

Short Communication

Evaluation of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Rio Grande do Sul, Brazil

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Abstract

Introduction: The rapid global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is a threat to the health system. **Methods:** We evaluated the antimicrobial susceptibility profiles of 70 CRE isolated in a tertiary hospital in Brazil between August and December 2015, and determined their resistance mechanisms. **Results:** The most prevalent microorganism was *Klebsiella pneumoniae* (95.7%); it showed high-level resistance to carbapenems (>98%), with sensitivity to colistin (91.4%) and amikacin (98.6%). The *bla*_{KPC} gene was detected in 80% of the CRE isolates. **Conclusions:** Evaluation of bacterial resistance contributes to an appropriate treatment, and the reduction of morbimortality and dissemination of resistance.

Keywords: *Enterobacteriaceae*. *Klebsiella pneumoniae* carbapenemase. Multiresistance.

Bacterial resistance is a serious health problem, leading to relapsing nosocomial infections, reduced drug efficacy, and expensive and imprecise treatment, and resulting in high morbidity and mortality rates¹. The spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is threatening the health system, since the bacteria show multiple resistance mechanisms, such as the production of carbapenemases [e.g., *Klebsiella pneumoniae* carbapenemase (KPC); metallo- β -lactamases (MBL); and oxacillinases (OXA)] and efflux pumps, and porin loss².

Since the spread of CRE is a worldwide problem, studies focusing on the characterization of resistance mechanisms and on the understanding of epidemiology at infection sites are required to inform an adequate empirical therapy and control the dissemination of resistance³. Therefore, the aim of the present study was to evaluate the antimicrobial susceptibility profiles of 70 CRE isolates obtained between August and December 2015 at the Hospital Universitário de Santa Maria (HUSM) (Santa Maria, Rio Grande do Sul, Brazil) and to determine their resistance mechanisms. The isolates were obtained from several clinical specimens and identified using the Vitek[®] 2 automated system (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial resistance of the isolates was evaluated against antimicrobial agents described in **Table 1**. The minimum

inhibitory concentration (MIC) values were determined using the Advanced Expert System (AES) program in Vitek[®] 2; these values were interpreted according to the criteria established by the Clinical and Laboratory Standards Institute (CLSI) and the technical standard no. 01/2013 from the *Agência Nacional de Vigilância Sanitária* (ANVISA)^{4,5}. Phenotypic methods, such as disc diffusion tests with enzymatic inhibitors [phenylboronic acid (PBA); ethylenediaminetetraacetic acid (EDTA)], biochemical tests Carba NP [Carbapenemase Nordmann-Poirel (CN)] and Blue-Carba (BC), and molecular methods [polymerase chain reaction (PCR)] were used to evaluate the resistance mechanisms. The disk diffusion method identifies KPC-producing strains using carbapenem disks supplemented with PBA; it identifies MBL-producing strains using the chelating agent EDTA⁴. The CN biochemical test is based on the hydrolysis of the β -lactam ring of imipenem. The strains were considered to be carbapenemase producers when a color change of a solution containing the phenol red indicator was observed. The BC test is based on the same principle as CN, except that the bromothymol blue indicator is used⁶. To confirm the production of carbapenemases, PCR was performed to detect the presence of the *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{NDM}, and *bla*_{GES} genes⁷. The sensitivity and specificity of the different phenotypic methods were calculated considering PCR as the standard methodology⁶.

During the study period, 70 isolates were obtained from patients infected or colonized by CRE, being that each isolate corresponded to only one clinical specimen per patient. *K. pneumoniae* was the prevailing microorganism (67 isolates; 95.7% of all CRE isolates), followed by *Escherichia coli* (1; 1.4%), *Citrobacter freundii* (1; 1.4%), and *Enterobacter cloacae* (1;

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TABLE 1
Antimicrobial susceptibility profile of 70 CRE isolated between August and December 2015 at the HUSM, Santa Maria/RS, Brazil.

Antimicrobial agents	MIC range ($\mu\text{g/mL}$)	MIC50 ($\mu\text{g/mL}$)*	Resistant n (%)
Amikacin	2-64	≤ 16	1 (1.42)
Ampicillin	1-32	≥ 32	70 (100.0)
Cefepime	1-64	≥ 64	68 (97.1)
Ceftazidime	1-64	≥ 64	69 (98.6)
Ceftriaxone	1-64	≥ 64	67 (95.7)
Ciprofloxacin	0.25-16	≥ 4	66 (94.3)
Colistin	0.5-16	≤ 0.5	6 (8.6)
Ertapenem	0.5-8	≥ 8	70 (100.0)
Gentamicin	1-16	≥ 16	46 (65.7)
Imipenem	0.25-16	≥ 16	69 (98.6)
Meropenem	0.25-16	≥ 16	69 (98.6)
Piperacillin-tazobactam	4-128	≥ 128	69 (98.6)
Tigecycline	0.25-8	≥ 8	66 (94.3)

CRE: carbapenem-resistant *Enterobacteriaceae*; **HUSM:** Hospital Universitário de Santa Maria; **MIC:** minimum inhibitory concentration. *MIC50, concentration required to inhibit the growth of 50% of microorganism.

1.4%). The specimen type from which the CRE were isolated most frequently was the rectal swab used in the epidemiological surveillance research (32; 45.7% of 70 clinical specimens), followed by the urine (21; 30%), sputum (3; 4.3%), feces (3; 4.3%), blood (2; 2.9%), sacral lesion biopsy (1; 1.4%), fibrin (1; 1.4%), bronchoalveolar lavage (1; 1.4%), leg lesion (1; 1.4%), abdominal fluid (1; 1.4%), catheter tip (1; 1.4%), axillary secretion (1; 1.4%), surgical wound fluid (1; 1.4%), and tracheal secretion (1; 1.4%). Considering the patient gender and age, there was a prevalence of males (50; 71.4% of 70 patients) over-60-year-olds (37; 52.9% of 70 patients), 21-59-year-olds (29; 41.4%), and zero-20-year-olds (4; 5.7%). The adult intensive care unit (ICU) was the hospital unit with the highest CRE isolation rate (19; 27.2% of 70 patients), followed by the general surgery unit (18; 25.7%), the semi-intensive care unit (8; 11.4%), adult emergency room (8; 11.4%), the clinic (6; 8.6%), nephrology unit (3; 4.3%), treatment center for children and adolescents with cancer (3; 4.3%), cardiac intensive care unit (2; 2.9%), the maternal-infant and women's health unit (1; 1.4%), psychiatric unit (1; 1.4%), and surgical block (1; 1.4%). Antimicrobial susceptibility profiles of the isolates are shown in **Table 1**. Genotypic analysis based on PCR revealed the presence of the *bla*_{KPC} gene in 56 isolates (56/70; 80%), with the KPC activity also phenotypically demonstrated by the disk diffusion, CN, and BC techniques (**Table 2**). The OXA (*bla*_{OXA-48}) and MBL (*bla*_{NDM} and *bla*_{GES}) genes were not detected. All phenotypic methods (disk diffusion, CN and BC) detected the KPC enzyme with 100% sensitivity and specificity.

Carbapenem-resistant gram-negative bacilli may exhibit low susceptibility to antimicrobials, mainly to β -lactam antibiotics, because the production of carbapenemases is associated with

porin loss, overexpression of efflux pumps, and the production of another β -lactamase [e.g., extended-spectrum β -lactamases (ESBL) and AmpC β -lactamase]⁸. This may explain why the *bla*_{KPC} gene was not detected in 14 (out of 70) isolates in the present study. Similarly, Ribeiro et al., observed high-level resistance against β -lactams and ciprofloxacin, and sensitivity to polymyxin B and amikacin in *Enterobacteriaceae* isolates in southern Brazil. In the same study, the *bla*_{KPC} gene was identified in only 14 (14/345) isolates and, among the non-carbapenemase-producing isolates, ESBL and AmpC phenotypes were observed⁹. Differentiating a carbapenemase-producing CRE from a non-carbapenemase-producing CRE is important. The selective pressure caused by indiscriminate use of broad-spectrum antibiotics in treating hospital infections may lead to resistance of endogenous bacteria (from the intestinal microbiota), but the reduction of susceptibility may also be associated with a rapid horizontal transmission (e.g., via healthcare workers, equipment contamination, and mobile genetic elements), contributing to the spread of heterogeneous resistance¹⁰.

In recent years, multiple resistance mechanisms have reduced the available therapeutic options, leading to an extensive use of aminoglycosides and polymyxins¹¹. Although colistin showed good activity against CRE, 6 (6/70) *K. pneumoniae* isolates were resistant to this antimicrobial agent (MIC $\geq 16\mu\text{g/mL}$). Santos et al. described a similar resistance profile of colistin-resistant *Pseudomonas aeruginosa* (9/231) isolated at the same hospital (HUSM) as in the present study¹². This phenotype may be due to the lipopolysaccharides changes, such as modifications in lipid A by the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N)

TABLE 2
Phenotypic and molecular evaluation of the isolated clinical specimens.

Microorganism	Unit	Specimen	n	Phenotype				Genotype
				PBA	EDTA	CN	BC	PCR
<i>Citrobacter freundii</i>	Clinic	Urine	1	+	-	+	+	<i>bla</i> _{KPC}
<i>Enterobacter cloacae</i>	Adult emergency room	Urine	1	-	-	-	-	-
<i>Escherichia coli</i>	Semi-intensive care unit	Rectal swab	1	+	-	+	+	<i>bla</i> _{KPC}
		SLB	1	+	-	+	+	<i>bla</i> _{KPC}
	Clinic	Feces	1	-	-	-	-	-
		Rectal swab	1	+	-	+	+	<i>bla</i> _{KPC}
			1	-	-	-	-	-
		Urine	1	+	-	+	+	<i>bla</i> _{KPC}
	Surgical block	Fibrin	1	+	-	+	+	<i>bla</i> _{KPC}
	TCCAC	Axillary secretion	1	+	-	+	+	<i>bla</i> _{KPC}
		Rectal swab	1	+	-	+	+	<i>bla</i> _{KPC}
			Urine	1	-	-	-	-
	Nephrology	Rectal swab	2	+	-	+	+	<i>bla</i> _{KPC}
			1	+	-	+	+	<i>bla</i> _{KPC}
	Adult emergency room	Sputum	1	+	-	+	+	<i>bla</i> _{KPC}
		Blood	1	+	-	+	+	<i>bla</i> _{KPC}
		Urine	3	+	-	+	+	<i>bla</i> _{KPC}
			2	-	-	-	-	-
	Psychiatric	Urine	1	-	-	-	-	-
	Semi-intensive care unit	Sputum	1	+	-	+	+	<i>bla</i> _{KPC}
		Feces	1	+	-	+	+	<i>bla</i> _{KPC}
		Rectal swab	1	+	-	+	+	<i>bla</i> _{KPC}
3			+	-	+	+	<i>bla</i> _{KPC}	
Urine		1	+	-	+	+	<i>bla</i> _{KPC}	
<i>Klebsiella pneumoniae</i>	MIWHU	Feces	1	-	-	-	-	-
		Sputum	1	+	-	+	+	<i>bla</i> _{KPC}
	General surgery unit	BAL	1	-	-	-	-	-
		Catheter tip	1	+	-	+	+	<i>bla</i> _{KPC}
		Blood	1	-	-	-	-	-
		SWF	1	+	-	+	+	<i>bla</i> _{KPC}
		Rectal swab	6	+	-	+	+	<i>bla</i> _{KPC}
			3	+	-	+	+	<i>bla</i> _{KPC}
			3	+	-	+	+	<i>bla</i> _{KPC}
	Cardiac intensive care unit	Rectal swab	1	+	-	+	+	<i>bla</i> _{KPC}
			1	+	-	+	+	<i>bla</i> _{KPC}
	Intensive care unit	Leg lesion	1	+	-	+	+	<i>bla</i> _{KPC}
		Abdominal fluid	1	+	-	+	+	<i>bla</i> _{KPC}
		Tracheal secretion	1	+	-	+	+	<i>bla</i> _{KPC}
			7	+	-	+	+	<i>bla</i> _{KPC}
			2	+	-	+	+	<i>bla</i> _{KPC}
		Urine	1	-	-	-	-	-
2			+	-	+	+	<i>bla</i> _{KPC}	
2			+	-	+	+	<i>bla</i> _{KPC}	
2	-	-	-	-	-			

n: number of clinical specimens; PBA: phenylboronic acid; EDTA: ethylenediaminetetraacetic acid; CN: carba NP; BC: blue-carba; PCR: polymerase chain reaction; SLB: sacral lesion biopsy; TCCAC: treatment center for children and adolescents with cancer; MIWHU: maternal-infant and women's health unit; BAL: bronchoalveolar lavage; SWF: surgical wound fluid; +: positive; -: negative.

and phosphoethanolamine (pEtN), and *mgrB* gene mutations, responsible for the regulation of the PmrAB and PhoPQ two-component systems, as well as through plasmid-mediated *mcr-1* gene, responsible for horizontal transfer of colistin resistance, necessitating monitoring of colistin use during therapy and reinforcing control practices during hospital infections to avoid the dissemination of resistance and loss of efficacy of this drug¹³.

In the present study, all phenotypic methods (disk diffusion, CN and BC) detected the KPC enzyme activity with 100% sensitivity and specificity. Similarly, Giske et al. reported 100% sensitivity and 98% specificity of an assay that relied on the 3-aminophenylboronic acid for the detection of KPC in *K. pneumoniae* strains¹⁴. Further, García-Fernández et al. reported 100% specificity of both methodologies, with 100% and 98% sensitivity of CN and BC, respectively¹⁵.

As demonstrated, the disk diffusion (PBA and EDTA) method, and CN and BC tests are characterized by high sensitivity and specificity, similarly to PCR. They might be incorporated into the workflow of a clinical microbiology laboratory, as they are quick, practical, and low-cost.

Simultaneous use of two or more of these methods increases the reliability of CRE detection and, together with the antimicrobial susceptibility profile data, they may inform the adequate treatment, assisting in the reduction of mortality and dissemination of resistance. The phenotype found in the Institution is worrying and the adoption of nosocomial infection control and prevent measures are necessary.

This research presented limitations of the study in terms of non-detection of other carbapenemases enzymes (OXA, MBL), which could interfere with sensitivity and specificity. Posteriorly, mechanisms such as efflux pump and porin loss will be evaluated, as well as molecular typability for outbreak analysis.

Ethical considerations

This research was approved by the Ethical Research Committee, *Universidade Federal de Santa Maria*, under approval number 38850614.4.0000.5346.

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Conflict of interest

The authors declare that there is no conflict of interest.

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