polymyxin-susceptible and KPC-producing
		(R)	(R)	(R)	(S)	(S)	*K. pneumoniae* and *Acinetobacter baumannii* (surgical wound)

MIC: minimum inhibitory concentration; **PB:** polymyxin B; **CL:** colistin (i.e., polymyxin E); **IPM:** imipenem; **AMK:** amikacin; **TGC:** tigecycline; **KPC:** *Klebsiella pneumoniae* carbapenemase; **S:** susceptible; **R:** resistant; **I:** intermediate susceptibility; [a]Determination of MIC by Etest; [b]MIC obtained by VITEK 2.

monolactams and beta-lactams combined with beta-lactamase inhibitors are associated with colistin-resistant *K. pneumoniae* infection[12]. The previous colonization or infection with polymyxin-susceptible *K. pneumoniae* detected in three of the four cases was probably related to the development of strains resistant to these antibiotics.

Polymyxin-resistant *K. pneumoniae* strains may disseminate via horizontal transmission[5]. In the present series, three patients were admitted to the hematology or bone marrow transplantation wards, which are close to each other and share some healthcare professionals. Dissemination of the extensively resistant bacterial strain probably occurred from case 1 to cases 2 and 3 via the actions of healthcare professionals or by undetected colonization of other patients. Regarding case 4, although the patient was admitted to a ward far from the others, she spent two days in the ICU where she had been admitted seven days after case 2 had left the unit; case 2 was probably already infected with the polymyxin-resistant strain, because she had a bloodstream infection by this microorganism eight days after leaving the ICU. Therefore, transmission to case 4 must have occurred in this unit.

Extensive antimicrobial resistance is not necessarily indicative of a high-virulence *K. pneumoniae* phenotype. Case 1 only exhibited mucosal colonization, while case 4 presented with fever and cellulitis around the venous catheter. However, the remaining cases had bloodstream infections, which contributed to mortality. Polymyxin-resistant and KPC-producing *K. pneumoniae* has caused more deaths than KPC producers susceptible to polymyxins[15].

The treatment of patients infected with KPC-producing *K. pneumoniae* is impaired by the limited number of effective antimicrobials, which include polymyxins, tigecycline, and aminoglycosides[3]. In addition to being resistant to polymyxins, three of the four bacterial isolates studied herein were non-susceptible to tigecycline *in vitro*, exhibiting susceptibility only to amikacin. A similar susceptibility profile has been observed in other studies, suggesting a global trend toward even greater resistance of KPC clones[15].

The present findings suggest the isolation of polymyxin-resistant and KPC-producing *K. pneumoniae* is associated with previous colonization by polymyxin-susceptible and KPC-producing strains in patients submitted to prolonged antibiotic therapy and previous administration of polymyxin B. Furthermore, there is additional evidence of horizontal transmission of this extensively drug-resistant clone.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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