

Title: MOLECULAR EPIDEMIOLOGY OF NDM-1-PRODUCING *ENTEROBACTERIACEAE* IN SOUTHERN BRAZIL

Authors: Rozales, F.P.^{1,2}, Magagnin, C.M.^{1,2}, Nunes, L.S.^{1,3}, Pagano, M.^{1,2}, Pancotto, L.R.^{1,2}, Antochévis, L.C.^{1,2}, Zavascki, A.P.^{1,4}, Barth, A.L.^{1,2}.

Institution ¹LABRESIS- Laboratório de Pesquisa em Resistência Bacteriana, Hospital de Clínicas de Porto Alegre (Ramiro Barcelos 2350, Porto Alegre, RS), ²UFRGS– Universidade Federal do Rio Grande do Sul (Av Paulo Gama 110, Porto Alegre, RS), ³ UNISC – Universidade de Santa Cruz do Sul, Departamento de Biologia e Farmácia (Av. Independência, 2293, Santa Cruz do Sul, RS), ⁴HCPA- Hospital de Clínicas de Porto Alegre, Serviço de Doenças Infecciosas (Ramiro Barcelos 2350, Porto Alegre, RS),

Abstract:

The emergence of carbapenemase-producing *Enterobacteriaceae* is a major public health issue due to their high dissemination capacity and limited treatment options. The spread of the Class B carbapenemase New Delhi metallo- β -lactamase gene (NDM-1)-producing bacteria has become a growing concern worldwide. The aim of this study was to evaluate the presence of *bla*_{NDM} gene in *Enterobacteriaceae* isolates with decreased susceptibility to carbapenems. A total of 4631 isolates were obtained from April 2013 to April 2015 in a molecular epidemiology study that aimed to evaluate isolates with reduced susceptibility to carbapenems in Rio Grande do Sul, Brazil. The isolates were obtained from 28 hospitals. The bacterial DNA was extracted by thermal lysis and detection of carbapenemases (*bla*_{IMP}, *bla*_{KPC}, *bla*_{GES}, *bla*_{NDM}, *bla*_{OXA-48-like} and *bla*_{VIM}) was performed by PCR multiplex real-time HRM. The presence of *bla*_{NDM} gene was confirmed by conventional simplex PCR followed by sequencing. The nucleotide sequences were compared with those available in GenBank using the BLAST program. The clonal profile of NDM-producing isolates was evaluated by the DNA macrorestriction technique followed by PFGE and minimum inhibitory concentrations (MICs) for carbapenems (imipenem, meropenem and ertapenem) and polymyxin B were analyzed by E-Test®. Among the 4631 isolates, 3032 were positive for at least one carbapenemase, and 108 presented the *bla*_{NDM} gene. Sequence analyses revealed the presence of *bla*_{NDM-1} gene. The main NDM-1-producing isolates were *Enterobacter cloacae* complex (39%), *Klebsiella pneumoniae* (20%), *Klebsiella oxytoca* (19%), *Escherichia coli* (7%), *Citrobacter freundii* (6%), *Morganella morganii* (5%), *Providencia rettgeri* (3%) and *Proteus mirabilis* (1%). The majority of clinical samples were urine (35%) and rectal swab (22%). NDM-positive isolates presented high carbapenem-resistance level but were fully susceptible to polymyxin B. NDM-positive *Klebsiella* sp presented a variety of macrorestriction profiles indicating a polyclonal distribution. The results demonstrated the rapid spread of the NDM gene, reinforcing the importance of surveillance studies in order to evaluate the epidemiology of this resistance mechanism in order to promote effective infection control measures.

Keywords: *Enterobacteriaceae*, Carbapenemase, New Delhi metallo- β -lactamase

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