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ANTIBIOTIC SUSCEPTIBILITY OF *S. aureus* EXCEEDS CLINICAL BREAKPOINTS OF RESISTANCE DURING COEXISTENCE WITH *Enterobacter* IN AN INTRAMAMMARY INFECTION

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The World Health Organization recently reported an alarming increase in antibiotic resistance. In the veterinary sector, this issue is exacerbated by the improper use of antibiotics, often due to unclear treatment guidelines. Bovine mastitis, a major cause of economic loss in the dairy industry and the primary reason for antibiotic use in cattle, facilitates the emergence of multi-resistant pathogens. Independent culture techniques indicate that low cure rates might be due to the presence of multiple causative agents, complicating treatment predictions. This study assessed the interactions between *Staphylococcus aureus* L33 and *Enterobacter* sp. L34, isolated from a subclinical intramammary co-infection at the Experimental Dairy Farm of Estancia Yucat. The strains were obtained following veterinary evaluation and California Mastitis Test (CMT), with the mixed culture isolated from a milk sample of a cow with CMT(+) in all quarters. The interactions between these strains were evaluated *in vitro*, focusing on the virulence potential of *S. aureus* in monoculture and in combination with *Enterobacter*. This evaluation included the hydrophobicity index, staphyloxanthin production, biofilm formation, and the ability to internalize in bovine epithelial cells. Results showed that *Enterobacter* sp. L34 did not affect *S. aureus* L33's ability to invade mammary tissue but reduced staphyloxanthin production. *Enterobacter's* hydrophobicity and potential to damage eukaryotic cell surfaces appeared to be transferred to the mixed culture. Additionally, the total biomass of the mixed *Enterobacter-S. aureus* biofilm was significantly lower than the monospecies *S. aureus* biofilm. A significant finding was the reduced antibiotic susceptibility of *S. aureus* L33 in the presence of *Enterobacter* sp. L34. The Minimum Bactericidal Concentration (MBC) for *S. aureus* L33 increased up to a thousand-fold in the mixed culture compared to the pure culture: from 0.5 to 512 mg/L for cloxacillin, from 1 to 128 mg/L for erythromycin, and from 0.02 to >512 mg/L for penicillin. These MBC values, above clinical resistance breakpoints, indicate a shift in *S. aureus* L33 phenotype from sensitive to resistant. This highlights the importance of understanding microbial interactions in intramammary infections to improve treatment outcomes and reduce antibiotic use. The findings underscore the need for therapies that consider microbial ecology to enhance success rates and mitigate resistance issues.

Palabras clave: *S. aureus*-*Enterobacter*-mixed intramammary infection-antibiotic susceptibility-antimicrobial resistance