TITLE: ANALYSIS OF THE INFLUENCE OF SUBINHIBITORY CONCENTRATIONS OF CIPROFLOXACIN, THE INVOLVIMENT OF VIRULENCE GENES AND THE UROPATHOGENIC POTENTIAL OF STRAINS OF *Staphylococcus saprophyticus*

AUTHORS: DE PAIVA SANTOS, W.; ARGENTINO, I.C.V.; VASCONCELOS, A.B.; GIAMBIAGI-DEMARVAL, M.

INSTITUTION: UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, CEP 21941590, RIO DE JANEIRO – RJ, BRAZIL)

ABSTRACT:

Staphylococcus saprophyticus is the most frequent Gram-positive causative agent of urinary tract infections. However, the role of some virulence genes and their distribution among *S. saprophyticus* strains are poorly understood. It should be noted that the capacity of subinhibitory concentrations of antimicrobials (sub-MIC) to modulate bacterial virulence has been reported, raising concerns over the appropriateness of low-dose therapies for recurrent urinary tract infection management. Thereby, the aim of this study was to analyze the influence of sub-MIC of ciprofloxacin on *S. saprophyticus* strains, evaluate the participation of ssp and sdrI genes in virulence phenotypes and investigate the distribution of virulence genes among strains isolated from different sources. Firstly, biofilm analysis in the absence of the antimicrobial revealed that 26 strains were considered strong, 4 moderate and 15 were classified as weak biofilm producers. When exposed to sub-MIC of ciprofloxacin, biofilm formation for 16 strains demonstrated a strain-dependent behavior in which 10 strains showed an increase in biofilm production. SEM and CLSM assays confirmed these results showing an increase in the number and size of cell aggregates in the presence of the antimicrobial. For S. *saprophyticus* 7108 strain, qRT-PCR assays showed that expression of ureC, ssp and sdrI genes increased in response to ciprofloxacin. The involvement of ssp and sdrI genes in virulence phenotypes was assessed using mutant and complemented strains. In this case, quantitative biofilm assays and adhesion/internalization with the human bladder carcinoma cell line showed no difference between the wild-type and mutant strains. Finally, the distribution of virulence genes was assessed by PCR in clinical, colonizing, environmental and foodborne strains. In every tested strain the presence of genes encoding the surface proteins UafA, Aas, Ssp and SssF and the DsdA and UreC enzymes was detected while the gene encoding SdrI surface protein was not found. We consider that the obtained results may alert about a potential virulence increase for strains of *S. saprophyticus* exposed to sub-MIC of ciprofloxacin. In addition, analysis of the participation of ssp and sdrI genes in virulence provides new information on the role of virulence determinants of this species. Finally, our data suggests that *S. saprophyticus* strains from different sources have the necessary prerequisites for colonization of the urinary tract.