Title: UNIVERSAL PRIMER FOR DETECTION OF *mec*A GENE AND VARIATIONS IN *Staphylococcus* spp. FROM DIFFERENT HOSTS

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Abstract:

Staphylococcus spp. is the most common cause of bovine mastitis, presenting a high level of antimicrobial resistance, especially to beta-lactams. This resistance is related to the expression of *mec*A gene, which encodes an alternative penicillin-binding protein, PBP2' or PBP2a. Currently, the detection of the *mec*A gene is performed by PCR (Polymerase Chain Reaction) using primers described in literature, based on sequences of *Staphylococcus* spp. of human origin. However, a variant of the *mec*A gene was detected in *Staphylococcus* spp. from bovine, which has mutations over the gene, impossible to detection with primers already described. Based on these findings, this work aimed to develop a universal primer for the simultaneous detection of *mec*A gene from different species of animals and human. *Staphylococcus* spp. *mec*A sequence from bovine was compared to *Staphylococcus* spp. *mec*A sequence from different hosts, animals and human available in Genbank database. New primers named "universal" were designed based on the comparison of the conserved regions of *mec*A gene from distinct host origins. A PCR was performed using either primers based on *Staphylococcus* spp. *mec*A sequence from human and on the variant of *mec*A gene sequence from bovine. Four isolates of *Staphylococcus aureus* and 11 Coagulase-Negative *Staphylococcus* from milking production line were used in this study. Six isolates that only amplified *mec*A gene with human sequences based primers plus two isolates that amplified *mec*A gene with primers based on sequence of *mec*A variant from bovine, tested positive when using the “universal” primer. It proves that the proposed primer allowed the detection of *mec*A gene in isolates from distinct origins. Three isolates only amplified with universal showing the existence of variations in *mec*A gene sequence that prevent their detection with primers already described. Besides this four strains of Coagulase-Negative *Staphylococcus* amplified only with primer based on *mec*A variant. These preliminary results revealed that should be differences in composition of nucleotides of this gene in Coagulase-Negative *Staphylococcus* from bovine, and confirms the importance of more detailed studies of the *mec*A gene in order to propose primers to ensure adequate coverage of all variations, to predict *mec*A-mediated resistance in *Staphylococcus* spp. from different hosts.

Keywords: bovine mastitis, *mec*A gene, universal primer.

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