EP0042
ePoster Session
Microbial pathogenesis reloaded

RpiRc is a regulator of Staphylococcus aureus biofilm susceptibility to antibiotics and virulence

Adrien Fischer*[1], Myriam Girard[1], Anne-Kathrin Woischnig[2], Gael Panis[3], Nina Khanna[4], Patrice François[1], Jacques Schrenzel[5]

[1]*Geneva University Hospitals, Department of Medical Specialties, Geneva, Switzerland*

[2]*Departement of Biomedicine, Infection Biology, Lab315, Basel, Switzerland*

[3]*Faculty of Medicine / Cmu, Geneva, Switzerland*

[4]*Infektiologie & Spitalhygiene, Universitätsspital Basel, Basel, Switzerland*

[5]*Geneva University Hospitals, Department of Medical Specialties, Geneve 14, Switzerland*

Background: Biofilm is the most common mode of bacterial growth on medical devices and has also been reported on human tissues, e.g. during lung infection by *Pseudomonas aeruginosa*. Bacteria living within a biofilm do not become resistant to antibiotic; but the biofilm appears recalcitrant to antibiotics. Some antibiotics are inefficient against susceptible bacteria growing in biofilm, even in the presence of very high drug concentration, either because molecules are too large to penetrate the biofilm matrix or the antibiotic has higher affinity for matrix components (e.g. vancomycin and extracellular DNA, eDNA), but other mechanisms likely remain to be deciphered. Matrix is composed of proteins, glucids (PIA for *Staphylococcus aureus*) and eDNA. eDNA provides structuration and stability in mature biofilms and is degraded by DNase. In many bacterial biofilms, eDNA originates from cell lysis although eDNA can also be actively secreted or exported by bacterial membrane vesicles.

Material/methods: By screening the Nebraska transposon library, we identified *rpiRc* as a biofilm regulator involved in eDNA regulation. Biofilm formation was monitored using crystal violet staining and eDNA was quantified by qPCR. Biofilm susceptibility to antibiotics was tested in 96 well-plates using a similar protocol as the Calgary biofilm device. Virulence of different strains was assessed in a mouse model of subcutaneous catheter infection. 10^4 CFU of wt and *rpiRc* mutant strains were introduced in the catheter. Catheter and capsule were explanted after 5 days and CFU counts determined in the catheter, in the capsule and in the surrounding tissue. Statistical analysis was performed using *t*-test or 2-way ANOVA for antibiotic susceptibility using GraphPad Prism 6 software.

Results: RpiRc is a transcription factor from the pentose phosphate pathway whose product is a PIA precursor. However, *rpiRc* mutant strain showed neither susceptibility to DispersinB (a commercially available enzyme disrupting PIA biofilms) nor alteration of *ica* transcription (the operon regulating PIA production). While MICs of planktonic cells were not affected in the mutant strain, we observed increased biofilm susceptibility to almost all tested antibiotics, regardless of their mode of action. More importantly, the *rpiRc* mutant showed reduced virulence in the mouse model of catheter infection.

Conclusions: RpiRc is an important regulator involved in eDNA degradation inside the matrix of mature PIA independent biofilms. Our results illustrate that RpiRc contributes to increased antibiotic tolerance in mature bacterial biofilm and also to *S. aureus* virulence during subcutaneous infection.