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

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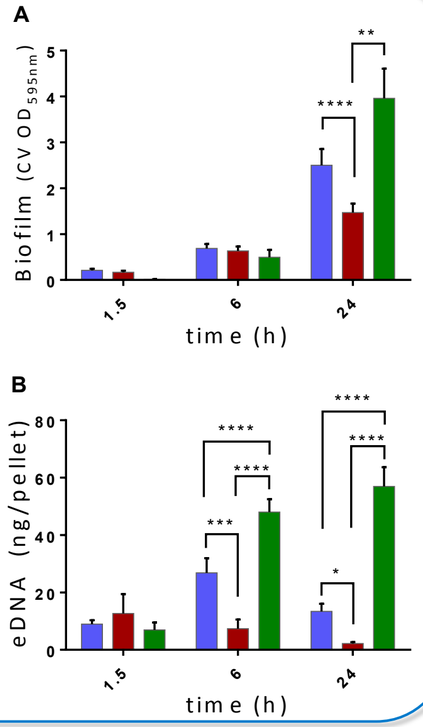
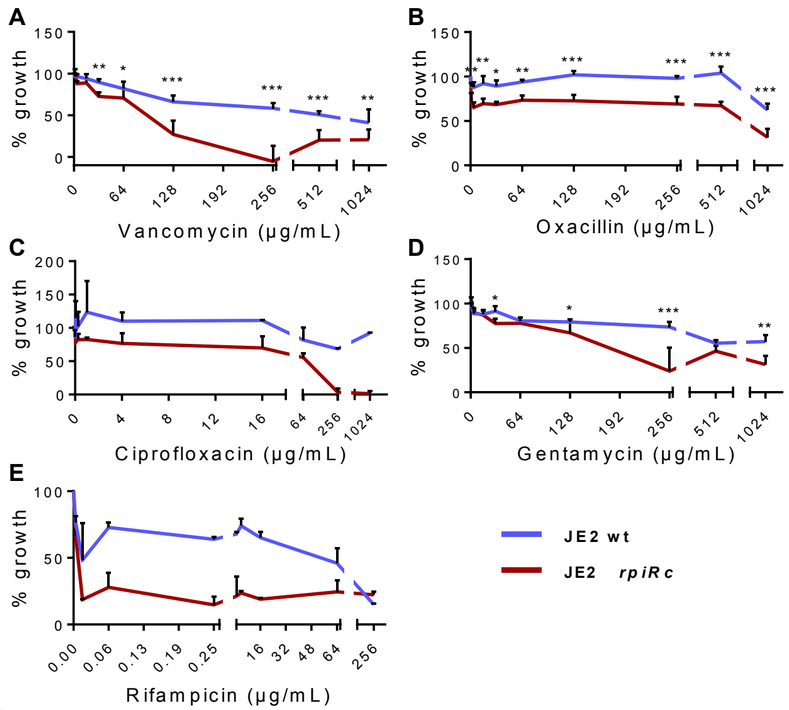
Background: Biofilms are the most common bacterial mode of growth on medical devices and soft tissues during infections, e.g. by *Pseudomonas aeruginosa*. Bacteria living within a biofilm do not become resistant to antibiotic; the biofilm is rather considered as recalcitrant to antibiotics. Some antibiotics are inefficient against susceptible bacteria growing in biofilm phase even in the presence of very high drug concentrations, either because molecules are too large to penetrate the biofilm matrix or the antibiotic has higher affinity for matrix compounds (e.g. vancomycin and eDNA, extracellular DNA) or other mechanisms that 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 membrane vesicles.

A RpiRc is involved in eDNA dependent biofilm formation:

Biofilm formation of USA300 JE2 wt (blue), Δ*rpiRc* (red) and complemented strain (green) was assessed using crystal violet staining at different time points (1.5h, 6h and 24h), B) depicts the corresponding eDNA amounts.

RpiRc deletion caused a 2 fold decreased biofilm formation in mature biofilms related to decreased eDNA amounts. eDNA begins to decrease in the mature strain after 6hours of biofilm growth without affecting the biofilm, suggesting a regulation of nucleases by RpiRc.

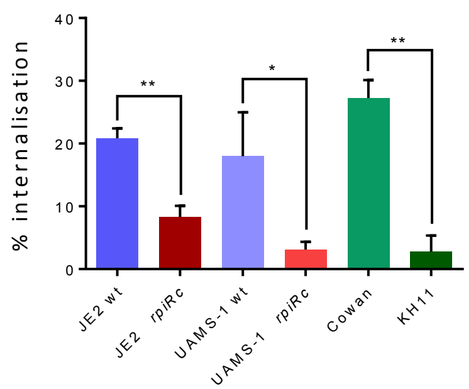




B Biofilm susceptibility to antibiotics:

24h old biofilms were challenged with increasing antibiotic concentrations for another 24h. Antibiotic susceptibility was represented as OD difference between t6h and t0 after antibiotic challenge (reference 100% = control biofilm without antibiotic). C) and E) were performed in duplicates, therefore no statistical analysis was applied.

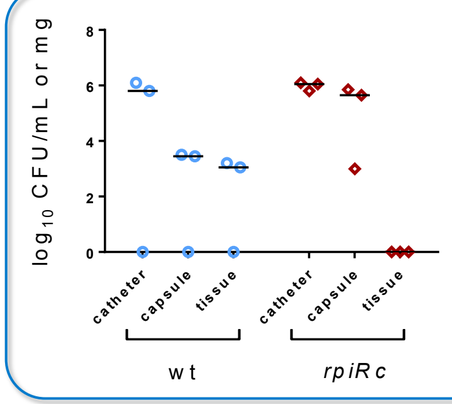
We tested antibiotics targeting different pathways in bacterial metabolism or directed against bacterial components. While E-test showed similar MICs between wt and mutant strains, *rpiRc* deletion always showed decreased biofilm recalcitrance to all these antibiotics.



C Internalization:

Overnight culture of JE2 wt and mutant strains were stained with TRITC, incubated 3 hours in presence of HEK293 cells and internalized bacteria were estimated using a Moxi Flow™ cytometer. Cowan and KH11 were used as positive and negative control respectively.

We observed a decreased expression in *fnbpA* and *B* for the mutant strain after 3 hours of biofilm formation. This decreased expression was linked to a decreased adhesion to fibronectin and a decreased internalization in HEK293 cells.



D *In vivo* virulence:

10^4 CFU of wt and *rpiRc* mutant strains were introduced perioperatively in the catheter enclosed in a capsule. Catheter and capsule were explanted after 5 days and CFU counts were determined in the catheter, in the capsule and in the surrounding tissue.

One mice was not infected with the wt strain, but JE2 Δ*rpiRc* seems to be less virulent compared to wt strain because *rpiRc* mutant was not able to colonize tissues.

Conclusion: RpiRc regulates eDNA production and *fnbpA-B* transcription leading to increased *S. aureus* biofilm formation and antibiotic recalcitrance. In addition, *rpiRc* is an important regulator for *S. aureus* interaction with host cells and *in vivo* virulence.