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Activity of Moxifloxacin against *Staphylococcus aureus* in Models of Persistent Infections (Intracellular Survival, Biofilms)

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Abstract (edited)

Background:
Staphylococcus aureus (SA) causes difficult-to-treat infections, partly due to its capacity to adopt modes of life recalcitrant to antibiotics after phagocytosis and in biofilms. Our aim was to compare the activity of a bactericidal antibiotic (moxifloxacin; MXF) against SA intracellularly and in biofilms using a reference strain and 7 isolates collected from patients with persistent infections.

Methods:
Strain and isolates: ATCC 25923 and 7 SA isolates collected from patients hospitalized at the Bach Mai Hospital, Ha Noi, Viet Nam who were still infected after 5 days of treatment with an active antibiotic or presented a recurrence from a previous infection.
Intracellular infections: infected THP-1 cells exposed to MXF (0.003 -100 X MIC) for 24 h. Intracellular residual inoculum determined by CFU counting (normalized by mg cell protein). E_{max} determined from the Hill equation of the concentration-response curve as the decrease in intracellular inoculum extrapolated for an infinitely large antibiotic concentration.
Biofilms: grown during 24 h in 96-well plates and exposed during 24 h to MXF (0.001-1000 mg/L). Residual viability quantified by CFU counting with E_{1000} being the actual reduction in bacterial counts observed at a concentration of 1000 mg/L (highest).

Results:
A bactericidal effect (-3 log_{10} CFU) was never reached against any strain and in both models although MXF is a highly bactericidal antibiotic in broth. Furthermore, MXF efficacy was lower against the two resistant isolates included in this study.

Conclusion:
MXF efficacy is markedly reduced and to a similar extent in models of persistent infections (intracellularly and in biofilms). As MXF efficacy is more reduced against resistant isolates in both models, these modes of life may further contribute to therapeutic failures.

References

[1] Carryn et al., Infect Dis Clin North Am (2003) 17: 615-34
[2] Archer et al., Virulence (2011) 2:445–59.
[3] Barcia-Macay et al., AAC (2006) 50:841-51
[4] Bauer et al., AAC (2013) 50:2726-37

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Background and Aims

Staphylococcus aureus (SA) is an important human pathogen causing chronic and recurrent infections that are difficult to treat. Biofilm formation and intracellular survival contribute to the persistence of infections, by protecting bacteria from the immune system and most antimicrobial agents [1, 2].

In this context, our laboratory developed a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments [3, 4].

Our aim was to compare the activity of a bactericidal antibiotic (moxifloxacin; MXF) against SA intracellularly and in biofilms, using a reference strain and 7 clinical isolates collected from patients with persistent or recurrent infections.

Methods

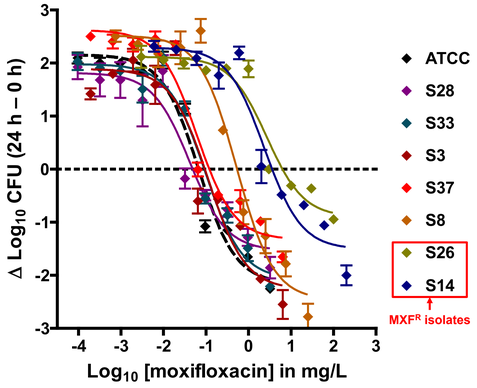
1. Strains: ATCC 25923 and 7 isolates *S. aureus* collected from patients hospitalized at the Bach Mai Hospital, Ha Noi, Viet Nam and still infected after 5 days of treatment with an active antibiotic or presenting a recurrence from a previous infection.
2. MIC determinations: microdilution (CLSI recommendations) with susceptibility assessed according to the EUCAST interpretive criteria (http://www.eucast.org).
3. PCR detection of *mecA* and *mecC* for MRSA.
4. Typing phylogeny: *spa* typing (Staphylococcus protein A gene typing) by PCR and sequencing.
5. Antibiotic activity against intracellular bacteria: Phagocytosis of bacteria by human THP-1 monocytes. Elimination of non-internalized bacteria by exposure to gentamicin. Incubation with a wide range of extracellular concentrations (0.003-100 x MIC) of MXF for 24 h to obtain full concentration-dependent responses. Intracellular activity evaluated as the change in CFU) (log_{10} units) from the initial inoculum at 24h. Maximal efficacy (E_{max}) is the change in CFU as extrapolated for an infinitely large antibiotic concentration (calculated using a Hill equation fitted to concentration-response data [3]).
6. Antibiotic activity against biofilms: Bacterial growth in TGN (Tryptic soy broth + 1% Glucose + 2% NaCl) in 96-well plates for 24 h and thereafter exposed during 24 h to MXF (0.001-1000 mg/L). Residual viability quantified by CFU counting. As the plateau value of the sigmoidal regression was unreached in most cases, we used and report E_{1000} at 24 h (actual difference in CFU [log_{10} units] between untreated controls and biofilms exposed to the highest concentration of MXF tested [1000 mg/L])

Main message and Key Conclusion

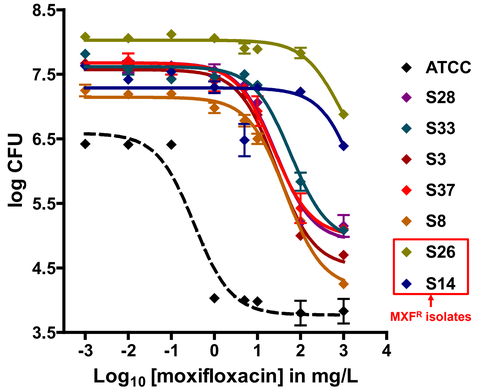
- MXF efficacy against SA is markedly reduced intracellularly and in biofilms (a bactericidal effect [-3 log CFU] was never reached against any strain in both models).
- E_{max} (intracellular infections) and E_{1000} (biofilms) were more reduced against resistant isolates in both models, suggesting that these modes of life could contribute to therapeutic failures and persistence of the infection beyond what could be due to resistance alone.

Results

1. Activity of moxifloxacin against intracellular *S. aureus*



2. Activity of moxifloxacin against *S. aureus in biofilms*



Data analysis (Figs in 1 and 2 and Table in 3)

1. Intracellular activity
- E_{max} reaches a maximum of -2.5 ($log\ CFU_{10}$) (*vs.* -3.9 for extracellular bacteria [3]), failing to show bactericidal effect against any strain.
- MXF^R isolates show both a loss of potency (shift of the curves to the right) and a considerably lower (less negative) E_{max} compared to MXF^S isolates.

2. Biofilms
- All clinical isolates are less susceptible than the reference strain ATCC 25923 with no reduction in CFUs for or concentrations lower than 10 mg/L.
- As for intracellular bacteria, MXF^R strains show both a loss of potency (right shift) and lower (less negative) E_{1000} compared to MXF^S strains

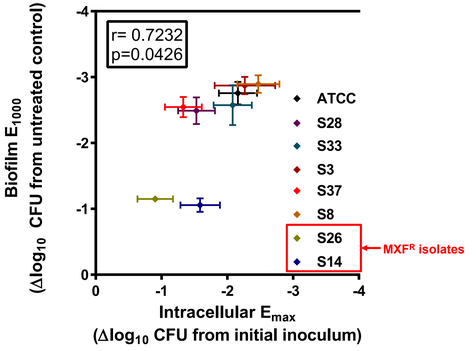
The figure in 4 shows the correlation between the activity in biofilms (E_{1000}) and the activity against intracellular forms (E_{max}) for ATCC 25923 and all isolates. This suggests that both are reduced together in MXF^R isolates.

3. Resistance, *spa* type and efficacy of moxifloxacin in models of intracellular infections and biofilms

Strains		Resistance phenotype [a]	*spa* type	MIC_{MXF} (mg/L)	E_{max} Intracellular[b]	E_{1000} Biofilm[c]
ATCC 25923		-	-	0.032	-2.16 ± 0.29	-2.75 ± 0.17
Clinical isolates	S28	MRSA,KLM^R	t437	0.032	-1.53 ± 0.26	-2.52 ± 0.20
	S33	MSSA, P^R	t304	0.032	-2.08 ± 0.32	-2.57 ± 0.30
	S3	MSSA, KLM^R	t021	0.064	-2.27 ± 0.46	-2.87 ± 0.13
	S37	MRSA,MLT^R	t1250	0.064	-1.33 ± 0.28	-2.55 ± 0.15
	S8	MSSA, KLM^R	t657	0.25	-2.47 ± 0.32	-2.89 ± 0.13
	S26	MSSA, FLM^R	t189	1	-0.90 ± 0.27	-1.15 ± 0.10
	S14	MSSA, F^R	t437	2	-1.51 ± 0.33	-1.06 ± 0.01

[a] F: fluoroquinolone; K: ketolide; L: lincosamide; M: macrolide; P: Penicillin; T: tetracycline
[b] Maximal CFU decrease (log_{10} units) at 24 h as extrapolated from the Hill equation for an infinitely large concentration
[c] Actual CFU decrease (log_{10} units) at 24 h calculated as the difference between the control value (without antibiotic) and the value measured in biofilms exposed to the highest concentration tested (1000 mg/L).

4. Correlation between E_{1000} (biofilms) and E_{max} (intracellular)



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