Título: Multiplex-PCR to *mecA*, *mecC* and *BlaZ* genes detection in *Staphylococcus* spp.

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Abstract
The presence of β-lactam resistance in *Staphylococcus* is a worldwide problem both in human and veterinary science. The main form of resistance to β-lactam in staphylococci is mediated by the *mecA* gene although another genes may be involved as *mecC* and *BlaZ* genes. The presence of *mecA* or *mecC* genes in *Staphylococcus* regards this strain by MRS (Methicillin-resistant *Staphylococcus* spp.). There was standardized a multiplex-PCR to detect and differ *mecA* (amplification of 313 bp), *mecC* (138 bp) and *BlaZ* (516 bp) genes with an intern control with 16S gene (139 bp), using literature and designed primers to be compatible to multiplex form. DNA extraction were performed with purified colonies (10^7 CFU) fresh or frozen, deproteinated with chloroform/isoamyl alcohol (24:1) and concentrated with alcohol 70%. The concentrations of reagents and cycles of amplification were tested. The best amplifications were accomplished by following conditions: 0.4 pmol of each primers, 0.4 mM of each dNTP, 2 mM of $MgCl_2$, 1x PCR Buffer and 1.25 units of *Platinum*® *Taq* DNA polymerase. The amplification were performed in a thermocycler with following steps: a first step of denaturation at 94° C for 7 min; 40 cycles of amplification at 94° C for 1 min, 54 ° C for 1 min and 72 ° C for 1 min; and a last step for final extension at 72 ° C for 7 min. The amplified products of PCR were submitted to electrophoresis in agarose gel (1.5%) staining with SYBR® Safe DNA gel stain. To standardize the reaction were used *Staphylococcus aureus* standard strain (ATCC 43300) and 10 *Staphylocccus* spp. of animal clinical samples resistant to oxacillin or cefoxitin by disc-diffusion technique. In the ATCC 43300 strain was amplified 3 amplicons (16S, *mecA* and *BlaZ* genes). Two clinical samples did not amplified resistant genes; three samples amplified just *BlaZ* gene; three samples amplified both *mecA* and *BlaZ* genes; one sample amplified just *mecA* gene; and one sample amplified both *mecA*, *mecC* and *BlaZ* genes; all samples amplified internal control. The use of an internal control concomitant with the detection of resistant genes promotes greater reliability to results of PCR reaction excluding false negatives samples. The research of different genes of resistance in the same reaction results in improved detection of resistance to β-lactam drugs to *Staphylococcus* strains.

Key-words: β-lactam; staphylococci; MRS