TITLE: CHARACTERIZATION OF STAPHYLOCOCCAL SECRETORY ANTIGEN A (SsaA) IN THREE *Staphylococcus saprophyticus* CLINICAL STRAINS

AUTHORS: OLIVEIRA, A. S.[1]; ROSA, I. I. R.[1]; BAEZA, L. C.[1]; BORGES, C. L.[1]; MARLINGHAUS, L.[3]; PACCEZ, J. D.[1]; SOARES, C. M. A.[1]; GIAMBIAGI-DEMARVAL, M.[2]; PARENTE-ROCHA, J. A[1].

INSTITUTION:
1. UNIVERSIDADE FEDERAL DE GOIÁS, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, LABORATÓRIO DE BIOLOGIA MOLECULAR (AVENIDA ESPERANÇA, S/N, CAMPUS SAMAMBAIA, CEP 74-690-900, GOIÂNIA-GO, BRAZIL)
2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, INSTITUTO DE MICROBIOLOGIA PROF. PAULO DE GÓES, LABORATÓRIO DE MICROBIOLOGIA MOLECULAR (ILHA CIDADE UNIVERSITÁRIA, S/N, CEP 21941-970, RIO DE JANEIRO-RJ, BRAZIL)
3. RUHR-UNIVERSITY BOCHUM, DEPARTMENT OF MEDICAL MICROBIOLOGY (BOCHUM, GERMANY).

ABSTRACT:

Staphylococcus saprophyticus is coagulase-negative staphylococci (CoNS) that is responsible for urinary tract infections (UTI), and mainly affects young sexually active women. Together with *Escherichia coli*, it accounts for 90% of infections in fertile women; however, by colonizing the gastrointestinal and genitourinary tracts of humans, it can cause infections in men and women of all ages. Staphylococcal secretory antigen A (SsaA) is a secreted protein highly antigenic and conserved in pathogenic bacteria of the *Staphylococcus* genus. In this sense, the main objective of the present work was to characterize SsaA in clinical strains ATCC 15305, 7108, 9325 of *S. saprophyticus*. Extract of secreted proteins from the three strains were obtained and submitted to Ultra Performance Liquid Chromatography coupled to in-tandem Mass Spectrometry (UPLC-MS/MS^{E}), for total protein identification. In a comparative proteomics study, the immunogenic protein SsaA was found to be abundant in strains ATCC 15305 and 9325, but in strain 7108 it was not detected. PCR reactions, gene sequencing and Western-blot assays, were performed to confirm the data. The production of serum containing polyclonal antibodies for Western-blot assays was performed by heterologous expression of the SsaA protein in a bacterial system and subsequent mouse inoculation. PCR reactions with specific primers and genomic DNA showed the presence of the *ssaA* gene in the three strains, whereas the gene sequencing indicated that the gene of the three strains showed complete similarity and lack of mutation. Western-blot assays using secreted and total protein extracts and recombinant anti-SsaA polyclonal antibodies, have shown that the SsaA classical antigen is produced and secreted by strains ATCC 15305 and 9325, but not by 7108, although this strain possesses the *ssaA* gene in identical conditions to the others. Thus, the characterization of SsaA in clinical strains of *S. saprophyticus*, pointed out a possible diversity in the infective ability of strain 7108, indicating that this strain may utilize different strategies to escape from immune system and cause infection in the human host. In this way, it is essential to study of the specific role of SsaA in the pathogenesis of urinary infections caused by *S. saprophyticus*.

Keywords: *S. saprophyticus*, urinary infection, antigen, pathogenesis, proteomic.
Development Agency: INCT-IPH, CNPq, FAPEG, CAPES