

Title: PLASMID CHARACTERIZATION OF NDM-1-PRODUCING *ENTEROBACTERIACEAE* IN BRAZIL

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Abstract:

The emergence of carbapenemase producing *Enterobacteriaceae* isolates is an important public health problem since the treatment of carbapenem-resistant isolates is extremely difficult owing to the few options that remain available for clinical use. The New Delhi Metallo beta-lactamase (NDM-1) is the most common class B carbapenemase in *Enterobacteriaceae*. The aim of this study was to evaluate the characteristics of *bla*_{NDM} plasmids, detected in different bacterial species. Isolates were obtained from a molecular epidemiology study that aimed to evaluate *Enterobacteriaceae* with reduced susceptibility to carbapenems in Rio Grande do Sul State, Brazil. We analyzed 10 isolates: 3 *Enterobacter cloacae* complex, 3 *Klebsiella oxytoca*, 1 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 1 *Escherichia coli* and 1 *Citrobacter freundii*. The plasmids were extracted by alkaline lysis and were transformed into a *E. coli* TOP10 electrocompetent cells. Transformants were selected on LB agar with ceftazidime 4 mg/L. The presence of *bla*_{NDM} was confirmed by conventional PCR and the amplicons were purified and sequenced. The nucleotide sequences were compared with those available in the GenBank using the BLAST program. Minimum inhibitory concentrations (MIC) of carbapenems were evaluated by Etest®. EDTA was used as a phenotypic method to detect metallo-beta-lactamases. The transformants obtained from each isolate presented higher MICs than the original *E. coli* TOP10 for β -lactams. In fact, the MIC of transformants were similar to the donor NDM-positive isolates. Moreover, the inhibition test using EDTA was positive for transformants. The sequencing confirmed the presence of *bla*_{NDM-1} gene. Plasmid analysis demonstrated the presence of a 52-kb plasmid in a *K. oxytoca*, a 66-kb plasmid in an *E. cloacae* complex, a 154-kb plasmid in a *K. oxytoca* and *K. pneumoniae*. In addition, most transformants presented a 110-kb plasmid (2 *E. cloacae* complex, 1 *K. oxytoca*, 1 *M. morganii*, 1 *E. coli* and 1 *C. freundii*). The variety of plasmids observed in the transformants suggests that the strains producing *bla*_{NDM-1} harbor plasmids of different sizes, demonstrating the plasticity of these mobile genetic elements.

Keywords: Carbapenem resistance, Plasmid, New Delhi metallo- β -lactamase

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