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Activity of fusidic acid (FUS) alone or in combination with daptomycin (DAP), vancomycin (VAN), or linezolid (LDZ) in an *in vitro* model of *Staphylococcus aureus* Biofilm

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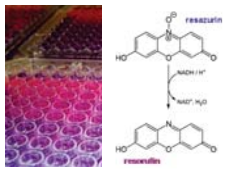
Introduction

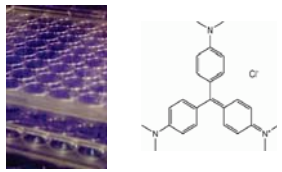
Staphylococcus aureus is an important human pathogen causing chronic infections that are difficult to treat. Biofilm contributes to the persistence of infections, by protecting bacteria from immune system and antimicrobial agents. We showed that many antibiotics are poorly active on biofilms [1], especially when using clinical isolates from persistent infections [2]. Fusidic acid (FUS) may constitute a useful alternative for treatment of *Staphylococcus aureus* infections (in regions with low resistance rates) but shows moderate activity against biofilms [1]. Since FUS is commonly used in combination to avoid resistance selection, we examined which other antistaphylococcal antibiotics could at the same time improve, in vitro, its activity against mature biofilms of clinical isolates.

Materials and Methods

S. aureus reference strain ATCC25923 and 5 clinical strains isolated on medical devices or from chronic tissue infections were used. Biofilms were grown for 24 h in 96-wells plates and then exposed for 48 h to increasing concentrations (0.25-64 mg/L) of FUS (to obtain full concentration-response curves), combined with concentrations corresponding to the human fC_{min} or the fC_{max} of the associated drug. Bacterial viability in biofilms was quantified using the redox indicator resazurin (reduced to fluorescent resorufin by viable bacteria); biofilm biomass was evaluated using crystal violet absorbance.



Redox indicator rezasurin assay:
blue-colored wells: dead bacteria ;
pink-colored wells: viable bacteria
(blue rezasurin reduced in the pink, fluorescent
)compound resorufin



Quantification of matrix
by non-specific staining of biofilm constituents
with crystal violet

References

1-Bauer, J., Siala, W., Tulkens, P. M. & Van Bambeke, F. (2013) *Antimicrob. Agents Chemother.* **57**, 2726-2737.

2-Siala, W., Mingeot-Leclercq, M. P., Tulkens, P. M., Hallin, M., Denis, O. & Van Bambeke, F. (2014) *Antimicrob. Agents Chemother.* **58**, 6385-6397.

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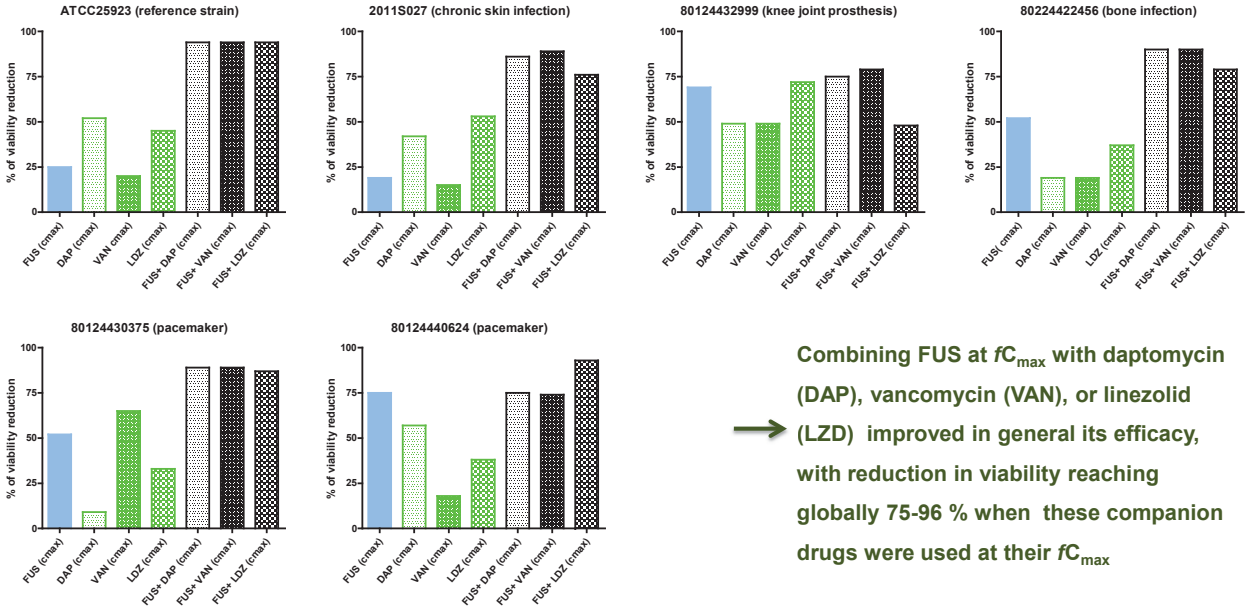
Results



Combining FUS at fC_{max} with daptomycin (DAP), vancomycin (VAN), or linezolid (LZD) improved in general its efficacy, with reduction in viability reaching globally 75-96 % when these companion drugs were used at their fC_{max}

Fig 1 : **% reduction in viability within biofilms for antibiotics alone or for FUS at *f*Cmax combined with other antibiotics at their respective fC_{min}/fC_{max}.** Reduction in viability was compared to untreated control.
FUS fC_{max}: 35 mg/L; DAP fC_{min}/fC_{max}: 0.7/9.4 mg/L; VAN fC_{min}/fC_{max}: 2.5/20 mg/L; LZDfC_{min}/fC_{max}: 9/17 mg/L

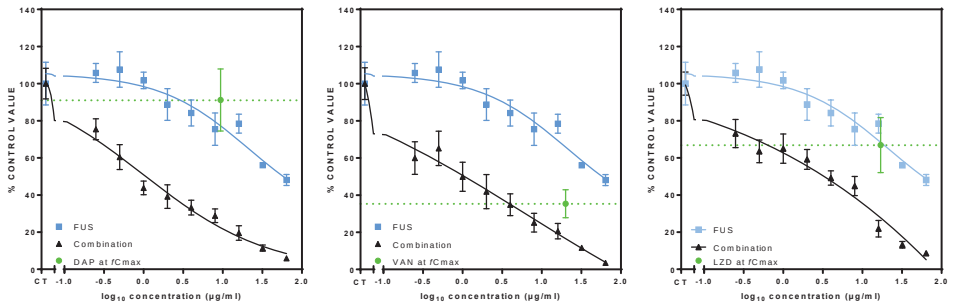


Fig 2: Concentration-response curves for a typical clinical isolate (80124430375)
Viability (% control values) in biofilms exposed to FUS alone over a wide range of conc. (blue); *f*cmax of DAP,VAN,LDZ alone (green) or in combination with FUS at increasing conc. (black)

Conclusions

Combining FUS with DAP, VAN, or LZD appears as a useful strategy to increase its antibacterial activity against biofilms. These data support the evaluation of these combinations in biofilm-related infections in vivo.