Relationship between biofilm formation and antibiotic resistance in commensal isolates of *Staphylococcus epidermidis*

F. Oliveira, **L. D. R. Melo** and **N. Cerca**

[1]Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4700-057 Braga, Portugal

Staphylococcus epidermidis is a common bacterial coloniser of the normal human microflora and usually have a benign relationship with the host. For several years, *S. epidermidis* was regarded as a harmless commensal microorganism. However, this bacterium is now recognised as an opportunistic pathogen, representing a leading cause of healthcare-associated infections. The major recognised determinants in the pathogenesis of *S. epidermidis* infections are its ability to form thick and multilayered biofilms along with high resistance to several classes of antibiotics. Biofilms are defined as structured communities of microorganisms embedded in a self-produced matrix of extracellular polymeric. It is well established that bacteria exhibiting a biofilm phenotype are more recalcitrant to antibiotic therapy. Hence, these two pathogenic features stated above appear to be intimately related.

The present study aimed to evaluate the pathogenic potential of commensal *S. epidermidis* isolates through the assessment of their biofilm formation ability and antibiotic susceptibility profiles, as well as to analyse the relationship between biofilm formation and antibiotic resistance. To achieve that, thirty-one *S. epidermidis* isolates from Portuguese healthy volunteers (obtained from September 2012 to April 2013) were tested for biofilm formation ability, carriage of biofilm-associated genes (*icaA*, *aap* and *bhp*) and antibiotic susceptibility to six antibiotics (clindamycin, erythromycin, gentamicin, penicillin, rifampicin and vancomycin).

The study of biofilm formation revealed that 20 (65%) isolates were able to produce biofilm at different levels, while 11 (35%) did not form biofilm. Moreover, 12 (39%) isolates were positive for *icaA*, 18 (58%) for *aap*, and 6 (19%) for *bhp*. With regards to the results of antibiotic susceptibility assays, the highest rates of resistance were detected for penicillin (n = 16, 52%), followed by erythromycin (n = 15, 48%) and gentamicin (n = 13, 42%), while the lowest rate was exhibited for ciprofloxacin (n = 2, 6%). All isolates were susceptible to rifampicin and vancomycin. By comparing the data on biofilm formation and antibiotic susceptibility assays, we found a significant higher frequency of antibiotic resistance in biofilm-formers than in non-biofilm formers (p = 0.02). Additionally, we also found a significant higher proportion of multidrug-resistant isolates among biofilm formers comparing with non-biofilm formers (p = 0.03), demonstrating a clear trend of isolates with biofilm formation ability to be resistant to two or more antibiotics simultaneously. In order to elucidate the nature of the relationship between biofilm formation and antibiotic resistance, we also compared the data on detection of biofilm-associated genes with the data on antibiotic susceptibility assays. In general, we also observed a tendency of isolates that carry the *icaA* and/or *aap* genes to be resistant to two or more antibiotics simultaneously. Interestingly, and when analysing the frequency of resistance for each of the antibiotics tested, the association between the presence of icaA gene and antibiotic resistance was particularly evident for gentamicin, with a p-value much close to the significance level (p = 0.07). Conversely, and with regards to *aap* gene, the same association was not observed.

Overall, our findings provide evidence that commensal *S. epidermidis* strains are well equipped with biofilm formation determinants as well as are resistant to different groups of antibiotics. Moreover, our results support the existence of an association between biofilm formation and antibiotic resistance. Nevertheless, and taking into account other previously published results, our findings lead us to challenge the hypothesis that this association is common throughout all antibiotic classes. We rather hypothesise that this association might be restricted to some classes of antibiotics, especially aminoglycosides, and that it may be primarily associated with *icaADBC* operon and not with the biofilm phenotype *per se*.

Keywords: antibiotic resistance; biofilm formation; biofilm-associated genes, commensal bacteria, *Staphylococcus epidermidis*