Title: HOW TO DETECT THE Tn*3000* BY PCR

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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. In Brazil, the first case of *bla*$_{NDM-1}$ was identified in Rio Grande do Sul, and then in Rio de Janeiro and São Paulo. These cases have no epidemiological link with India. Using next generation sequencing we have recently described a new compound transposon, designated Tn*3000*, as responsible for *bla*$_{NDM-1}$ gene dissemination among *Enterobacteriaceae* in Brazil. In order to easy its detection we designed six PCR reactions. They were carried out using a final concentration of 0.2 mM of each primer and Platinum Taq DNA Polymerase (Invitrogen) to amplify specific genes present in Tn3000. After optimization, amplification conditions were: denaturation of 94°C for 10 min.; 35 cycles at 94°C for 40s, 56°C for 40s and 72°C for a variable time depending on the fragment length; and a final extension for 72°C for 7 min. The Tn*3000* was amplified with the six pairs of primers: (1) Tn3000-34232-13FW 5'GGGGCAGTTCAGACGAAGAA and Tn3000-28160-79RV 3'ATGAAAGCCGGGATTCAGCA (5 min. for extension, 6.073 bp); (2) Tn3000-dsbD31638-19-RV 5'CTCGGGTGAAGTCGGGAAAA and Tn3000-28160-79RV 3'ATGAAAGCCGGGATTCAGCA (3 min. for extension, 3.478 bp); (3) Tn3000-ndm33418-399-FW 5'GGCCAGCAAATGGAAACTGG and Tn3000-dsbD30657-75-FW 3'ATGACCGCATCCACGATCC (3 min. for extension, 2.761 bp); (4) Tn3000-34323-13FW 5'GGGGCAGTTCAGACGAAGAA and Tn3000-ndm32781-800-RV 3'ATCACGATCATGCTGGCCTT (2 min. for extension, 1.452 bp); (5) meth-IS3000-38627-08FW 5'AGCTGCTGTTCCTTCCTGTG and meth-IS3000-35460-79RV 3'TTTTTCGCCAATGTTCCCCG (3 min. for extension, 3.168 bp); (6) meth-IS3000-37263FW 5'CCAGTCATTGCCAAACGCAG and meth-IS3000-36748RV 3'ATGAAAGCCGGGATTCAGCA (1 min. for extension, 515 bp). The fragments were sequenced with the Big Dye Terminator kit version 3.1 in a 3130xl Genetic Analyzer (Applied Biosystems) with the same primers. We designed a set of primers that can easily be used to detect the Tn*3000* transposon in Enterobacteriaceae without the use of next generation sequencing.

Keywords: Tn*3000*, *bla*$_{NDM-1}$, PLASMID, *Enterobacteriaceae*.

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