Comparison of anti-MRSA antibiotics (vancomycin, linezolid, daptomycin, rifampin) and anti Gram-positive fluoroquinolones (moxifloxacin, delafloxacin) against MSSA and MRSA in models of young and mature biofilms

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INTRODUCTION

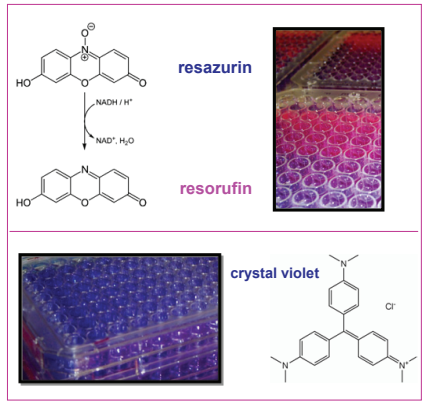
Biofilm-related infections by *S. aureus* represent a major problem in the hospital. Antibiotic activity is however poorly characterized against this particular form of infection.

We have set up a model of young and mature biofilms allowing comparison of the activity of anti-staphylococcal antibiotics on a pharmacodynamic basis.

We selected conventional anti-MRSA agents and compared them to fluoroquinolones, for which high diffusibility and strong bactericidal character may offer advantages in this respect. Moxifloxacin was compared to the investigational fluoroquinolone delafloxacin, which is highly potent against Gram-positive organisms (1).

METHODS

- **Bacterial strains**: MSSA ATCC25923 and MRSA ATCC 33591
- **Biofilm culture:** bacteria were cultivated at 30°C in TSB complemented with 1% glucose and 2% NaCl for 6 h (young biofilm) or 24 h (mature biofilm) in 96-wells plates.
- **Antibiotic exposure**: biofilm culture medium was removed and replaced by the same medium (control) or medium containing antibiotics at increasing concentrations (0.5 to 256-fold their MIC in broth). Biofilms were then re-incubated for 24h (6h biofilms) or 48h (24h biofilms) at 30°C .
- **Assessment of bacterial viability within biofilms** : fluorescence signal (λexc 560 nm; λem: 590 nm) associated to the reduction of resazurin (blue, non-fluorescent) into resorufin (pink) by viable bacteria (2).
- **Determination of total biofilm mass:** crystal violet staining and measure of absorbance at 570 nm (3).

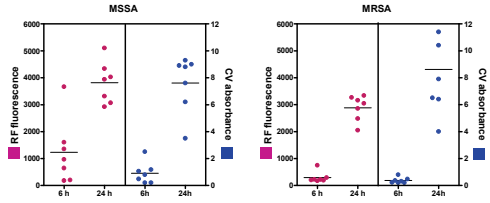


RESULTS

Antibiotic intrinsic activity

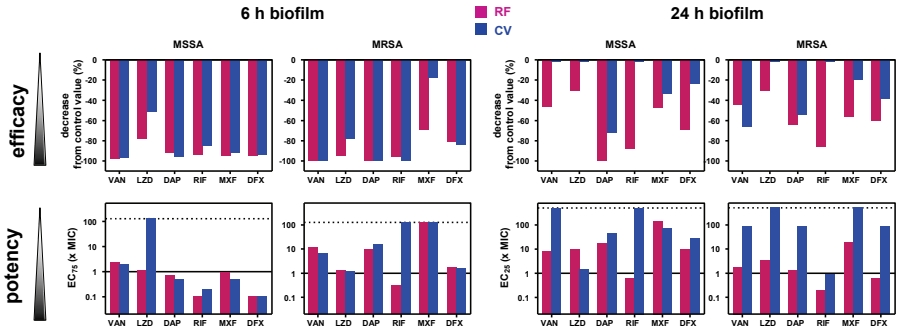
antibiotic	MIC (µg/ml) MSSA ATCC 25923	MIC (µg/ml) MRSA ATCC 33591
Vancomycin	1	1
Daptomycin	1	1
Rifampin	0.032	0.032
Linezolid	1	1
Moxifloxacin	0.032	0.032
Delafloxacin	0.004	0.004

Biofilm growth over time



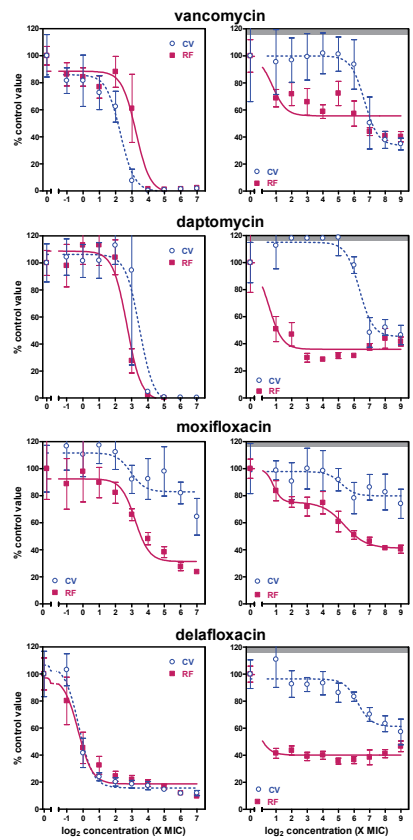
RF fluorescence signal (left panel) and CV absorbance (right panel) during biofilm growth with MSSA or MRSA. Each data point is the mean of 8 wells for an independent experiment.

Pharmacodynamic parameters



Pharmacological descriptors of concentration-effect relationships.
Upper panel: maximal efficacy (E_{max}): maximal decrease in signal extrapolated for an infinitely large concentration.
Lower panel: relative potency (EC): drug concentration needed to reach a 75 % decrease in the signal (6h-biofilm) or a 25 % of the signal (24h-biofilm), as interpolated from the sigmoidal regression of the concentration-effect curve. Dotted line: highest concentration tested; EC values are > to this value

Concentration – effect curves for MRSA



Concentration-response activity of selected antibiotics against 6h biofilms of MRSA. 6-h and 24-h biofilms were incubated with increasing concentrations of antibiotics for 24 h and 48 h respectively. The ordinate shows the change in resorufin fluorescence (RF; pink) or in crystal violet absorbance (CV; blue) in percentage of the control value (no antibiotic present). Values that are above controls have been set to a value of 120 % (highlighted by the grey zone on the graphs). N=8.

- Biofilms grew over time, with a 4- fold and 14-fold increase in viable bacteria, and a 14-fold and 26-fold increase in biofilm mass between 6 and 24 h for MSSA and MRSA, respectively.
- Antibiotic activity was concentration-dependent against both viable bacteria and biofilm mass, and was globally lower against 24 h-biofilms than against 6 h-biofilms.
- For 6 h-biofilms, maximal efficacy (E_{max}) towards viable bacteria and biofilm mass reached > 80 %, except for linezolid against MSSA and moxifloxacin against MRSA. With respect to potency, a 75 % reduction in the signal (EC_{75}) was obtained at concentrations close to the MIC or even lower for rifampin and delafloxacin, except for linezolid which did not reach this effect against the matrix.
- For 24 h–biofilms, E_{max} values were reduced, with only daptomycin, rifampin, and delafloxacin reaching a 50 % reduction in viability and only daptomycin (towards MSSA) and vancomycin (against MRSA) reaching this effect towards the matrix. With respect to potency, a 25 % reduction in viability (EC_{25}) was obtained for concentrations of 1-10 X MIC against MSSA and 0.1-1 X MIC against MRSA, for all drugs except moxifloxacin, which required higher concentrations. High concentrations were needed to act upon the matrix.

CONCLUSIONS

- Activity of antibiotics is markedly defeated against mature biofilms and is strain-dependent, probably reflecting differences in the nature and/or the physicochemical properties of the biofilm produced.
- Daptomycin, rifampin, and delafloxacin were the most effective drugs tested in this model. The high intrinsic activity of these antibiotics, especially that of delafloxacin (lowest MICs), may offer an additional advantage in combating biofilms with respect to the concentrations that could be achieved in vivo.

REFERENCES

(1) Lemaire et al, 2011, AAC 55:649-58.
(2) Toté et al. 2008, Lett Appl Microbiol. 46:249-54
(3) Christensen et al. 1985, J Clin Microbiol.22:996-1006

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INNOVIRIS EMPOWERING RESEARCH

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