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Activity of moxifloxacin in a model of intracellular infection by *Staphylococcus aureus* clinical isolates from patients suffering from persistent infections in Vietnam.

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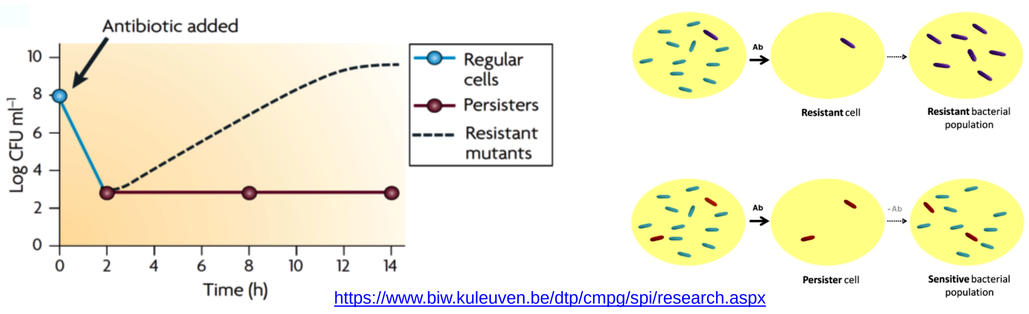
Background

Clinical antibiotic failure may result from bacterial resistance but also from persistent forms of infection.

Persisters are antibiotic-exposed bacteria that have become refractory to antibiotic killing. In contrast to resistance, persistence is neither genetically-inherited nor associated to genomic mutations. It is reversible upon antibiotic removal and is associated to the adoption of a transient dormant lifestyle [1,2].



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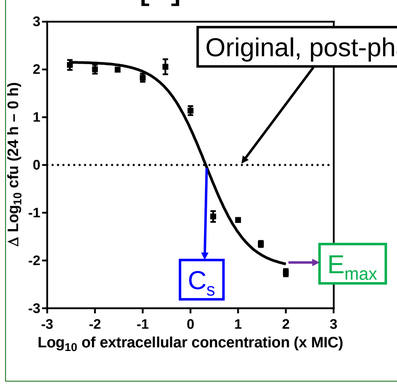
Intracellular survival may constitute one of these persistent forms of infections in which bacteria are protected from host immune defense and, partly also, from antibiotic action.

In this context, our laboratory has developed an *in vitro* pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in this environment [3].

Here, we study the activity of moxifloxacin in a model of intracellular infection by *Staphylococcus aureus* (localized in phagolysosomes), comparing a reference strain to clinical isolates collected from patients hospitalized in the Bach Mai hospital (Hanoi, Vietnam) and presenting infections persisting after at least 5 days of treatment with an active antibiotic.

Methods

- **Isolates:** Clinical *S. aureus* isolates collected at the Bach Mai Hospital (Hanoi) from patients still infected after 5 days treatment with an active antibiotic or with recurrence of a previous infection and for whom data on antibiotic treatment were available. Reference strain: ATCC 25923.
- **Typing:** *spa* typing (*Staphylococcus* protein A gene typing); PCR detection of *mecA* and *mecC* for MRSA.
- **MIC determinations:** microdilution (CLSI recommendations) with susceptibility assessed according to EUCAST criteria.
- **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 antibiotics for 24 h to obtain full concentration-dependent responses. Intracellular activity evaluated as the change in CFU from initial inoculum at 24h. Static concentrations (C_s) and maximal efficacy (E_{max}): calculated from Hill equation fitted to concentration-response data [3].



C_s (relative potency): extracellular concentration resulting in no apparent bacterial growth from CFU at 0h (post-phagocytosis inoculum)

E_{max} (maximal efficacy): CFU change (in $\log_{10}$ units) at 24h from the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration.

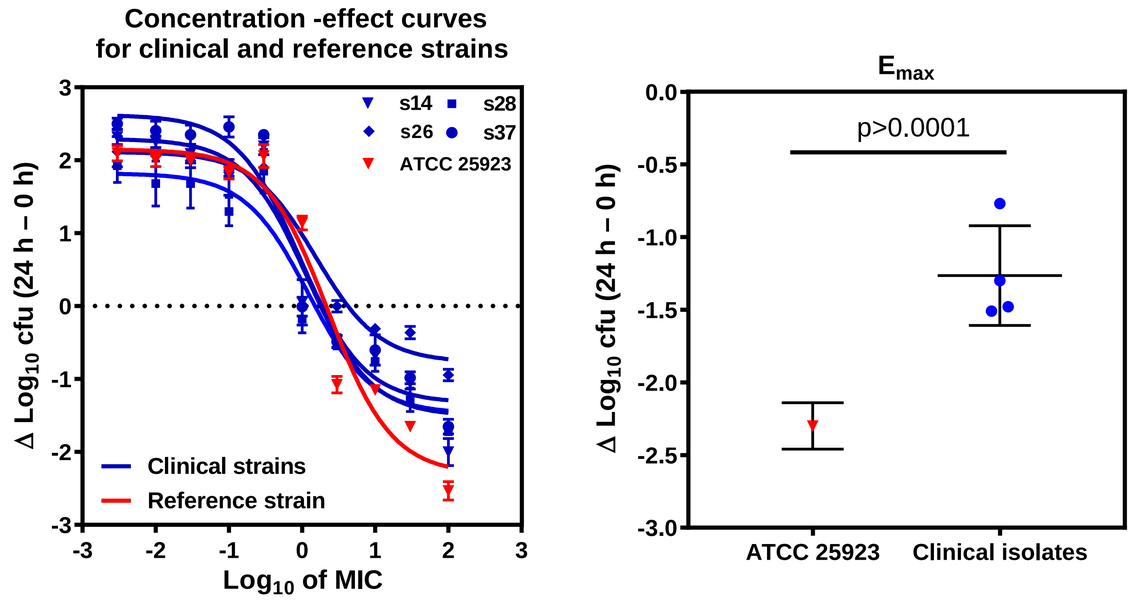
Results

The Table shows the phenotype of resistance and *spa* type as well as the moxifloxacin MIC and the pharmacodynamic parameters of intracellular activity for the reference strain ATCC 25923 and 4 clinical isolates from persistent infections.

Strains		Resistance phenotype [a]	*Spa* type	Moxifloxacin MIC (mg/L)	C_s [b]	E_{max} [c]
ATCC 25923		-	-	0.032	2.14 ± 0.20	-2.30 ± 0.16
Clinical isolates	S28	MRSA, MKL^R	t437	0.032	1.50 ± 0.32	-1.48 ± 0.26 *
	S37	MRSA, MLT^R	t1250	0.064	1.87 ± 0.27	-1.33 ± 0.28 *
	S26	MSSA, FML^R	t189	1	4.23 ± 0.43 *	-0.77 ± 0.15 *
	S14	MSSA, F^R	t437	2	1.80 ± 0.36	-1.51 ± 0.33 *

[a] M: macrolide; K: ketolide; L: lincosamide; T: tetracycline; F: fluoroquinolone;
[b] extracellular antibiotic concentration (in x MIC) resulting in no apparent intracellular growth;
[c] maximal CFU decrease ($\log_{10}$ units) at 24 h as extrapolated from the Hill equation;
* $p<0.05$ vs ATCC25923 (one-way ANOVA, Dunnett post-hoc test)

The figures show concentration-effects relationships for moxifloxacin against intracellular bacteria (left) and the corresponding E_{max} values calculated from the Hill equation (right).



- C_s was always close (1.5 to 4.2 x) to moxifloxacin MIC for all isolates, whatever their susceptibility in broth.
- E_{max} was significantly lower (less negative) against clinical isolates than against the reference strain, whatever their intrinsic susceptibility to moxifloxacin (MIC), their resistance phenotype or their *spa* type.

Conclusion

Intracellular forms of the clinical isolates obtained from patients with persistent infections are significantly less eradicated compared to the reference strain, suggesting a state of lower responsiveness to moxifloxacin in the phagolysosomal environment. A potential link with therapeutic failure i*n vivo* remains to be established.

References

1. Bigger *et al*, *Lancet* (1944) **244**: 497-500
2. Cohen *et al*, *Cell Host Microbe* (2013) **13**: 632-642.
3. Barcia-Macay *et al. Antimicrob Agents Chemother* (2006) **50**:841-851

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