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Avaliação de Heterorresistência ao Imipenem em Enterobactérias Produtoras e Não-Produtoras de KPC

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Imipenem heteroresistance: evaluation of *Klebsiella pneumoniae* carbapenemase-producing and non-producing *K. pneumoniae* and *E. coli*

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Abbreviations: ESBL, extended spectrum beta-lactamase; IPM, imipenem; KPC, *Klebsiella pneumoniae* carbapenemase; MEM, meropenem; MIC, minimum inhibitory concentration.

SUMMARY

The misdetection of *Klebsiella pneumoniae* carbapenemase (KPC) may lead to treatment failure and favour the spread of this resistance mechanism. Heteroresistance is an antimicrobial resistance in a subset of an isolate considered to be susceptible by conventional testing. The aim of this study was to evaluate the presence of heteroresistance to imipenem in KPC-producing and non-producing *K. pneumoniae* and *E. coli.* After the determination of the minimum inhibitory concentration (MIC) to imipenem by broth microdilution, each isolate with MIC lower than 4 mg L^{-1} was subcultured in plates containing imipenem ranging from 0.25 mg L^{-1} to 32 mg L^{-1}. Isolates with growth in imipenem concentrations two fold dilutions above the original MIC were considered heteroresistant. The KPC group consisted of 10 isolates, while the non-KPC group consisted of four isolates and distinct results were observed among them: all KPC-producing isolates presented heteroresistance to imipenem, while for the KPC-non-producing isolates this phenomenon was not observed. Interestingly, we also found heteroresistance in *E. coli* isolates, which, to best of our knowledge, was not reported as yet. Our results indicated a possible relationship between heteroresistance and KPC production. The clinical implications of appearing of heteroresistant subpopulations remains to be investigated, considering that this phenomenon seems to be frequent in Gram-negative rods, including members, such as *E. coli,* in which this phenomenon is still rarely investigated.

INTRODUCTION

Carbapenems are currently the treatment of choice for severe infections due to Enterobacteriaceae producing extended spectrum β-lactamases (ESBLs) (Pitout *et al.*, 2008). *Klebsiella pneumoniae* carbapenemase (KPC) has already been described in virtually all members of *Enterobacteriaceae* (Nordmann *et al.*, 2009) since its first description in a *K. pneumoniae* isolate (Yigit *et al.*, 2001). The KPC enzyme is an Ambler class A β-lactamase that hydrolyses not only carbapenems, but also other β-lactams, such as penicilins, cephalosporins and monobactams (Alba *et al.*, 2005).

The detection of KPC-producing isolates based solely in susceptibility tests may be difficult, as KPC may confer only low-level carbapenem resistance in vitro (Anderson *et al.*, 2007). The misdetection of KPC-producing strains will not only lead to a treatment failure but also favour the spread of this resistance mechanism.

Heteroresistance is defined as an antimicrobial resistance expressed by a subset of a microbial population that is considered susceptible to an antibiotic by traditional in vitro susceptibility testing (Falagas *et al.*, 2007). This phenomenon is already well known in Gram-positive bacteria. Among the Gram-negative rods, heteroresistance to carbapenems was observed in *Pseudomonas aeruginosa* (Oikomonou *et al.*, 2011) and *Acinetobacter baumannii* (Cuenca *et al.*, 2012; Ikonomidis *et al.*, 2009). In *Enterobacteriaceae*, the reports of heteroresistance are still rare. The aim of this study was to evaluate the presence of imipenem heteroresistant subpopulations in KPC-producing and non-producing *K. pneumoniae* and *E. coli*.

METHODS

Bacterial isolates. We selected a total of 33 KPC-producing *K. pneumoniae* and *E. coli* (KPC group) and four KPC-non-producing isolates (non-KPC group) susceptible to imipenem and/or meropenem by disc-diffusion, from February to July 2012. The KPC production was previously confirmed by a multiplex real-time PCR with specific primers, including bla_{KPC}, bla_{GES}, bla_{IMP}, bla_{NDM}, bla_{VIM} and bla_{OXA-48} genes (Monteiro *et al.*, 2012). No other carbapenemase was found in both groups. The isolates from KPC group were collected in the same

hospital in Florianópolis, Brazil, whereas the isolates from the non-KPC group were obtained from three distinct institutions in two capitals southern Brazil (Florianópolis and Porto Alegre). All isolates were recovered from urine samples, with exception of 5C isolate, which was recovered from a rectal swab.

Susceptibility testing. The minimum inhibitory concentration (MIC) to imipenem for both groups was determined by broth microdilution according to CLSI (2012). Isolates with MIC lower than 4mg L^{-1} were further evaluated for the presence of heteroresistant populations.

Population analysis. For each isolate, an inoculum of approximately 10^8 UFC mL^{-1} was prepared. A volume of 20µL were plated in Mueller-Hinton agar containing imipenem ranging from 0.25 mg L^{-1} to 32 mg L^{-1} and incubated at 37°C. The inoculum was also incubated in an imipenem-free plate. The procedures were performed in duplicate. After 48 hours, the presence of bacterial growth was observed for each concentration of imipenem. The isolates were considered heteroresistant when they grew in plates with imipenem concentrations at least two fold dilutions of MIC.

RESULTS

From the 33 KPC-producing isolates, only 10 (five *K. pneumoniae* and five *E. coli*) presented MIC lower than 4mg L^{-1} to imipenem (Table 1). In addition, all isolates from the non-KPC group (three *K. pneumoniae* and one *E. coli*) (Table 1) also presented MIC lower than 4mg L^{-1}.

The isolates above were evaluated for the presence of heteroresistance. A distinct profile was observed among the groups, considering that only the KPC-producing isolates presented heteroresistant subpopulations. The population analysis, as well as other information about the isolates can be found in Table 1.

DISCUSSION

In this study, we compared the presence of heteroresistant subpopulations in two distinct groups, including KPC-producing and KPC non-producing isolates. In KPC group, all isolates presented colonies growing at imipenem concentrations at least four times the original MICs. On the other hand, the

heteroresistance phenomenon was not observed in isolates from non-KPC group.

The heteroresistant subpopulations of *K. pneumoniae* isolates reached growth up to concentrations of 16 times the original MIC, with exception of the isolate 2C. Considering the *E. coli* isolates, the growth was at most eight times the MIC (Table 1). These results may contribute to the understanding of the fact that *Klebsiella* genus is significantly more involved with multiresistance and therapeutic failure (Nordmann *et al.*, 2011). Indeed, to the best of our knowledge, this is the first report of heteroresistance among *E. coli*.

According to our results, heteroresistance was only observed in the KPC-producing isolates. Although heteroresistance reports among *Enterobacteriaceae* are still uncommon, some studies reported the presence of meropenem heteroresistant subpopulations in VIM-1- and KPC-producing *K. pneumoniae* isolates (Tato *et al.*, 2010; Pournaras *et al.*, 2010). On the other hand, the relationship between the presence of carbapenemase genes and heteroresistance in non-fermenters Gram negative rods is still not clear. In a study with *A. baumannii*, Ikonomidis *et al.* (2009) found no carbapenemase gene in meropenem heteroresistant isolates, while Cuenca *et al.* (2012) detected the $bla_{\text{OXA-58-like}}$ gene in 57% of the heteroresistant isolates studied.

Heteroresistance may not be detected by conventional susceptibility methods and isolates may be reported as susceptible to antibiotics which may lead to carbapenem treatment failure. Since the heteroresistance, at least to imipenem, seems to be related to the presence of an enzymatic resistance mechanism, it is well recommended that other methodologies than the susceptibility standard methodologies need to be used to identify the presence of such phenomenon. The clinical implications of appearing of heteroresistant subpopulations remains to be investigated, considering that this phenomenon seems to be frequent in Gram-negative rods, including members, such as *E. coli,* in which this phenomenon is still rarely investigated.

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Table 1. Clinical and laboratorial data of the isolates analysed for the presence of heteroresistance.

Strain	Hospital	Specie	IPM MIC[#]	Highest IPM concentration with growth[#,*]
		KPC group		
1C	A	*K. pneumoniae*	2	**≥32**
2C	A	*K. pneumoniae*	2	**8**
3C	A	*K. pneumoniae*	2	**≥32**
5C	A	*K. pneumoniae*	≤0.5	**≥32**
10C	A	*K. pneumoniae*	2	**≥32**
4C	A	*E. coli*	1	**8**
6C	A	*E. coli*	1	**8**
7C	A	*E. coli*	1	**8**
8C	A	*E. coli*	≤0.5	**8**
9C	A	*E. coli*	2	**16**
		Non-KPC group		
12C	A	*K. pneumoniae*	1	2
14C	A	*K. pneumoniae*	2	1
16C	C	*K. pneumoniae*	2	4
13C	B	*E. coli*	≤0.5	1

MICs were obtained by broth microdilution. Concentration in mg L^{-1}.
[#] IPM, imipenem.
[*] The presence of heteroresistant subpopulations is indicated in bold.