Is there a relationship between biofilm forming-capacity and antibiotic resistance in *Staphylococcus* spp.? *In vitro* results

Matthew Gavino Donadu[1,2], Marco Ferrari[2], Vittorio Mazzarello[2], Stefania Zanetti[2], Ivan Kushkevych[3], Simon K.-M.R. Rittmann[4], Anette Stájer[5], Boglárka Ónódi[5], Zoltán Baráth[6], Edit Urbán[7], Mário Gajdács[8,*]

[1]Hospital Pharmacy, Azienda Ospedaliero Universitaria di Sassari, 07100 Sassari, Italy; [2]Department of Biomedical Sciences, University of Sassari, 07100 Sassari, Italy; [3]Department of Experimental Biology, Faculty of Science, Masaryk University, 62500 Brno, Czech Republic; [4]Archaea Physiology & Biotechnology Group, Department of Functional and Evolutionary Ecology, Universität Wien, 1090 Wien, Austria; [5]Department of Periodontology, Faculty of Dentistry, University of Szeged, Tisza Lajos körút 62-64, 6720 Szeged, Hungary; [6]Department of Prosthodontics, Faculty of Dentistry, University of Szeged, Tisza Lajos körút 62–64, 6720 Szeged, Hungary; [7]Department of Medical Microbiology and Immunology, University of Pécs Medical School, Szigeti út 12, 7624 Pécs, Hungary; [8]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Tisza Lajos krt. 63, 6720 Szeged, Hungary

Correspondence to: gajdacs.mario@stoma.szote.u-szeged.hu; 6720 Szeged, Tisza Lajos krt. 63., Hungary

Introduction

Staphylococcus species are considered as important members of the normal skin microbiota, in addition to being common pathogens in human and animal infections. In addition to *S. aureus*, other members of the genus are now widely-recognized as pathogens, especially in immunocompromised individuals. One of the most important virulence factors of staphylococci is the formation of biofilm (slime), which enhances their survival on inanimate surfaces, in addition to providing protection against immune cells and antibiotics *in vivo*. There has been considerable interest in the study of the relationship between biofilm formation and the antibiotic resistant phenotype, however, the results in the available literature are inconsistent. Thus, this study aims to investigate the correlation between biofilm formation and antibiotic resistance in *Staphylococcus* spp. isolates using phenotypic methods.

Materials and methods

A total of n=180 *Staphylococcus* spp. isolates were included in this study (the species-distribution is presented in **Table 1**). *S. epidermidis* ATCC 35984 (positive for biofilm-formation) and ATCC 12224 (non-biofilm-producer) were used as control strains. Sample processing was carried out according to established protocols (**Fig. 1**) Susceptibility-testing (AST) was performed using standardized disk diffusion (Oxoid, Basingstoke, UK) or E-test (Liofilchem, Roseto degli Abruzzi, Italy) methodologies on Mueller–Hinton agar. Biofilm-formation was evaluated using a crystal violet microtiter plate-based method. Absorbance at 570 nm (OD_{570}) was measured in the plates using a spectophotometric plate reader, with OD_{570} values expressed as mean ± SD. Statistical analyses were carried out using SPSS 22.0.

Results and Discussion

Table 1. Species-distribution of *Staphylococcus* spp. included in the study (n=180)

Species name	n	%
S. epidermidis	72	40.00
S. lugdunensis	18	10.00
S. haemolyticus	16	8.89
S. capitis	12	6.67
S. hominis	9	5.00
S. xylosus	9	5.00
S. cohnii	8	4.44
S. saprophyticus	8	4.44
S. intermedius	8	4.44
S. pseudointermedius	8	4.44
S. schleiferi	6	3.33
S. warneri	6	3.33

Based on the results of the AST, resistance rates in these isolates were the following: erythromycin 48.9% (n=88), clindamycin 51.1% (n=92), norfloxacin 27.8% (n=50), gentamicin 26.1% (n=47), trimethoprim-sulfamethoxazole 51.1% (n=92), rifampin 24.4% (n=44), tigecycline 1.1% (n=2), fusidic acid 1.7% (n=3); isolates were all susceptible to linezolid, synercid, ceftaroline and vancomycin. Methicillin-resistance was observed in 47.2% (n=85) isolates.

In the biofilm-formation assay, the OD_{570} values of the controls ATCC 12224 and ATCC 35984 were 0.145±0.018 and 0.608±0.045, respectively. Classification breakpoints for our isolates (based on Stepanovic et al., 2007) were the following: non-biofilm producer: OD≤0.199, weak biofilm producer: 0.398≥OD>0.199, medium biofilm producer: 0.796≥OD>0.398, and strong biofilm producer: OD>0.796. Based on this classification n=13 (7.2%), n=13 (7.2%), n=42 (23.3%) and n=113 (62.3%) staphylococcal isolates were non-biofilm-producing, weak, moderate and strong biofilm producers, respectively. For biofilm-formation, no significant association was noted on the basis of methicillin-resistance (sensitive: 0.881±0.309 vs. resistant: 0.890±0.347; p=0.133). In addition, no significant differences were seen for resistance towards erythromycin, clindamycin, norfloxacin 27.8%, gentamicin 26.1% and trimethoprim-sulfamethoxazole. Rifampin-resistant isolates were more potent biofilm-producers, than their susceptible counterparts (S: 0.802±0.296 vs. R: 1.194±0.221; p=0.024).

The association of the antibiotic-resistant phenotype and biofilm-formation is still inconclusive, due to the heterogeneity of the results in the presently available studies, however, the understanding of these mechanisms in *Staphylococcus* spp. is crucial to appropriately address the therapy and eradication of these pathogens.

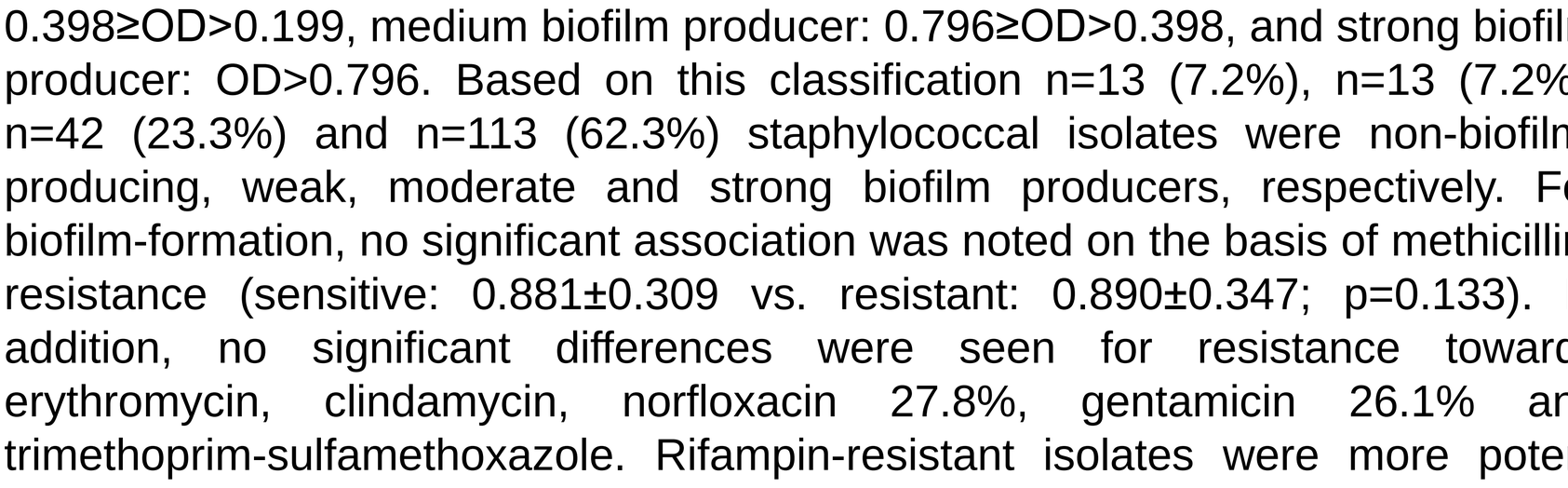

Figure 1. Schematic algorithm for sample processing in the study

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