J Bras Patol Med Lab. 2018 Apr; 54(2): 95-98.

10.5935/1676-2444.20180017

Prevalence of carbapenemase in Enterobacteriaceae with decreased susceptibility to carbapenems isolated in a tertiary referral hospital

Prevalência de carbapenemases em enterobactérias com sensibilidade diminuída aos carbapenêmicos isoladas em um hospital de referência terciária

Diana M. Viegas; Valéria M. Soares

Fundação Hospitalar do Estado de Minas Gerais (FHEMIG), Minas Gerais, Brazil.

ABSTRACT

Infections caused by carbapenem-resistant Enterobacteriaceae are a serious public health issue. This work aims to analyze the resistance mechanisms related to Enterobacteriaceae with decreased susceptibility to carbapenems isolated at the Hospital Júlia Kubitschek (HJK) of the Minas Gerais State Founding Hospital [Fundação Hospitalar do Estado de Minas Gerais (FHEMIG)], Brazil. Seventy-one strains with reduced susceptibility to carbapenems were found, and 45/71 (63.4%) were *Klebsiella pneumoniae* carbapenems (KPC) producers.

Key words: Enterobacteriaceae; carbapenems.

INTRODUCTION

Infections caused by carbapenem-resistant Enterobacteriaceae are a serious public health issue due to high morbidity and mortality worldwide[1]. Among the mechanisms of resistance to carbapenems (doripenem, ertapenem, imipenem and meropenem), the production of carbapenemases, either by their hydrolytic efficiency, their codification by genes located in mobile genetic elements, such as plasmids and transposons, or by their rapid worldwide dissemination, it has the most significant impact on human health[2].

Three major classes of carbapenemases are currently found in Enterobacteriaceae worldwide: metalobetalactamases, including New Delhi metallobetalactamase (NDM) OXA-carbapenemases, with OXA48 as the most widespread among Enterobacteriaceae and carbapenemases type *Klebsiella pneumoniae* carbapenemase (KPC)[2].

Since the initial description of KPC in Brazil and around the world, several authors have demonstrated its presence and dissemination in several bacterial genera and species[2]. In the Hospital Júlia Kubitschek (HJK) of the Minas Gerais State Founding Hospital [Fundação Hospitalar do Estado de Minas Gerais (FHEMIG)], the first report of KPC-producing Enterobacteriaceae isolation was in 2009 in strains of *Klebsiella pneumoniae*, with constant isolations since then, including other genera of the family[3].

Regarding NDM, the first report in Brazil was in 2013, and sporadic cases have been described, as well as the emergence of isolates with the detection of the gene for OXA48-type enzymes, previously less disseminated with circulation restricted in the Middle East and some European countries[2, 4, 5].

The reduction of susceptibility to carbapenems in Enterobacteriaceae may also be caused by the presence of extended-spectrum beta-lactamases (ESBL) associated with class C beta-lactamases (AmpC), generating low potential of carbapenems hydrolysis and alterations in porins channels, which modify drugs action and penetration[2, 3].

OBJECTIVES

This work aims to analyze the resistance mechanisms related to Enterobacteriaceae with decreased susceptibility to carbapenems

First submission on 09/18/17; last submission on 01/24/18; accepted for publication on 03/01/18; published on 04/20/18

isolated in the HJK of the FHEMIG, a regional and statewide general hospital located in Belo Horizonte, Brazil. Furthermore, we evaluated which genera and species were involved, as well as the general antimicrobial susceptibility profile.

The work was approved by the Research Ethics Committee of the FHEMIG, under technical report no. 62710016.6.0000.5119 (CAAE).

MATERIAL AND METHOD

This is a retrospective descriptive study. We included 71 strains of Enterobacteriaceae with decreased susceptibility to carbapenems isolated between January 2014 and September 2016. Isolates from clinical samples from the same patients were excluded.

In the Microbiology Laboratory of the HJK, the identification of the bacterial species was carried out through traditional biochemical tests and through the semi-automated system BBL Crystal® (Enteric/Nonfermenter ID System/Becton, Dickinson and Company). Carbapenemase-producing Enterobacteriaceae were screened according to the annual report of the Clinical and Laboratory Standard Institute (CLSI)[6] and technical note no. 01/2013 of the National Sanitary Surveillance Agency [Agência Nacional de Vigilância Sanitária (Anvisa)][2].

When performing the susceptibility test, using the disc diffusion test methodology, for the Enterobacteriaceae isolated, the laboratory simultaneously tested ertapenem, imipenem and meropenem. In case the isolate was susceptible to the three carbapenems, the result was released as such, and it was not necessary to search for carbapenemases.

Regarding the screening for carbapenemase producers in isolates of the groups *Citrobacter freundii*, *Enterobacter* spp., *Serratia* spp., *Providencia* spp., *Morganella morganii* and *Hafnia alvei* (CESP) only the results of imipenem and meropenem were considered.

Isolates with inhibition zone diameter ≤ 22 mm for imipenem and/or meropenem and isolates with inhibition zone ≤ 24 mm were considered suspected to be carbapenemase producers. The strain was sent to the reference laboratory of the Fundação Ezequiel Dias (FUNED) for the performance of the multiplex polymerase chain reaction (PCR) for the genes coding for KPC-type, NDM-type and OXA-type carbapenemases. At the same time, the microbiology sector performed the determination of the minimum inhibitory concentration (MIC) for imipenem, meropenem and polymyxin, only for the isolates that showed resistance to carbapenem by disk diffusion, using the Etest® method (AB Biodisk, Solna, Sweden).

Data collection was carried out from the internal records of the microbiology sector, for the following variables: genus and species of the isolated bacteria, clinical sample, place of hospitalization, susceptibility profile to carbapenems and other antimicrobials released and molecular test result.

The data collected were analyzed using the Epi Info™, a public domain suite of software tools designed by the Centers for Disease Control and Prevention (CDC)[7].

In the study period 71 strains with decreased susceptibility to carbapenems were found, with 45/71 (63.4%) KPC producers, through molecular confirmation, and 26/71 (36.6%) were negative for KPC, OXA and NDM.

Regarding the isolation site, most of these strains were isolated in urine samples (50.6%), followed by blood samples (19%), endotracheal aspirate (12.6%) and others. Regarding the place of hospitalization, most of the strains were isolated from patients in the wards (46.8%), emergency unit (27.4%), intensive care center (19.4%) and outpatient clinic (6.4%).

The **Table** presents the prevalence of different species of Enterobacteriaceae in the sample and the positivity for KPC.

TABLE – Prevalence of Enterobacteriaceae species in clinical specimens and KPC positivity

		Positivity for carbapenemases *n* (%)	
Microorganism	Isolated *n* (%)	KPC	Negative
Klebsiella pneumoniae	36 (50.7)	25 (69.4)	11 (30.6)
Citrobacter freundii	1 (1.4)	1 (100)	0
Enterobacter aerogenes	7 (9.9)	1 (14.3)	6 (85.7)
Enterobacter aglomerans	1 (1.4)	1 (100)	0
Enterobacter cloacae	12 (16.9)	7 (58.3)	5 (41.7)
Escherichia coli	2 (2.8)	2 (100)	0
Klebsiella oxytoca	1 (1.4)	1 (100)	0
Morganella morganii	3 (4.2)	1 (33.3)	2 (66.7)
Proteus mirabilis	1 (1.4)	1 (100)	0
Providencia stuartii	1 (1.4)	1 (100)	0
Serratia marcescens	5 (7)	4 (80)	1 (20)
Serratia sp.	1 (1.4)	0	1 (100)

KPC: Klebsiella pneumoniae *carbapenemase.*

Regarding the susceptibility profile to other antimicrobials, the data for amikacin and gentamicin were recovered in 42/45 isolates, with susceptibility of 38.1% (16/42) and 45.2% (19/42), respectively. For ciprofloxacin and sulfazotrim, the data were recovered in 41/45 and 39/45 isolates with susceptibility of 24.4% (10/41) and 38.5% (15/39), respectively.

RESULTS AND DISCUSSION

The only carbapenemase found among isolates was KPC. No carbapenemases of the NDM-type and OXA48-type were found.

The most frequently identified species was *K. pneumoniae*. These data highlight the worrying spread of KPC in Brazil and worldwide. Despite the prominent role of *K. pneumoniae*, regarding their predisposition to cause hospital-acquired infections and their ability to accumulate and transfer mechanisms of resistance[5], the study showed the presence of the enzyme in other bacterial genera in our institution. Although several publications demonstrate the spread of KPC throughout Brazil[2], data for the state of Minas Gerais are scarce.

These isolates were obtained from clinical samples. We did not include isolates obtained from surveillance rectal swabs, since the reference laboratory (FUNED) does not recommend forwarding strains from sites where previous confirmation of KPC detection has already taken place. Such a measure limits the epidemiological study, since the colonized patient is a reservoir of bacterial resistance, through mobile genetic elements such as plasmids and transposons, not clarifying which type of carbapenemase involved.

No carbapenemase was found in 27/79 (34.2%). As mentioned earlier, among the mechanisms of resistance that may interfere with the action of carbapenems, we can mention the combination of membrane impermeability, caused by the deficiency of a major outer membrane protein associated with strains producing chromosomal (AmpC) or extended-spectrum beta lactamases (ESBL) according to the study of Martinez *et al.* (1999)[8].

According to the literature, the rates of resistance to polymyxins in strains producing carbapenemases may vary from 20%-40% in Brazilian hospitals[9]. Although microdilution in broth was the only acceptable laboratory methodology for the evaluation of the susceptibility to polymyxins, it was not possible to standardize it for routine use in the institution, like other laboratories in the country because it is a laborious method.

A limitation of this study was the lack of collection of clinical and epidemiological data from the patients to verify possible risk factors associated with the production of carbapenemases. In addition, it was not possible to evaluate the MIC values found against resistance mechanisms due to loss in data collection. Further studies are needed to correlate the MIC values and the presence of carbapenemases.

CONCLUSION

Epidemiological studies are shown as necessary tools to establish strategies and management methods to prevent infections caused by these multiresistant microorganisms[10]. As shown, the main mechanism related to decreased susceptibility to carbapenems for the Enterobacteriaceae isolated in the HJK is the production of KPC-type carbapenemases. Moreover, the study shows that the screening of resistance mechanisms has been well conducted by our institution.

ACKNOWLEDGEMENTS

To FHEMIG, for the support in conducting this study.

RESUMO

As infecções causadas por enterobactérias com resistência aos carbapenêmicos são um grave problema de saúde pública. Este trabalho tem como objetivo analisar os mecanismos de resistência relacionados com as enterobactérias com sensibilidade diminuída aos carbapenêmicos isoladas no Hospital Júlia Kubitschek (HJK) da Fundação Hospitalar do Estado de Minas Gerais (FHEMIG), Brasil. Foram encontradas 71 cepas com sensibilidade diminuída aos carbapenêmicos, sendo 45/71 (63,4%) produtoras de Klebsiella pneumoniae *carbapenemase (KPC).*

Unitermos: Enterobacteriaceae*; carbapenêmicos.*

REFERENCES

1. Duin DV. Carbapenemresistant enterobacteriaceae: what we know and what we need to know. Virulence. 2017 Mar; 8(4): 379-82.

2. Anvisa. Agência Nacional de Vigilância Sanitária. Nota técnica no. 1/2013. Medidas de prevenção e controle de infecções por enterobactérias multiresistentes. Brasília (DF); 2013.

3. Soares VM. Emergência de Klebsiella pneumoniae produtora de carbapenemase (KPC) em um hospital terciário. J Bras Patol Med Lab. 2012; 48(4): 251-3.

4. Sampaio JL, Ribeiro VB, Campos JC, et al. Detection of OXA370, an OXA48 related class D βlactamase, in enterobacter hormaechei from Brazil. Antimicrob Agents Chemother. 2014; 58(6): 3566-7.

5. Pinto FM, Sima SDM, Baldin CP, et al. Prevalência de carbapenemases em enterobactérias resistentes a carbapenêmicos em quatro hospitais terciários de Porto Alegre. Clin Biomed Res. 2014; 34(1): 47-52.

6. CLSI. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; table: M100S25; 950 West Valley Road, Suite 2500. Wayne, PA; 2015.

7. CDC. Center for Disease Control and Prevention. Epiinfo program version 7.2.1.0 of january 2017. Atlanta; 2004. Available at: https://www.cdc.gov/epiinfo/index.html. Access in: March, 2017.

8. Martinez LM, Pascual A, HernándesAlle S, et al. Roles of betalactamases and porins in activities of carbapenems and cephalosporins against Klebsiella pneumoniae. Antimicrob Agents Chemother. 1999; 43(7): 1669-73.

9. Anvisa. Agência Nacional de Vigilância Sanitária. Comunicado de risco no. 01/2016 –GVIMS/GGTES/ANVISA. Detecção do gene responsável pela resistência à polimixina mediada por plasmídeos (mcr1) no Brasil. Brasília (DF); 2016.

10. Rugini CL, Sobottka AM, Fuentefria DB. Occurrence and sensitivity profile of extended spectrum betalactamaseproducing Enterobacteriaceae at a tertiary hospital in Southern Brazil. Rev Soc Bras Med Trop. 2015; 48(6): 692-8.

CORRESPONDING AUTHOR

Diana Manzi Viegas
Rua Francisco Campos, 146; Trevo; CEP: 31545-210; Belo Horizonte-MG, Brasil; e-mail: dianaatropos@gmail.com.