Insufficient Activity of most Common Antistaphylococcal Antibiotics towards Intraphagocytic Forms of S. aureus from a Patient with Persistent Bacteriemia : a Cause for Treatment Failure ?

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Abstract

Objectives: Therapy failures in *S. aureus* endocarditis have often been ascribed to emergence of resistance combined with survival of intracellular forms. The intracellular activity of most antistaphylococcal antibiotics towards fully susceptible *S. aureus* is markedly lower than their extracellular activity (AAC 2003, 47:2283-92; 2006, 50:841-51). We have measured the intraphagocytic activity of common antistaphylococcal antibiotics towards *S. aureus* isolates obtained from a patient with endocarditis and therapeutic failure upon therapy (ICAAC 2006, E-727 and C1-685).

Methods: MSSA ATCC 25923 and two isolates from the patient (HMC 546 [aortic valve; vanco S]; HMC 549 [blood; VISA]) were tested for susceptibility in broth (MIC [micro-dilution]) and in human THP-1 macrophages (24 h change in post-phagocytosis inoculum [delta log CFU] at an extracell. concentr. corresponding to human Cmax (AAC 2006; 50:841-851).

Results:

Drugs	Cmax (mg/L)	Strains: ATCC 25923 MIC (mg/L)	ATCC 25923 Δ Log cfu (24h)	HMC 546 AORTIC VALVE MIC (mg/L)	HMC 546 AORTIC VALVE Δ Log cfu (24h)	HMC 549 BLOOD MIC (mg/L)	HMC 549 BLOOD Δ Log cfu (24h
Rifampicin (RIF)	4	0.03	-1.4 ± 0.1	> 4	+2.2 ± 0.1	> 4	+2.5 ± 0.1
Oxacillin (OXA)	8	0.25	-0.7 ± 0.1	16	+2.0 ± 0.1	32	+1.9 ± 0.1
Ciprofloxacin (CIP)	4	0.125	-1.3 ± 0.1	32	+2.0 ± 0.0	64	+2.5 ± 0.0
Vancomycin (VAN)	50	1	-0.6 ± 0.1	2	-0.4 ± 0.1	4-8	+0.4 ± 0.1
Fusidic acid (FUS)	4	0.5	-0.7 ± 0.1	0.5	-0.8 ± 0.1	0.5	-0.7 ± 0.0
Gentamicin (GEN)	18	0.25	-0.8 ± 0.1	1	-0.9 ± 0.1	1-2	-0.7 ± 0.1
Linezolid (LNZ)	20	0.5-1	-0.7 ± 0.1	1-2	-1.0 ± 0.1	1	-0.5 ± 0.1
Moxifloxacin (MXF)	4	0.03	-2.1 ± 0.1	2	-1.0 ± 0.1	2	-0.6 ± 0.0
Quinupristin-dalfopristin (Q-D)	10	0.5	-1.8 ± 0.1	0.5	-1.8 ± 0.1	0.5	-1.6 ± 0.1

Clinical isolates showed marked loss of intracellular susceptibility towards RIF, OXA, CIP, VAN, and MXF. GEN, LNZ, FUS, and Q-D were unaffected.

Conclusions : Changes in MIC are only partly predictive of loss of intracellular activity. Direct measurements of intracellular activities may be necessary to obtain correct antibiotic ranking, rationalize therapeutic failures, and suggest alternatives.

Methods

Strains : We used a fully susceptible *S. aureus* (ATCC 25923) and two clinical isolates recovered from a patient with persistent bacteriemia and endocarditis (HMC 546, aortic valve; HMC 549, blood)[1]

MICs : Susceptibility testing was performed by micro-dilution method in Mueller-Hinton broth.

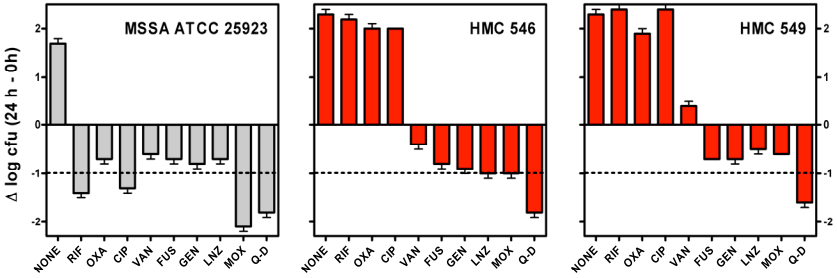
Determination of the intracellular antibiotic activity[2-3] : Cells were infected with preopsonized bacteria (1 h; 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/Liter) to eliminate non-adherent and non-internalized bacteria. Infected cells were exposed for 24 h to antibiotics at a concentration corresponding to the plasma C_{max} reached in patients treated with conventional dosages (control cells were maintained in the continuous presence of gentamicin [0.5 x MIC] to prevent the extracellular growth of bacteria released from cells).

Results

1 Susceptibility testing

Drugs	Abbreviation	MICs (mg/L) ATCC25923	HMC 546	HMC 549
Rifampicin	RIF	0.03	> 4	> 4
Oxacillin	OXA	0.25	16	32
Ciprofloxacin	CIP	0.125	32	64
Vancomycin	VAN	1	2	4-8
Gentamicin	GEN	0.5	0.5	0.5
Fusidic acid	FUS	0.25	1	1-2
Linezolid	LNZ	0.5-1	1-2	1
Moxifloxacin	MOX	0.03	2	2
Quinupristin-dalfopristin	Q-D	0.5	0.5	0.5

2 Comparative intracellular activity of antibiotics



The ordinate shows the change in cfu (log_{10}) per mg of cell protein observed after 24 hours of incubation, in comparison with the original inocula (mean ± SEM [n=3]), in cells incubated with a drug equivalent to their human C_{max} (in mg/L: RIF, 4; OXA, 8; CIP, 4; VAN, 50; FUS, 4; GEN, 18; LNZ, 20; MOX, 4; Q-D, 10)

Clinical isolates showed a marked loss of intracellular susceptibility to RIF, OXA, CIP, VAN and MXF as compared to the ATCC strain, while FUS, GEN, LNZ and Q-D were unaffected. Q-D proved most active towards both clinical isolates

Acknowledgments

S. L. is *Boursière* of the Belgian *Fonds pour la Recherche dans l'Industrie et l'Agriculture* (*FRIA*), and F.V.B. *Maître de Recherches* of the Belgian *Fonds de la Recherche Scientifique* (FRS-FNRS). This work was supported by the Belgian *Fonds de la Recherche Scientifique Médicale* (grants no. 3.4.639.04 et 597.06).

Background

S. aureus **is an aggressive pathogen creating significant public health threat. In the setting of endocarditis, therapy failures[4] have been well chronicled and ascribed to emergence of antibiotics resistance combined with survival of intracellular forms.[5]**

Routine evaluation of antibiotic activity is performed against extracellular bacteria only. Yet, the intracellular activity of most antistaphylococcal antibiotics is markedly lower compared to what is observed extracellularly.[2] Models of intracellular infection may prove critical for a correct appraisal of antibiotic efficacy in situations such as endocarditis.

In this context, we have measured the intraphagocytic activity of several common anti - ***S. aureus*** **agents towards two isolates recovered from a patient with endocarditis and therapy failure upon therapy[1].**

Conclusions

- **Our results suggest that most antibiotics commonly recommended for the management of** ***S. aureus*** **infections are poorly active intracellularly, with only MXF and Q-D showing significant reduction in bacterial counts for a fully sensitive strain.**
- **Against our clinical isolates, only Q-D displayed marked intracellular activity. Direct measurements of intracellular activity of antibiotics may, therefore, be required, in addition of MIC evaluations, for optimizing therapy of staphylococcal infections with multiresistant isolates.**

References

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