

TITLE: BORDERLINE OXACILLIN-RESISTANT *Staphylococcus aureus* (BORSA) STRAINS ISOLATED FROM BOVINE SUBCLINICAL MASTITIS

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ABSTRACT:

Staphylococcus aureus is one of the most important etiological agent in bovine mastitis. Preventive management practices are usually recommended to reduce the likelihood of mastitis infections caused by contagious or environmental pathogens. Dry cow therapy is a preventive intramamarian method that may reduce subclinical infections persisting from the previous lactation. It is carried out using long acting antibiotics, such as combinations of β -lactams that can act in the udder up to seven weeks. We report borderline oxacillin-resistant *S. aureus* (BORSA) strains isolated from cows with subclinical mastitis during dry cow therapy. The aim of this study was to investigate resistance mechanisms that might lead to the emergence of resistance to oxacillin. We investigated 20 *S. aureus* strains isolated from milk samples of cows submitted to preventive treatments using a combination of ampicillin and sodium cloxacillin. Conventional methods and PCR test using primers for the *nuc* gene were performed in order to confirm *S. aureus*. The susceptibility to oxacillin was tested using disk diffusion method, and screening on Mueller-Hinton agar (4% NaCl) containing 4 μ g/ml of oxacillin according to the Clinical Laboratory Standards Institute (CLSI). Minimum inhibitory concentration (MIC) to oxacillin was determined by broth microdilution testing (CLSI). The hyper-production of β -lactamase was tested using clavulanic acid (10 μ g) in combination with two β -lactam antibiotics: cefotaxime (30 μ g) and ceftazidime (30 μ g) (CLSI). In addition, nitrocefin stick test was also used (Oxoid). The presence of the *mecA* gene was investigated by PCR. Fourteen *S. aureus* strains exhibited oxacillin MICs from 1 to 8 μ g/ml, while six *S. aureus* strains exhibited higher oxacillin MIC values (12-16 μ g/ml). All were *mecA* negative. The adding of a β -lactamase inhibitor resulted in a significant change in the halo size of cefotaxime and ceftazidime (≥ 5 mm), suggesting hyper-production of β -lactamases in these *S. aureus* strains. However, the high levels of oxacillin MIC observed in some strains do not exclude the occurrence of mutations in PBP. Multiple and/or inadequate therapies using β -lactam antibiotics in dairy cattle might lead to the emergence and spread of BORSA strains.

Keywords: mastitis, *Staphylococcus aureus*, resistance, oxacillin.

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