

TITLE: Influence of antibiotics on biofilm formation of invasive strains *Staphylococcus haemolyticus* from bloodstream infection neonates.

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ABSTRACT:

Oxacillin-resistant *S. haemolyticus* (ORSH) strains have been also associated with foreign body infections. *S. haemolyticus* is also among the species of coagulase-negative staphylococci (CoNS) that colonize and cause bacteremia in neonatal intensive care units (NICUs) in many countries. The most important virulence factor of this species is its ability to biofilm formation. This study evaluated the adhesion and biofilm formation in the presence of the antibiotics oxacillin, vancomycin and linezolid of *S. haemolyticus* strains isolated from infections of neonates. Thirty-one blood isolates were confirmed as *S. haemolyticus* by Maldi-tof method. Clonal distribution was evaluated using the pulsed-field gel electrophoresis method (PFGE). The capacity of adhesion and biofilm formation was investigated by the *slime* production method (Congo Red), adherence to glass and polystyrene in the presence of the antibiotics oxacillin, vancomycin and linezolid, and polyurethane and silicone catheters surfaces analyzed by semiquantitative roll plate and quantitative techniques and scanning electron microscopy (SEM). The polymerase chain reaction (PCR) method was performed to detect the *icaA*, *aap*, *fbp* and *atl* biofilm genes. ORSH strains were clustered in 6 pulsed-field gel electrophoresis types (with 58% of the strains belonging to 2 predominant types B and D). ORSH exhibited *slime* production on Congo-Red agar (67.7%), adherence to glass (87%) and polystyrene (96.7%) surfaces. ORSH strains showed viability and adherence in the catheter segments, revealing $> 5.0 \times 10^4$ CFU of viable cells exhibiting bacterial aggregation and microcolonies formation. Antibiotics did not inhibit biofilm formation on abiotic surfaces. The *ica*-operon was detected in 58% of the strains,

indicating that *ica* operon and biofilm-forming ORSH are endemic in Brazilian nosocomial environment, and the *aap*, *fbp* and *atl* genes were positive in 38.7%, 90.3% and 96.7%, respectively. ORSH strains are potentially associated with infections in patients with invasive medical procedures. *S. haemolyticus* were able to produce biofilm on abiotic surfaces including in the presence of vancomycin. The clonality of these strains in the Brazilian hospital revealed that some clones are endemic to the hospital environment. The molecular mechanism of biofilm formation in this species has not been elucidated, particularly in the presence of antimicrobial agents.

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