Imipenem heteroresistance: high prevalence among Enterobacteriaceae Klebsiella pneumoniae carbapenemase producers

Carbapenems are currently the treatment of choice for severe infections caused by extended-spectrum β-lactamase (ESBL)-producing isolates (Pitout & Laupland, 2008). Klebsiella pneumoniae carbapenemase (KPC) represents one of the major carbapenem resistance mechanisms in Gram-negative rods, particularly in Enterobacteriaceae (Nordmann et al., 2009). KPC, an Ambler class A β-lactamase, hydrolyses not only carbapenems, but also other β-lactams, such as penicillins, cephalosporins and monobactams (Alba et al., 2005). Since its first description in 2001 (Yigit et al., 2001), KPC has already been described in virtually all members of this family, including species not commonly related to multidrug resistance (Nordmann et al., 2011; Ribeiro et al., 2012).

The detection of KPC-producing isolates based solely on susceptibility tests may be difficult, as KPC can confer only low-level resistance to carbapenems in vitro (Anderson et al., 2007). The misdetection of these strains could lead to relevant consequences, including treatment failure and infection control failure, which may 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., 2008). Studies evaluating this phenomenon in Gram-negative rods are less frequent than those in Gram-positive bacteria.

Heteroresistance to carbapenems has been mainly accessed among non-fermenters, such as Pseudomonas spp. and Acinetobacter spp. (Pournaras et al., 2005; Pournaras et al., 2007; Ikonomidis et al., 2009; Oikonomou et al., 2011; Fernández Cuenca et al., 2012), but reports in Enterobacteriaceae are still very rare (Pournaras et al., 2010; Tato et al., 2010). The aim of this study was to evaluate the presence of imipenem heteroresistant subpopulations in KPC-producing and non-producing K. pneumoniae and Escherichia coli.

We selected a total of 10 KPC-producing (KPC group) and seven KPC-non-producing (non-KPC group) isolates with MICs lower than 4 mg l\(^{-1}\) to imipenem by broth microdilution (CLSI, 2012). KPC production was evaluated by multiplex real-time PCR with a specific primer for this gene (blaKPC) as well as primers for blaGES, blaIMP, blaNDM, blaVIM and blaOXA-48 genes (Monteiro et al., 2012). The isolates in the KPC group were from a single hospital, whereas the isolates from the non-KPC group were obtained from five different institutions. All isolates were recovered from urine samples, with the exception of 5C and 18C isolates, which were recovered from rectal swabs. The presence of heteroresistant populations was evaluated as follows: an inoculum of approximately 10\(^8\) c.f.u. ml\(^{-1}\) was prepared, and a volume of 20 μl was plated onto 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 medium. The procedures were performed in duplicate. After 48 h, the presence of bacterial growth was observed. The isolates were considered heteroresistant if they grew on plates with imipenem concentrations higher than 4 mg l\(^{-1}\) and at least twofold dilutions of the original MIC. The frequency of heteroresistant subpopulations at the highest drug concentration was estimated by dividing the number of colonies grown on antibiotic-containing plates by the colony counts of the same bacterial inoculum plated onto antibiotic-free plates.

None of the isolates (KPC-producing and non-producing) produced other carbapenemases. The group of KPC producers comprised five K. pneumoniae and five E. coli while the non-KPC producers included four K. pneumoniae and three E. coli. Heteroresistance was observed only among the KPC producers. Most heteroresistant subpopulations of K. pneumoniae isolates reached growth up to concentrations of 16 times the original MIC, whereas the growth for E. coli was, at most, eight times the MIC (Table 1).

According to our results, it was possible to observe a clear distinction between the KPC producers and non-producers in relation to presence of resistant subpopulations (heteroresistance).

Previous studies among Enterobacteriaceae have already reported the presence of heteroresistant subpopulations in carbapenemase producers (Tato et al., 2010; Pournaras et al., 2010), but they were not able to clarify if this phenomenon was characteristic of the production of an enzyme, as non-carbapenemase producers were not evaluated.

This relationship between the presence of carbapenemase genes and heteroresistance is also not clear in non-fermenting Gram-negative rods. In a study with A. baumannii, Ikonomidis et al. (2009) found no carbapenemase gene in meropenem-heteroresistant isolates, while Fernández Cuenca et al. (2012) detected the blaOXA-58-like gene in 57% of the heteroresistant isolates studied.

It should be noted that genus Klebsiella is significantly more multiresistant and usually involved in infections with therapeutic failure (Nordmann et al., 2011). This was also observed in our study, as most heteroresistant subpopulations from this genus presented growth at higher imipenem concentrations compared with E. coli. Indeed, to the best of our knowledge, this is the first report of heteroresistance among E. coli, which also
represents an important pathogen causing nosocomial infections.

As heteroresistance may not be detected by conventional susceptibility methods, isolates with resistant subpopulations may be reported as fully susceptible in vitro. As heteroresistance, at least to imipenem, seems to be related to the presence of an enzymic resistance mechanism (i.e. KPC), we consider that methodologies other than the antimicrobial susceptibility standards should be used in regions with a high prevalence of KPC.

The clinical implications of heteroresistance remain to be investigated, considering that this phenomenon might be related to treatment failure.

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Abbreviations: ESBL, extended-spectrum β-lactamase; KPC, Klebsiella pneumoniae carbapenemase.


