TITLE: *SUSCEPTIBILITY OF Staphylococcus SAPROPHYTICUS IN BIOFILMS TO ANTIBACTERIAL AGENTS*

AUTORS: Martins-Duarte, K.B.; Bonesso. M.F.; Pinheiro, L.; Ferreira, A.M.; Cunha, M.L.R.S.

INSTITUTION: UNIVERSIDADE ESTADUAL PAULISTA JÚLIO DE MESQUITA FILHO – Unesp BOTUCACU, BOTUCATU – SP – BRASIL.

S. saprophyticus is second only to *E. coli* as the most frequent causative organism of uncomplicated urinary tract infection (UTI). For the urinary tract can be established, the bacteria are required to adhere to the surface of epithelial cells. After the initial adhesion on the surface, there is the intercellular adhesion and biofilm formation. Biofilms are communities of microorganisms attached to a surface. It has become clear that biofilm-grown cells express properties distinct from planktonic cells, one of which is an increased resistance to antimicrobial agentes. This study aims to evaluate, in producing samples of biofilm, the sensitivity profile to vancomycin, oxacillin, sulfamethoxazole / trimethoprim, ciprofloxacin and norfloxacin in the biofilm. All isolates were collected from cases of urinary tract infection. They were previously confirmed as *S. saprophyticus* using Internal Transcribed Spacer PCR (ITS-PCR). Biofilm formation ability was determined by microtitre plate assay, and optical density (OD) was measured at 492 nm. The intensity of biofilm formation was categorized as strong (> 0,222) or weak (OD > 0,111 or ≤0,222). Conventional Minimum inibitory concentration (MIC) of vancomycin, norfloxacin, ciprofloxacin, trimethoprim/sulfamethoxazole were determined in all strains of *S. saprophyticus* biofilm-producing by serial broth microdilution (CLSI, 2012). *Staphylococcus aureus* ATCC 29213 was tested as a quality control. Minimum biofilm inhibitory concentrations (MBICs) were evaluated for their sensitivity profile to the same antimicrobial agents mentioned above, however in biofilm. In brief strains were cultured in TSB for 22 h with 2% of glicoseforam adjusted to a turbidity of 1.0 McFarland (corresponding to 1 x 10^{8} CFU / ml) diluted 1:50 in TSB with 2% glucose. Aliquots of 200µl were placed in flat bottomed 96 well plates and covered with a 96-pin cap (Nunc-TSP; Nunc) and incubated for 24 hr to allow biofilm formation on pins. Non-adherent cells were removed by gentle washing three times the pin cap with sterile saline solution (150 ml 0.9 % NaCl). The lid with the pegs is placed on the bottom plate in a plan prepared for microdilution broth susceptibility test, the wells contain a volume of 200 ml antimicrobial agent in cationadjusted Mueller–Hinton broth (CAMHB) were added to the microplates followed by incubation at 35 C for 24 h. MBICs was defined as the minimal antimicrobial concentration at which there was no observable bacterial growth in wells containing adherent pins. Among the 169 samples of *S. saprophyticus*, 119 (70.4%) produced biofilm, 88 (52.1%) samples classified as adherent strong and 31 (18.3%) adherent weak. Among 119 samples biofilm-producting all were susceptible to vancomycin, norfloxacin and ciprofloxacin. 21 (17,7%) were resistente to trimethoprim/sulfamethoxazole by MIC determination. The MBICs was determined and revealed that 19 (16,0%) presented intermediate resistance and 9 (7,6%) presented resistence to vancomycin; 15 (12,6%) presented intermediate resistance and 26 (21,9%) presented resistence to norfloxacin; 6 (5,1%)presented intermediate resistance and 24 (20,2%) presented resistence to ciprofloxacin; 58 (48,7%) presented resistence to trimethoprim/sulfamethoxazole. The isolates were subjected to antimicrobial susceptibility testing in biofilms showed an extremely high inhibitory concentration when compared to the MIC of those plankton samples. In addition, many samples increased from sensitive to intermediate resistant or resistant. It is becoming increasingly clear that biofilms have an enormous impact on medicine. It has become clear that biofilm-grown cells express properties distinct from planktonic cells, one of which is an increased resistance to antimicrobial agents. It has been suggested that this matrix, among other functions, prevents the access of antibiotics to the bacterial cells embedded in the community. The biofilm is an important virulence factor for *S. saprophyticus* as it protects the microrganisms from the action of the antibiotics tested.

Keywords: Antibacterial agentes; biofilm; Staphylococcus saprophyticus; urinary tract infection.

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