PADRONIZATION MULTIPLEX PCR FOR DETECTION KPC AND THE MAIN METALLO BETA-LACTAMASE TO GRAM-NEGATIVE BACILLI

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According to the brazilian technical note of National Health Surveillance Agency (No. 01/2013) metallo beta-lactamase of type IMP, VIM and NDM, carbapenemases KPC type are most often found in enterobacteria. Although, there are automated systems to detect genes such as KPC and NDM, these are not available in most laboratories because its expensive. The objective of this study was to standardize an available method and low cost of the polymerase chain reaction multiplex (multiplex-PCR) for detection of main genes of metallo beta-lactamases and KPC in the same reaction. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Acinetobacter baumannii* described in the literature to possess the gene bla_{IMP}, bla_{GIM}, bla_{SPM}, bla_{KPC}, bla_{NDM} were used as controls strain. Total DNAs bacterial isolates were extracted by boiling for 15 minutes followed by centrifugation (12000 rpm for 5 minutes). Primers for SPM, IMP and GIM used were previously described by Ellington et al. (2007) and primers for KPC and NDM were described by Poirel et al. (2011) to the reaction 2µL extracted DNA was added to a mix containing 1x Taq polymerase buffer, 0.2mM of each dNTP, 1.5mM $MgCl_2$, each primer 0.5µM, 1.5U Taq DNA polymerase and water to 25µL. The cycling used: one cycle of 10 minute at 94°C, followed by 36 cycles of 30 seconds at 94°C, 40 seconds 52°C and 50 seconds at 72°C and 5 final minutes at 72°C. Several reactions were performed for ten different laboratory technicians on different dates. All amplified products were analyzed on 1.5% agarose gel electrophoresis at 100V for 50 minutes. The products were photographed in fotodocumentador (LOCCUS Biotechnology®). The multiplex-PCR showed optimal results, all controls samples were amplified and allowed easy interpretation, since the amplicons have sizes in distinct pairs of bases (bp), (*bla*$_{IMP}$ 188bp, *bla*$_{SPM}$ 271bp, *bla*$_{GIM}$ 477bp, 621bp *bla*$_{NDM}$ and *bla*$_{KPC}$ 893bp). After standardizing, the test is already being used in routine laboratorial carbapenemases detection in clinical samples with positive or indeterminate phenotypic tests with excellent performance. We concluded that the padronization test is of great importance because it reduces the important detection time of carbapenem resistance genes and reduce laboratorial and hospital costs of isolation, monitoring of patients in these enzymes. The rapid simultaneous detection of these genes is an important tool to measure control of the spread of resistance genes.

Keywords: multiplex-PCR, metallo beta-lactamase, KPC

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