

Brief Original Article

KPC: an important mechanism of resistance in *K. pneumoniae* isolates from intensive care units in the Midwest region of Brazil

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Abstract

Introduction: Infections caused by multidrug-resistant *Klebsiella pneumoniae* are difficult to treat and pose a serious threat to public health worldwide. Here, we describe the presence of carbapenemase-producing *K. pneumoniae* in intensive care units (ICU) of three major Mato Grosso do Sul hospitals located in the Midwest region of Brazil.

Methodology: A total of 165 *K. pneumoniae* isolates with reduced susceptibility to carbapenems as identified by the VITEK-2 compact system were studied. Antimicrobial susceptibility testing was performed using the disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute, and the E-test method. The detection of carbapenemase was performed using the modified Hodge test and polymerase chain reaction.

Results: The *bla*_{KPC} gene was identified in 88.1% (n=89) of the selected *K. pneumoniae* isolates from Beneficent Association of Campo Grande, 94.9% (n=34) of the isolates from the Regional Hospital of Mato Grosso do Sul and 95.2% (n=26) of the isolates from Maria Aparecida Pedrossian University Hospital. Resistance greater than 80% was observed against cephalosporins, aztreonam, ciprofloxacin and piperacillin/tazobactam. Carbapenemase-producing *K. pneumoniae* (Kp-KPC) isolates were considered important causative agents of urinary tract infections, pneumonia and bloodstream infections in ICU patients. While rarely reported in the literature, we documented three cases of meningoencephalitis caused by Kp-KPC.

Conclusions: Our study documents the presence of Kp-KPC in three major Mato Grosso do Sul state hospitals, providing key national epidemiology data. This is an important mechanism of resistance in *K. pneumoniae* isolates from ICU patients and is associated with resistance to multiple classes of antimicrobial drugs.

Key words: intensive care units; *Klebsiella pneumoniae*; carbapenemase.

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Introduction

Meropenem and ertapenem are antibiotics of choice for the treatment of severe infections caused by multidrug-resistant *Klebsiella pneumoniae* (Kp-MR), and these drugs are widely used in intensive care units (ICU) [1].

The emergence of resistance to carbapenems among members of the *Enterobacteriaceae* family has become a major problem in the hospital environment [2,3]. In this context, different antimicrobial resistance mechanisms have been described, including metallo- β -lactamase (VIM type-1 and NDM), OXA-carbapenemases (OXA type-48) and carbapenemase-

producing *K. pneumoniae*. From an epidemiological point of view, KPC-type carbapenemases are extremely important, as they have rapidly spread throughout the world [4].

Since the first description of carbapenemase-producing *K. pneumoniae* (Kp-KPC) infection in the United States, this mechanism of resistance has been reported in various countries, including Puerto Rico, China, Israel, Palestine, Canada, France, Colombia, Greece, Italy, Argentina and Brazil [4-8]. From 2009-2010, outbreaks of Kp-KPC infections were documented in different regions of Brazil [9-11], but

few reports have been described in the Midwest region [12,13].

Microorganisms that express KPC are resistant to all β -lactam antibiotics and several other classes of antibiotics, limiting treatment options and contributing to a high mortality rate [1-2].

Considering the lack of published data on Enterobacteriaceae antimicrobial resistance in hospitals located in the Midwest region of Brazil, this research was conducted to verify whether the emerging carbapenem-resistance observed in *K. pneumoniae* isolates at these hospitals is mediated by KPC and to provide national epidemiology data.

Methodology

Bacterial strains

A total of 165 non-duplicate *K. pneumoniae* clinical isolates isolated from March 2013 to March 2014 that were non-susceptible to carbapenems ($\geq 1 \mu\text{g/mL}$ for ertapenem, $\geq 2 \mu\text{g/mL}$ for meropenem and imipenem) were selected. The isolates were recovered from ICU inpatients at three Brazilian hospitals: Beneficent Association of Campo Grande (BACG), the Regional Hospital of Mato Grosso do Sul (RHMS), and Maria Aparecida Pedrossian University Hospital of Mato Grosso do Sul (UH/FUMS), which have 707, 344, and 271 beds, respectively.

Susceptibility tests

The disk diffusion test was performed according to the methodology described in document M100-S23 of the Clinical and Laboratory Standards Institute (CLSI) guidelines [14] and was used to determine the antimicrobial susceptibility to amikacin (AMK), gentamycin (GEN), aztreonam (ATM), cefepime (FEP), ceftriaxone (CRO), ceftazidime (CAZ), ciprofloxacin (CIP), ertapenem (ETP), imipenem (IPM), meropenem (MEM), and piperacillin/tazobactam (TZP). The minimum inhibitory concentrations (MIC) of meropenem (MEM), ertapenem (ETP) and imipenem (IPM) against *K. pneumoniae* recovered from blood and cerebrospinal

fluid were determined using the E-test method (BioMérieux, Marcy-I’Etoile, France). *E. coli* ATCC 25922 was used as a quality control strain for the susceptibility tests.

The production of carbapenemases was detected by the modified Hodge test (MHT) as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [14].

Genotype test

Template DNA was extracted from *K. pneumoniae* using the boiling method, and the *bla*_{KPC} gene was detected using polymerase chain reaction (PCR).

One or two colonies were transferred to a microcentrifuge tube containing 100 μL of sterile MilliQ water. The suspension was subjected to treatment in a dry bath at 90°C for 1 min and was subsequently spun for 2 min at 12,000 rpm. The supernatant was carefully aspirated and transferred to a new sterile microtube.

The following primers were used to amplify the *bla*_{KPC} gene (800 bp): forward, 5’-TCGCTAAACTCGAACAGG-3’ and reverse, 5’-TTACTGCCCTTGACGCCCAATCC-3’ [13]. The conditions used in the thermal cycler (Biocycler - Hangzhou, Zhejiang, China) were initial denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 1 minute, and after the final cycle, a final extension at 72°C for 10 minutes. The PCR products were resolved using 1% agarose gel electrophoresis with Gel RED (Biotium, Hayward, USA).

Strategies for data analysis

Statistical analysis was performed using SPSS, version 22.0, assuming a significance level of 5%. The chi-square test was used to investigate the association between the presence of the *bla*_{KPC} gene and the study variables of interest (gender, age and clinical specimen).

Table 1. Distribution of 165 *K. pneumoniae* isolates in accordance with the positive phenotypic and genotypic testing used for detecting carbapenemases.

| Hospital | N | MHT % (n) | <i>bla</i> _{KPC} gene % (n) |
|----------|-----|------------|--------------------------------------|
| BACG | 101 | 94.4 (95) | 88.1 (89) |
| RHMS | 36 | 91.7 (33) | 94.4 (34) |
| UH/FUMS | 28 | 95.7 (27) | 91.3 (26) |
| Total | 165 | 93.9 (155) | 90.3 (149) |

BACG-Beneficent Association of Campo Grande; RHMS-Regional Hospital of Mato Grosso do Sul; UH/FUMS-Maria Aparecida Pedrossian University Hospital of Federal University of Mato Grosso do Sul.

Ethical considerations

This study was approved by the Research Ethics Committee of the Federal University of Mato Grosso do Sul, under number 230.169/2013.

Results

Among the 165 *K. pneumoniae* carbapenem non-susceptible isolates recovered from ICU, 101 (61.2%) were isolated from BACG, 36 (21.8%) from RHMS and 28 (17.0%) from UH/FUMS.

Table 1 presents the results of phenotypic (MHT) and genotypic tests (PCR, *bla*_{KPC} gene). The sensitivity and specificity of the phenotypic test in relation to the genotypic test were 96% and 25%, respectively.

The antimicrobial resistance profile of KPC-producing *K. pneumoniae* (n = 149) is shown in Table 2.

Nine Kp-MR strains were isolated from blood cultures and three from cerebrospinal fluid. The MIC of the *K. pneumoniae* strains isolated from these sterile sites is shown in Table 3.

Data from patients who were colonized/infected with Kp-MR are listed in Table 4. Overall, *K. pneumoniae* was isolated from patients of both genders with a male predominance and was most frequently isolated from urine samples, surveillance swabs and tracheal aspirates.

Discussion

Since the first description of Kp-KPC (2001, North Carolina, USA [5]), carbapenemase-producing bacteria have spread throughout the world [4,9]. In Brazil, the first reports of Kp-KPC infections were described in 2006 in Recife [10]. However, there is evidence that the *bla*_{KPC} gene has been circulating in Brazil since 2005 [15].

In Mato Grosso do Sul State; located in the Midwest region Brazil, the first Kp-KPC description was of an isolate from an elderly patient with a urinary tract infection hospitalized at UH/FUMS in 2010 [16]. However, Biberg *et al.* (2015) [13] showed that these microorganisms were present in RHMS since at least 2009. This period coincides with outbreaks documented in different regions of Brazil and reinforces the hypothesis that this resistance gene had been spreading throughout a large part of Brazil [11,12]. Our study documents the presence of Kp-KPC in three major hospitals in Mato Grosso do Sul State and provides national epidemiology data. The results of this study show that KPC is an important mechanism of resistance to carbapenems in large public hospitals in the state of Mato Grosso do Sul, but other carbapenemases should also be investigated. In future studies, sequencing will be used to determine the molecular type and compare

Table 2. Antimicrobial resistance profiles of 149 KPC-producing *K. pneumoniae* strains.

| Antibiotics | BACG % (n) | RHMS % (n) | UH/FUMS % (n) |
|-------------------------|---------------|---------------|------------------|
| Amikacin | 10.1 (9) | 2.9 (1) | 7.7 (2) |
| Gentamycin | 49.4 (44) | 55.9 (19) | 80.8 (21) |
| Aztreonam | 96.6 (86) | 97.1 (33) | 88.5 (23) |
| Cefepime | 83.2 (74) | 91.2 (31) | 80.8 (21) |
| Ceftriaxone | 97.7 (87) | 97.1 (33) | 92.3 (24) |
| Ceftazidime | 86.5 (77) | 97.1 (33) | 88.5 (23) |
| Ciprofloxacin | 70.3 (65) | 91.2 (31) | 88.5 (23) |
| Ertapenem | 87.6 (78) | 94.1 (32) | 88.5 (23) |
| Imipenem | 78.6 (70) | 88.2 (30) | 69.2 (18) |
| Meropenem | 86.5 (77) | 94.1 (32) | 96.1 (25) |
| Piperacillin/Tazobactam | 94.4 (84) | 94.1 (32) | 96.1 (25) |

BACG-Beneficent Association of Campo Grande; RHMS-Regional Hospital of Mato Grosso do Sul; UH/FUMS-Maria Aparecida Pedrossian University Hospital of Federal University of Mato Grosso do Sul.

Table 3. Variation in the minimum inhibitory concentrations of 12 multidrug-resistant *K. pneumoniae* isolates recovered from cerebrospinal fluid and blood samples.

| Antibiotic | MIC (μ g/mL)* | Susceptible %(n) | Resistant %(n) |
|------------|-----------------------|---------------------|----------------|
| Ertapenem | 0.75-32 | 0 (0) | 100 (12) |
| Meropenem | > 32 | 0 (0) | 100 (12) |
| Imipenem | 0.5-32 | 8.3 (1) | 91.7 (11) |

* MIC values were interpreted according to the CLSI 2013 guidelines.

Table 4. Demographic characteristics and clinical sample types of 165 patients with *K. pneumoniae* infections

| | Hospitals | | | <i>p</i> value* |
|---------------------|---------------|---------------|---------------|-----------------|
| | BACG % (n) | RHMS % (n) | UH/FUMS % (n) | |
| Sex | | | | |
| Female | 38.6 (39) | 27.8 (10) | 42.9 (12) | 0.542 |
| Male | 61.4 (62) | 72.2 (26) | 57.1 (16) | |
| Ages (years) | | | | |
| 12 to 18 | 2.0 (2) | 2.8 (1) | 00 | 0.381 |
| 19 to 59 | 55.4 (56) | 38.9 (14) | 39.3 (11) | |
| 60 or more | 42.6 (43) | 58.3 (21) | 60.7 (17) | |
| Sample type | | | | |
| Urine | 25.7 (26) | 33.3 (12) | 42.9 (12) | 0.633 |
| Swab surveillance | 37.6 (38) | 19.4 (7) | 00 | |
| Tracheal aspirate | 14.9 (15) | 30.6 (11) | 39.3 (11) | |
| Blood | 3.0 (3) | 5.6 (2) | 14.3 (4) | |
| Surgical wound | 3.0 (3) | 00 | 00 | |
| Catheter tip | 99 (10) | 5.6 (2) | 00 | |
| Ulcer | 1.0 (1) | 00 | 3.6 (1) | |
| Biological fluids | 1.0 (1) | 5.6 (2) | 00 | |
| Cerebrospinal fluid | 3.0 (3) | 00 | 00 | |
| Skin biopsy | 1.0 (1) | 00 | 00 | |

BACG-Beneficent Association of Campo Grande; RHMS-Regional Hospital of Mato Grosso do Sul; UH/FUMS-Maria Aparecida Pedrossian University Hospital of Federal University of Mato Grosso do Sul. * chi-square test.

the genetic similarity between the bacteria isolated in these three hospitals.

The results of this research show that MHT is a practical method for carbapenemase detection. The higher positivity of MHT compared to PCR in two of three studied hospitals suggests that other resistance mechanisms, such as AmpC β -lactamases and extended-spectrum β -lactamase (ES β L), may be involved [17,18].

In comparing the accuracy between the two tests (phenotypic and genotypic), we observed high sensitivity in MHT (96%), which is in agreement with recent publications [13,19]. Previous studies show specificity values ranging from 60 to 100% [20,21]. The low specificity observed in our study (25%) may be due to the presence of other carbapenemases that were not included in this study or may be due to false carbapenemase production using MHT, as suggested by Carvalhaes *et al.* (2010) [17]. The authors reported that of the 28 *K. pneumoniae* isolates studied 27 were false positives, which was most likely due to the presence of ES β L enzymes associated with porin loss. This finding was later confirmed by discovering presence of the *bla*_{CTX-M} gene.

The results of antimicrobial susceptibility tests are valuable for guiding treatment and for detection of emerging resistant bacterial strains. Considering that the bacterial resistance profile can vary from one institution to another, and even from one region to

another, the results presented here are relevant to Kp-MR in three hospitals in the Midwest region of Brazil, an area that currently lacks this information.

We observed elevated resistance (more than 80%) to multiple classes of antimicrobials (cephalosporins, aztreonam, ciprofloxacin and piperacillin/tazobactam), confirming previous studies claiming that carbapenemase-producing bacteria often carry other resistance genes [15,22,23]. The high MIC values of carbapenem antibiotics detected in this study can predict treatment failure. This is an alarming finding given the severity of the medical conditions of patients admitted to ICU.

In contrast to reports in other regions of Brazil [11], we observed low resistance to amikacin (6.8%). However, this drug is not recommended for monotherapy due to its pharmacokinetic and toxic properties [24]. A proposed alternative treatment for Kp-MR infections is combined meropenem and polymyxin or tigecycline and amikacin, which at appropriate doses have been shown to provide better therapeutic benefits, including a reduced mortality rate compared to monotherapy [2].

Data in the literature [7,25-27] indicate that patients at an advanced age and hospitalized in the ICU are more susceptible to infections by multidrug-resistant bacteria, which is in agreement with the results obtained in our study.

In the present study, KPC-producing *K. pneumoniae* isolates were considered important causative agents of urinary tract infections, pneumonia and bloodstream infections in ICU patients. Although there are only few reports in the literature of the isolation of Kp-KPC from cerebrospinal fluid (CSF), we documented three cases of meningoenzephalitis in our region. Prior neurosurgery and use of neurosurgical devices has been associated with adult *K. pneumoniae* meningitis [28].

Revisions to protocols associated with ventilator-associated pneumonia, indwelling catheters, central venous catheters and other preventive measures should be implemented in the studied hospitals to minimize the risk of infection by Kp-MR [29,30].

Conclusion

Carbapenem-resistance in *K. pneumoniae* isolated in ICU in the Midwest region of Brazil is mediated by the presence of the *bla*_{KPC} gene. Other resistance mechanisms must have been involved in the positive MHT samples that did not have the *bla*_{KPC} gene.

High resistance levels to multiple classes of antimicrobials were observed in KPC-producing *K. pneumoniae*. Similar to the other national and international reports, *K. pneumoniae* was largely isolated from elderly patients and urine samples.

The results of this study contribute to national epidemiology data and highlight the spread of *K. pneumoniae* strains producing the KPC enzyme throughout the state of Mato Grosso do Sul.

Future studies are needed to assess the genetic similarity between the isolates. Infection control strategies, including early diagnosis, review of the prevention protocols and effective treatment courses, should be adopted to control the spread of these multidrug-resistant pathogens in hospital environments.

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