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Intracellular Activity of Antibiotics against Methicillin-Sensitive, Hospital-Acquired Methicillin-Resistant, and Vancomycin-Intermediate *Staphylococcus aureus* (MSSA, HA-MRSA, and VISA) in Human Macrophages (THP-1)

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ABSTRACT

Background: Relapsing and chronic *S. aureus* infections have been ascribed to intraphagocytic bacterial persistence (resulting also in emergence of resistance). The activity of antibiotics against *S. aureus* markedly differs between the extracellular and intracellular milieus (Barcia-Macay et al. *Antimicrob Agents Chemother*. 2006;50:841-851). Intracellular activity, therefore, needs to be assessed in specific models. We have examined the activity of antibiotics commonly used for the treatment of *S. aureus* infections (oxacillin, vancomycin, linezolid, and rifampicin) in comparison with quinupristin-dalfopristin (30:70; [Synercid]), moxifloxacin, daptomycin (both recently approved for skin and soft-tissue infections), and telavancin (a lipoglycopeptide with multiple modes of action, under clinical development) in macrophages.

Methods: MSSA (ATCC 25923), HA-MRSA (ATCC 33591), and VISA (NRS23) were used. MICs were determined by broth microdilution method. Infection of THP-1 macrophages was obtained as described by Barcia-Macay and colleagues (*Antimicrob Agents Chemother*. 2006;50:841-851), and the intracellular localization of the bacteria was ascertained by confocal and electron microscopy. Activity was measured after 24 hours of exposure to a drug concentration corresponding to the C_{max} (control cells: gentamicin [0.5 x MIC] to prevent extracellular growth).

Results: Phagocytosed bacteria were unambiguously seen in membrane-bounded vacuoles (phagolysosomes) in controls. MICs and intracellular activities are shown in the table.

Antibiotic	C_{max}, mg/L	MSSA MIC, mg/L	MSSA THP-1*	HA-MRSA MIC, mg/L	HA-MRSA THP-1*	VISA MIC, mg/L	VISA THP-1*
None	–	–	0.7 ± 0.1	–	1.6 ± 0.1	–	1.0 ± 0.1
Oxacillin	8	0.25	-0.7 ± 0.1	256	0.6 ± 0.1	256	0.0 ±. 0.1
Vancomycin	50	0.5	-0.6 ± 0.1	0.5	-0.6 ± 0.1	2	-0.2 ± 0.1
Linezolid	20	0.5	-0.7 ± 0.1	1	-0.7 ± 0.0	1	-0.7 ± 0.1
Rifampicin	4	0.03	-1.3 ± 0.1	0.06	-1.5 ± 0.0	0.06	-1.6 ± 0.1
Daptomycin	77	0.12	-1.7 ± 0.1	0.125	-1.5 ± 0.1	0.5	-1.3 ± 0.1
Quinupristin-dalfopristin	10	0.5	-1.8 ± 0.1	0.5	-1.5 ± 0.1	1	-1.3 ± 0.0
Moxifloxacin	4	0.06	-2.0 ± 0.1	0.125	-1.3 ± 0.1	2	-1.1 ± 0.1
Telavancin	90	1	-2.3 ± 0.0	1	-1.8 ± 0.1	1	-1.4 ± 0.1

*$\log_{10}$ ± SD reduction in intracellular counts.

Conclusions: With the exception of rifampicin, the antibiotics commonly recommended for the management of *S. aureus* infections only poorly eradicate intracellular forms, but this can be improved with new molecules.

BACKGROUND

S. aureus can cause chronic and relapsing infections, probably because of its ability to survive and multiply within eukaryotic cells (Lowy. *Trends Microbiol*. 2000;8:341-343). Appropriate treatment should, therefore, aim at eradicating intracellular forms of infection. However, antibiotics are routinely evaluated against extracellular bacteria only. Thus, models of intracellular infection may not only prove useful but also be essential for a correct assessment of antibiotic efficacy.

AIMS OF THE STUDY

- To develop a 24-hour model of intracellular infection with *S. aureus* using various susceptibility phenotypes (MSSA, MRSA, and VISA) in human THP-1 macrophages.
- To compare the intracellular activities of commonly recommended antibiotics and newly developed anti–gram-positive molecules at an extracellular concentration corresponding to the level observed in patients receiving conventional dosages.

METHODS

1. SUSCEPTIBILITY TESTING

Susceptibility testing was performed by broth microdilution method in Mueller-Hinton broth.

2. INTRACELLULAR INFECTION

Cells were infected with preopsonized bacteria (1 hour; 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/L) to eliminate noninternalized bacteria. Electron microscopy was performed as described by Ouadrhiri and colleagues (*Antimicrob Agents Chemother*. 1999;43:1242-1251).

3. DETERMINATION of INTRACELLULAR ANTIBIOTIC ACTIVITY

(Lemaire et al. *J Antimicrob Chemother.* 2005;55:897-904)

Infected cells were exposed for 24 hours to antibiotics at a concentration corresponding to the 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 extracellular growth of bacteria released from dead cells).

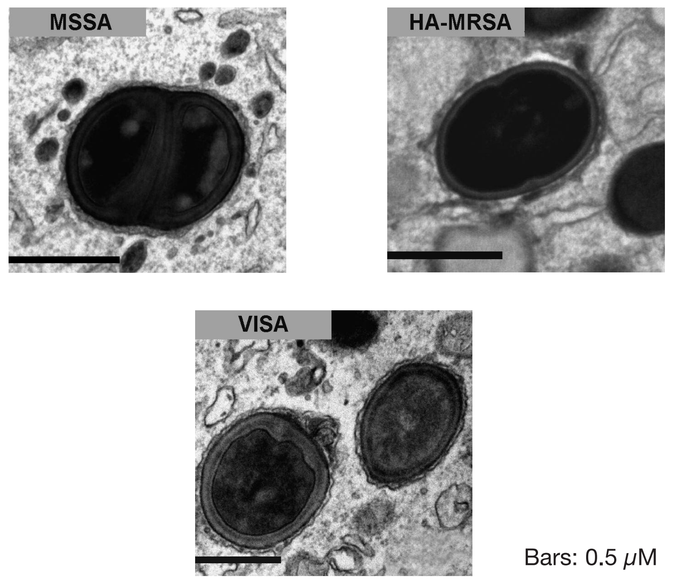
RESULTS

1 Susceptibility Testing

Antibiotic	Abbreviation	MICs (mg/L) MSSA*	MRSA†	VISA‡
Oxacillin	OXA	0.25	256	256
Vancomycin	VAN	0.5	0.5	2
Linezolid	LZD	0.5	1	1
Rifampicin	RIF	0.03	0.06	0.06
Daptomycin	DAP	0.12	0.125	0.5
Quinupristin-dalfopristin	Q-D	0.5	0.5	1
Moxifloxacin	MXF	0.06	0.125	2
Telavancin	TLV	1	1	1

*ATCC 25923.
†ATCC 33591 (HA-MRSA).
‡NRS23.

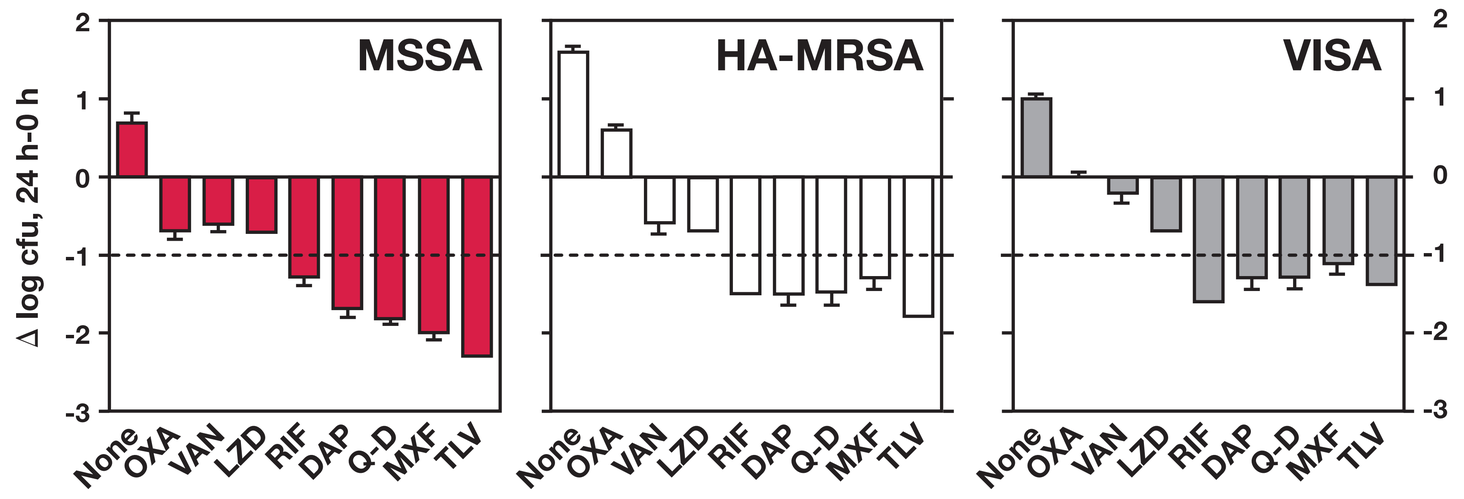
2 Electron Microscopy



Bars: 0.5 μM

After 24 hours cell-associated *S. aureus* appears clearly confined within membrane-bounded vacuoles, regardless of resistance phenotype.

3 Comparative Intracellular Activity Of Antibotics



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 concentration of drug equivalent to their human C_{max} (in mg/L: OXA, 8; VAN, 50; LZD, 20; RIF, 4; DAP, 77; Q-D, 10; MXF, 4; and TLV, 90).

CONCLUSIONS

- OXA, VAN, and LZD (commonly recommended for the management of *S. aureus* infections) are only poorly active against intracellular *S. aureus* (~–1 log cfu decrease).
- In contrast, RIF, DAP, Q-D, MXF, and TLV display more intense intracellular activity against intracellular MSSA, with lower but still highly significant activity against intracellular forms of resistant bacteria (MRSA and VISA).

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