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Increased frequency of *bla*$_{NDM}$ in a tertiary care hospital in southern Brazil

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Abstract

Resistance to carbapenems due to metallo-beta-lactamase NDM-1 was first described in Brazil in 2013. To date, only a few scattered reports of the prevalence of NDM-1 in the country have been reported, and most of them indicated a very low prevalence of this metalloenzyme. In the present study, we report a steady increase in the frequency of NDM among *Enterobacterales* resistant to carbapenems in a tertiary care hospital in southern Brazil. Carbapenemase genes were evaluated by multiplex real-time polymerase chain using high-resolution melting analysis among 3501 isolates of 8 different species of *Enterobacterales* recovered from January 2015 to May 2020. The *bla*$_{KPC-like}$ was identified in 3003 isolates (85.8%) and the *bla*$_{NDM-like}$ was the second most common gene (351 isolates—10%). There was a steady increase in frequency of *bla*$_{NDM-like}$, from 4.2% in 2015 to 24% in 2020. The increase of *bla*$_{NDM}$ frequency raises an important matter as novel therapeutic options are currently very limited for the treatment of patients infected by bacteria carrying the *bla*$_{NDM}$.

Keywords Carbapenemase · Dissemination · NDM-1 · New Delhi metallo-beta-lactamase · Resistance

In Brazil, resistance to carbapenems due to NDM-1 was first described in 2013 in a *Providencia rettgeri* in the city of Porto Alegre [1]. Since then, NDM-producing bacteria was heralded as the next public health threat in Brazil. However, this metallo-beta-lactamase (MBL) has been reported only sporadically in different regions of the country and in very low prevalence [2]. Nevertheless, *Klebsiella pneumoniae* carbapenemase type 2 (KPC-2) has been reported as the main carbapenemase in Brazil reaching the prevalence of more than 76% among carbapenem-resistant *Enterobacterales* [3, 4]. Similar to KPC, NDM has been associated with multidrug resistance and has been reported from various Brazilian states and in different gram-negative species, including *Enterobacter cloacae*, *E. hormaechei*, *P. rettgeri*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter pittii*, *K. oxytoca*, *Citrobacter freundii*, *A. bereziniae*, *Proteus mirabilis*, and *A. baumannii* [2, 3]. In 2013, we described the prevalence of NDM as only 0.97% among *Enterobacterales* with reduced susceptibility to carbapenems from several institutions in southern Brazil [4].

In order to monitor the frequency of the carbapenemase-encoding genes among carbapenem-resistant *Enterobacterales* in a tertiary teaching hospital located in the city of Porto Alegre, Rio Grande do Sul state, Brazil, we have evaluated carbapenem-resistant isolates since 2015. Isolates exhibiting resistance to at least meropenem were considered carbapenem-resistant isolates. Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Biomérieux, France) and antimicrobial susceptibility testing was performed by disk diffusion method as described by the Clinical and Laboratory Standards Institute guidelines [5].

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Carbapenem-resistant isolates were selected and carbapenemase genes were identified by multiplex real-time polymerase chain reaction (RT-PCR) using a high-resolution melting (HRM) for $bla_{\text{KPC-like}}$, $bla_{\text{NDM-like}}$, $bla_{\text{OXA-48-like}}$, $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, and $bla_{\text{GES-like}}$ as described previously [6]. Statistical analysis was performed using the Cochran-Armitage test.

A total of 3501 isolates of *Enterobacterales* including *Klebsiella pneumoniae* complex, *K. oxytoca*, *Enterobacter cloacae* complex, *E. aerogenes*, *E. gergoviae*, *E. hormaechei*, *Escherichia coli*, *Citrobacter freundii* complex, *C. braakii*, *C. koseri*, *C. werkmanii*, *Serratia marcescens*, *Morganella morganii*, and *Providencia rettgeri* were evaluated from January 2015 to May 2020. The $bla_{\text{NDM-like}}$ was the second most common gene (351 isolates—10%), whereas the $bla_{\text{KPC-like}}$ was identified in the majority of the isolates (3003 isolates—85.8%). The $bla_{\text{OXA-48-like}}$, $bla_{\text{GES-like}}$, $bla_{\text{IMP-like}}$, and $bla_{\text{VIM-like}}$ were also found among carbapenem-resistant *Enterobacterales* collection (6, 5, 2, and 2 isolates, respectively). A total of 48 isolates presented both $bla_{\text{KPC-like}}$ and $bla_{\text{NDM-like}}$ by HRM-RT-PCR (42 *K. pneumoniae*, 2 *Enterobacter hormaechei*, 2 *Enterobacter cloacae* complex, 1 *E. coli*, and 1 *S. marcescens*).

Relatively few studies have reported *Enterobacterales* isolates harboring more than one carbapenemase gene. Rozales et al. described the characteristics of 10 *Enterobacterales* co-harboring carbapenemase genes: 5 *E. cloacae* complex with $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-370}}$ genes, 3 *K. pneumoniae* and 1 *E. cloacae* complex with $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-2}}$ genes, and 1 *K. pneumoniae* with $bla_{\text{KPC-2}}$ and $bla_{\text{OXA-370}}$ genes [7]. Balm et al. and Both et al. reported a *K. pneumoniae* isolate co-harboring bla_{NDM} and $bla_{\text{OXA-181}}$ genes and a *E.coli* coproducing $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$, respectively [8, 9].

In this report, we observed a significant increase in frequency of $bla_{\text{NDM-like}}$, from 4.2% in 2015 to 24% in 2020 ($p < 0.001$; Fig. 1). The frequency of $bla_{\text{NDM-like}}$ per species was as follows: *Klebsiella pneumoniae* complex (224/3122 isolates), *Klebsiella oxytoca* (15/39 isolates), *Enterobacter* spp. (75/195 isolates), *Escherichia coli* (14/38 isolates), *Citrobacter* spp. (15/26 isolates), *Serratia marcescens* (5/31

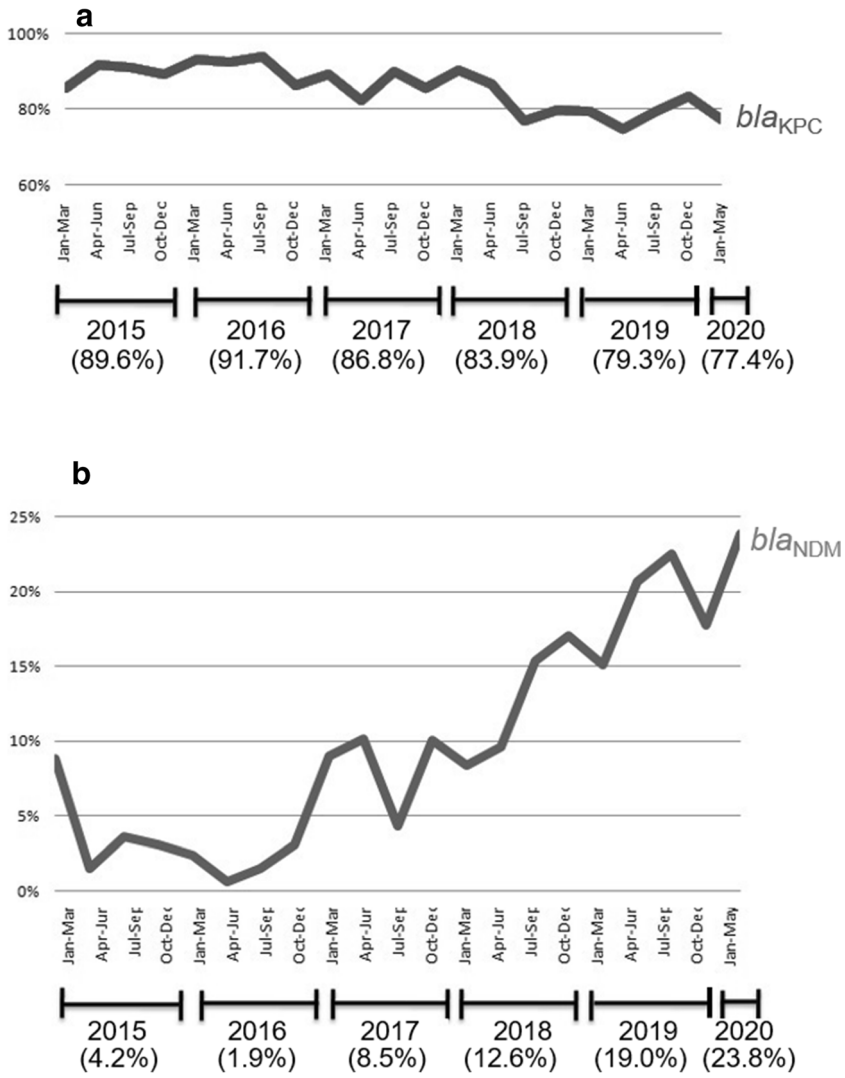

Fig. 1 $bla_{\text{KPC-like}}$ (**a**) and $bla_{\text{NDM-like}}$ (**b**) producing *Enterobacterales* distribution from January 2015 to May 2020

isolates), *Morganella morganii* (1/4 isolates), *Providencia rettgeri* (1/1 isolate), and *Providencia* spp. (1/1 isolate). In fact, to the best of our knowledge, this is the first report of the bla_{NDM} gene in a *S. marcescens*.

Conversely to the increase of $bla_{NDM\text{-}like}$, it was possible to note a trend of decrease of $bla_{KPC\text{-}like}$ frequency during the same period (from 90% in 2015 to 77% in 2020), which did not present a statistical significance ($p = 0.668$). It is worth to mention that only carbapenem-resistant isolates were included in this study, i.e., isolates with "intermediate" result of meropenem were not evaluated; thus, the frequency of $bla_{NDM\text{-}like}$ in our hospital may be even higher.

The increase of $bla_{NDM\text{-}like}$ frequency raises an important matter as therapeutic options are currently very limited for the treatment of patients infected by bacteria carrying $bla_{NDM\text{-}like}$ compared to $bla_{KPC\text{-}like}$ bacteria. MBLs are considered to increase the risk to public health because they hydrolyze all classes of beta-lactams except monobactams (aztreonam) and are not inhibited by the new beta-lactam/beta-lactamase inhibitor combinations, such as ceftazidime/avibactam; therefore, the predominance of NDM will affect the use of new therapeutic alternatives and treatment with new beta-lactam/beta-lactamase inhibitors targeting serine carbapenemases would need to be guided by the susceptibility test.

In this study, it was not possible to determine what led to an increase in the frequency of NDM-producing *Enterobacterales*. Since ceftazidime/avibactam has been introduced in our institution in early 2019 and it has been prescribed only to 6 patients in 2019 and 23 patients in 2020 (data not shown), we believe that selective pressure over KPC-producing isolates caused by a more extensive use of ceftazidime-avibactam does not explain the rise of isolates with this MBL noted since early 2017.

Another aspect to be considered is that NDM-positive *Enterobacterales* may co-harbor additional genes that can confer resistance by other routes. Unfortunately, aztreonam is readily inactivated by Ambler class A beta-lactamases, including extended-spectrum beta-lactamases and KPC, impairing the activity of aztreonam against these isolates [10].

A limitation of our study was that molecular typing of the bla_{NDM}-positive isolates was not performed. However, as the bla_{NDM} gene was identified in several different species of *Enterobacterales*, one could consider that the increased frequency of this MBL is not due to (at least not solely) the spread of a specific clone.

Our finds regarding the increase frequency of $bla_{NDM\text{-}like}$ in our institution highlight the importance of early detection of the molecular mechanism underlying carbapenem resistance to avoid the empirical treatment with ceftazidime/avibactam. Hence, there is an urgent need to establish recommendations for tackling NDM carbapenem-resistant infections in southern Brazil.

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Compliance with ethical standards

Conflict of interest APZ received a research grant from Pfizer.

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