

Correlation between biofilm-formation and antibiotic resistance in *Staphylococcus aureus*: an *in vitro* study using phenotypic methods[†]

Márió Gajdács^{1,2*}, Seyyed Askhan Senobar Tahaei¹, Anette Stájer³, Ibrahim Barrak³, Eszter Ostorházi², Dóra Szabó²

¹ Institute of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, 6720 Szeged, Eötvös utca 6., Hungary

² Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University, Nagyvárad tér 4., 1089 Budapest, Hungary

³ Department of Periodontology, Faculty of Dentistry, University of Szeged, Tisza Lajos körút 62–64., 6720 Szeged, Hungary

* Correspondence: mariopharma92@gmail.com; Tel.: +36-62-341-330

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Abstract: *Introduction:* *Staphylococcus aureus* (*S. aureus*) is an important causative agent in human infections. Biofilm-production is an important virulence factor of many pathogens, often leading to chronic infections. There has been significant interest in assessing the possible relationship between the multidrug-resistant (MDR) status and the biofilm-producer phenotype. *Aims:* In the present study, the biofilm-production rates in clinical methicillin-susceptible [MSSA] and resistant [MRSA] *S. aureus* isolates were characterized; in addition, the correlation between their antibiotic resistance and biofilm-forming capacity was also assessed. *Materials and methods:* A total of three hundred (n=300) *S. aureus* isolates (MSSA and MRSA isolates in equal measure) were included in this study. Identification of the isolates was carried out using the VITEK 2 ID/AST automated system and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method and E-tests. Biofilm-production was assessed using phenotypic methods, including the crystal violet (CV) tube-adherence method and the Congo red agar (CRA) plate method. *Results:* There were significant differences among MSSA and MRSA isolates regarding susceptibility-levels to commonly-used antibiotics (erythromycin, clindamycin and ciprofloxacin: p<0.001, gentamicin: p=0.023, sulfamethoxazole/trimethoprim: p=0.027, rifampin: p=0.037). In the CV tube adherence-assay, 37% (n=56) of MSSA and 39% (n=58) of MRSA isolates were positive for biofilm-production, while during the use of CRA plates, 41% (n=61) of MSSA and 44% (n=66) of MRSA were positive. Biofilm-positive isolates were most common from catheter-associated infections. Overall, no associations were found between methicillin-resistance and biofilm-production; however, resistance to erythromycin, clindamycin and rifampin was associated with biofilm-positivity (p=0.004, p<0.001 and p<0.001, respectively). *Conclusions:* The relationship between the MDR phenotype and biofilm-positivity in *S. aureus* has been studied extensively, but the results available in the literature are still inconclusive.

Keywords: *Staphylococcus aureus*; MSSA; MRSA; biofilm; antibiotic resistance; crystal violet; Congo red agar; phenotypic assay

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