

**Title:** Tn3000: a transposon responsible for *bla*<sub>NDM-1</sub> dissemination among *Enterobacteriaceae* in Brazil

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**Abstract:**

Carbapenems are the antimicrobials most widely used in the empirical treatment of severe infections caused by Gram-negative bacilli. The selective pressure generated by the use of these antibiotics over the last three decades has contributed to the spread of enterobacteria and Gram-negative non-fermenting producing carbapenemases, mainly KPC and NDM. Genes encoding these enzymes are usually located in plasmids and/or transposons. The NDM-1 was firstly described in a patient from India and subsequently was reported to be broadly disseminated in this country. In Brazil, the first cases were identified in Rio Grande do Sul, and then in Rio de Janeiro and São Paulo. Differently from the cases described in Europe and North America, the cases from Brazil have no epidemiological link with India. In this study we evaluated plasmids hosting *bla*<sub>NDM-1</sub>, isolated from different species: *Enterobacter hormaechei* “subsp. *oharae*”, *Enterobacter hormaechei* “subsp. *steigerwaltii*”, *Citrobacter freundii*, *Escherichia coli* and *Klebsiella pneumoniae*. They were isolated from August 2013 to November 2014, from the rectal swabs, blood and urine samples from patients from Rio de Janeiro and Salvador. Complete sequencing of plasmid DNA using Illumina platform and annotation of plasmids revealed that in all strains the *bla*<sub>NDM-1</sub> gene was located onto plasmids inside a new transposon designated Tn3000. This element is 11,823 bp long and is bracketed by two copies of IS3000. The first copy truncates the 5' portion of the IS*Aba125* upstream of the *bla*<sub>NDM-1</sub> gene. Downstream of the *bla*<sub>NDM-1</sub> gene, the bleomycin resistance gene *ble*<sub>MBL</sub> was identified, followed by genes encoding a phosphoribosylanthranilate isomerase (*trpF*), a twin-arginine translocation pathway signal protein gene (*tat*) and a divalent ion tolerance protein (*cutA1*). The *groEL* and *groES* genes were also part of Tn3000, but the *groEL* gene was truncated at its 3' extremity by insertion of a second copy of IS3000. The Tn3000 nucleotide sequences identified onto these plasmids were 100% identical. A BLAST search using the Tn3000 nucleotide sequence evidenced the presence of this structure in plasmids from Porto Alegre, Brazil and also countries from other continents as Nepal and India. Our findings show that the Tn3000 transposon is responsible for *bla*<sub>NDM-1</sub> gene dissemination among *Enterobacteriaceae* in different continents.

**Keywords:** *bla*<sub>NDM-1</sub>, *Enterobacteriaceae*, plasmids, Tn3000.

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