



Intracellular MRSA, VISA and VRSA Are Sensitive to Cloxacillin and Meropenem

S. Lemaire,¹ F. Van Bambeke,¹ Y. Glupczynski,¹ P. C. Appelbaum,² and P.M. Tulkens,¹

¹ Université catholique de Louvain, Brussels; ² Hershey Medical Center, Hershey, PA

Mailing address:
 P.M. Tulkens
 Pharmacologie cellulaire et moléculaire
 UCL 73.70 av. Mounier 73
 1200 Brussels - Belgium
 tulkens@facm.ucl.ac.be

A- 0634

Abstract

Objectives: Exposure of methicillin-resistant *S. aureus* (MRSA) to acid pH restores their susceptibility to beta-lactams (Sabath and al., AAC, 1972). In phagocytes, intracellular forms of *S. aureus* are largely restricted to the phagolysosomes, where pH is acidic (about 5.5). We have, therefore, examined the intraphagocytic activity of cloxacillin (CLX) and meropenem (MEM) against selected methicillin-sensitive *S. aureus* (MSSA), hospital-acquired MRSA, Vancomycin-intermediate (VISA) and vancomycin-resistant (VRSA) strains.

Methods: MICs were determined by micro-dilution method using pH-adjusted Mueller Hinton Broth (with added NaCl [2%]). Intracellular activity was assessed in human THP-1 macrophages exposed to extracellular concentrations equivalent to human C_{max} (total drug; MEM: 50 mg/L; CLX: 8 mg/L) and expressed as a difference in cell-associated CFU after 24h (DCFU) between controls (no antibiotic, approx. 2 log CFU) and tests.

Results: The table shows the MICs in neutral and acid broth and the intracellular activity for the 4 strains studied.

Strains	Cloxacillin				Meropenem	
	MIC (mg/L)		DCFU	MIC (mg/L)		DCFU
	pH 7.4	pH 5.5		pH 7.4	pH 5.5	
MSSA ATCC 25923	0.125	0.06	-2.5±0.1	0.125	0.125	-2.4±0.1
MRSA ATCC 33591	16	0.06	-2.6±0.1	16	0.125	-2.5±0.1
NRS18	8	0.06	-2.6±0.1	8	0.06	-2.6±0.1
VRS2	32	0.06	-2.7±0.1	16	0.06	-2.7±0.1

Acid pH made both CLX and MEM very active in broth against all strains. In THP-1 cells, CLX and MEM display also similar activities against all strains.

Conclusions: The similar intracellular activities of CLX and MEM against MRSA, VISA and VRSA in comparison with MSSA, in spite of their marked differences in susceptibility when tested at pH 7.4 in broth, may result from restoration of susceptibility due to acid pH in the phagolysosomal environment. Conventional MIC determinations are inadequate to accurately predict the susceptibility of the intracellular forms of resistant *S. aureus*.

Background

MRSA show a high level of resistance to β-lactams, in relation with the expression of a modified PBP (PBP2a). However, the activity of β-lactams against MRSA is restored in acidic conditions.¹ This may be of interest for staphylococcal infections developing in acidic environments. In particular, *S. aureus* has the potential of surviving within the phagolysosomal compartments of phagocytic cells (where pH is acidic). In macrophages, we recently showed that meropenem does exert intracellular activity against MSSA.²

Aim of the study

- To study the influence of acid pH on the activity of CLX and MEM against MSSA and MRSA (including a VISA and a VRSA strain) in broth
- To study the intracellular activity of CLX and MEM towards MSSA and MRSA in a model of infected macrophages

Results

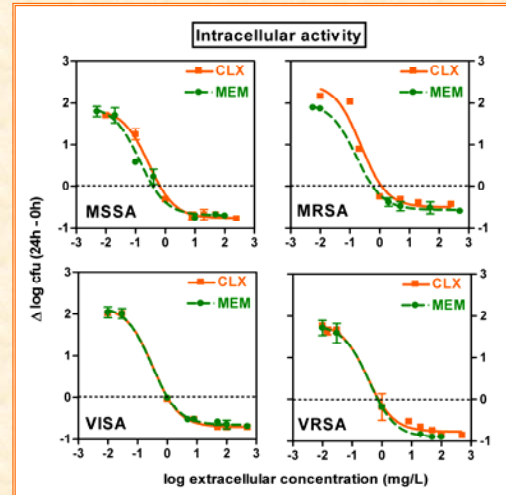
1 Susceptibility testing

In broth, acid pH restores the activity of CLX and MEM towards MRSA, including VISA and VRSA.

STRAINS	CLX		MEM	
	pH 7.4	pH 5.5	pH 7.4	pH 5.5
MSSA 25923	0.125	0.06	0.125	0.125
MRSA ATCC33591	16	0.06	16	0.125
& VISA NRS18	8	0.06	8	0.06
& VRSA VRS2	32	0.06	16	0.06

2 Intracellular activity

- CLX and MEM display a similar concentration-dependent activity against *S.aureus*
- Static concentrations and E_{max} (maximal effect) are similar whatever the resistance phenotype of the strain



Change in cfu (log₁₀) per mg of cell protein observed after 24 h of incubation of infected THP-1 macrophages exposed to extracellular concentrations of drugs ranging from 0.01 to 1,000-fold their MIC determined at pH 5.5. All values are mean ± SEM (n=3).

Methods

MICs were determined in broth by micro-dilution method, in MHB supplemented with NaCl 2 % and adjusted to pH 7.4 or 5.5.

Intracellular activity was studied in THP-1 macrophages.^{2,3} Briefly, macrophages were infected with preopsonised bacteria (1h, 37°C), washed with Phosphate-Buffered Saline (PBS) and incubated for 45 minutes with gentamicin to eliminate non-adherent and non-phagocytosed bacteria. Cells were then exposed for 24 h to antibiotics (extracellular concentrations ranging from 0.01 to 1,000-fold their MIC determined in acidic conditions).

Conclusions

Intracellularly, CLX and MEM are as active against MRSA (including VISA and VRSA) as against MSSA, probably in relation with their restored activity in the acidic environment of phagolysosomes

References

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