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Activity of anti-staphylococcal antibiotics in an in-vitro model of biofilm using clinical isolates of *S. aureus*

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ABSTRACT

BACKGROUND. Biofilms are associated with persistent infections refractory to most antibiotics. We lack however of data comparing them on a pharmacodynamic basis. This study used 7 clinical isolates of *S. aureus* from persistent infections to evaluate the activity on bacterial survival and matrix of anti-staphylococcal agents that are quickly (DAP, MXF) or slowly (VAN) bactericidal, or bacteriostatic (LZD).
METHODS. Biofilms were grown in 96-well plates for 6 and 24h. Total biofilm mass (matrix + cells) and bacterial viability were measured using crystal violet staining and the redox indicator resazurin (reduced to to resorufin [Lett. Appl. Microbiol. 2008, 49:249-54]).
RESULTS. See Table. Against 6h biofilms, antibiotics reduced viability of 50 % at low multiples of their MIC, except MXF the activity of which was highly variable among strains. Higher conc. were needed to act upon the matrix, with huge variations between strains. Against 24h biofilms, only DAP achieved a 50 % reduction in viability (not shown). Activity was much more variable among strains and required higher conc. to reach even a 20% reduction in viability. Marginal effects were observed on the matrix (not shown).

antibiotic	MIC (mg/L)[a]		AB conc. (mg/L) needed to reach		
			50 % reduction in signal [a] (6h-old biofilm[b])		20 % reduction in signal [a] (24h-old biofilm[c])
	Median	Range	Resorufin fluorescence	Crystal violet absorbance	Resorufin fluorescence
Moxifloxacin (MXF)	1	0.03-1	86 ± 188	84 ± 189	174 ± 235
Daptomycin (DAP)	1	1-1	3.1 ± 2.7	7 ± 4	49 ± 45
Vancomycin (VAN)	1	1-4	8.6 ± 5.4	27 ± 18	30 ± 195
Linezolid (LZD)	1	1-2	2.5 ± 1.5	93 ± 121	2.0 ± 1.0

[a] 3 MSSA and 4 MRSA
[b] calculated based on data expressed in percentage of the value recorded in the absence of antibiotic (control)
[b] biofilm grown in BHI added by 1 % glucose and 2 % NaCl for 6 h and then exposed during 24 h to antibiotics at concentrations ranging between 1 and 256 X their MIC
[c] biofilm grown in the same medium for 24 h and then exposed during 48 h to antibiotics at concentrations ranging between 1 and 512 X their MIC

CONCLUSIONS. Only DAP and VAN display activity towards bacteria viability and matrix at clinically-achievable conc. Variability among strains suggests that biofilm properties may influence antibiotic activity.

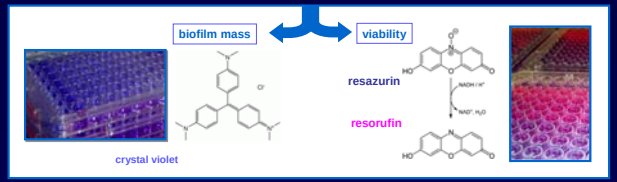
INTRODUCTION & OBJECTIVES

- Biofilm-related infections by *S. aureus* represent a major problem in the hospital. They play a preponderant role in persistent infections on external devices or in deep tissues [1]. Antibiotic activity is however poorly characterized against this particular form of infection.
- We have set up a model of young and mature biofilm allowing comparison of the activity of anti-staphylococcal antibiotics on a pharmacodynamic basis [2].
- The aim of the present study was to compare the activity of anti-staphylococcal agents against a series of clinical isolates collected from patients suffering from persistent infections and growing in biofilms.

STRAINS & MICs

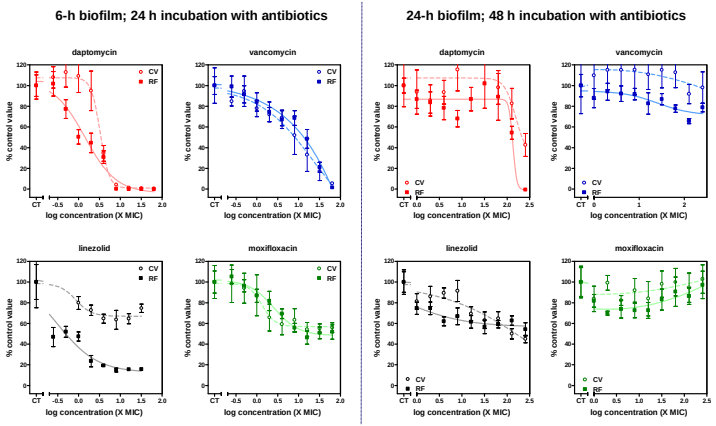
strain	MSSA MRSA	MICs (mg/L)			
		VAN	DAP	LZD	MXF
2003-1083	MSSA	1	1	2	0.032
2011-S027	MSSA	4	1	1	0.032
2009-S025	MSSA	2	1	2	0.032
2005-104	MRSA	4	1	2	1
2005-179	MRSA	2	1	1	1
2009-S028	MRSA	4	1	1	1
2003-651	MRSA	4	1	1	1

BIOFILM QUANTIFICATION



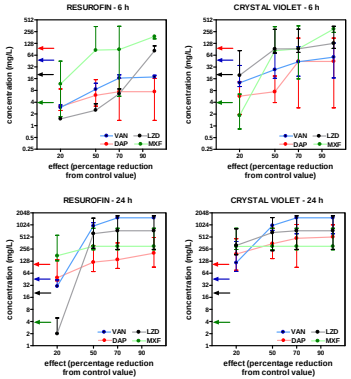
RESULTS

Pharmacodynamic evaluation of antibiotic activity against a biofilm of strain 2003-1083 exposed to increasing concentrations of drugs (0.25-256 X MIC)



Comparison of antibiotic effects on biofilm mass (crystal violet staining, CV) or bacterial viability within the biofilm (resorufin fluorescence; RF) after 24 (left) or 48 h (right) incubation with increasing concentrations.
Data are mean ± SD of 8 data points

Mean antibiotic concentrations needed to reach a predefined effect (reduction of 20, 50, 70, and 90 % of control) of biofilm mass or bacterial viability (all strains) for 6 and 24 h biofilms



Means (with 95 % confidence interval) of pooled data for the 7 investigated strains. The arrow on the Y axis points to the human Cmax of each drug observed in the serum of patients receiving conventional dosages.

METHODS

- **Bacterial strains:** clinical isolates collected from patients suffering from persistent infections (collection of the national *S. aureus* reference center).
- **Biofilm culture:** bacteria were cultivated at 30°C in TSB supplemented by 2 % glucose (strain 2005-179) or in BHI supplemented by 1% glucose and 2% NaCl (other strains) for 6 h (young biofilm) or 24 h (mature biofilm) in 96-wells plates. Medium was selected to optimize biofilm formation.
- **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 (λ_{exc} 560 nm; λ_{em} 590 nm) associated to the reduction of resazurin (blue, non-fluorescent) into resorufin (pink) by viable bacteria [3].
- **Determination of total biofilm mass:** crystal violet staining and measure of absorbance at 570 nm [4].

CONCLUSION

- Antibiotic activity against both the matrix and the viability within the biofilm are concentrations-dependent.
- Antibiotic efficacy and potency are markedly decreased over aging, especially against the matrix.
- MIC are not predictive of activity against biofilms.
- Among the antibiotics tested, only daptomycin, and to a lesser extent vancomycin, exert activity with respect to both matrix and bacterial viability at clinically-achievable concentrations.
- Activity is highly variable among strains, suggesting that biofilm properties affect antibiotic activity.

REFERENCES

1. Archer *et al*. 2011, Virulence; 2:445-59
2. Bauer *et al.* 2011, 51th ICAAC Poster C1-618
3. Toté *et al*. 2008, Lett Appl Microbiol. 46:249-54
4. Christensen *et al.* 1985, J Clin Microbiol.22:996-1006

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INNOVIRIS

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