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Menadione-Dependent Small-Colony Variants (SCV) of MRSA are Hypersusceptible to Beta-Lactams Intracellularly Due to Cooperation between Vacuolar Acidic pH and Oxidant Species

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ABSTRACT

BACKGROUND. SCVs persist intracellularly, which is associated with recurrent infections. Phagocytized MRSA are susceptible to β-lactams because the acid pH of phagolysomes induces a conformational change of PBP2a allowing its acylation by these antibiotics (AAC 51:1627-32; JBC 283:12769-76). This study now examines the cooperation of host defenses with β-lactams against a *menD* SCV of MRSA compared to the parental normal phenotype strain.

METHODS. Strains: MRSA COL and its *menD* disruptant. MICs: microdilution (MHB + 2% NaCl adjusted at pH 7.4 or 5.5); when specified, preincubation with H_2O_2. Intracellular activity in THP-1 cells: change in CFU from the post-phagocytosis inoculum after 24 h incubation with AB combined or not with N-acetylcysteine (NAC; scavenger of oxidant species); relative potency calculated from the Hill equation of concentration.-response curve.

RESULTS. See Table. Against COL strain, β-lactam MIC were markedly reduced at pH 5.5, with limited additional effect of preincubation with H_2O_2. Intracellular potency was not modified or slightly decreased (2-16 fold increase in Cs) by NAC. In contrast, for the *menD* mutant, MICs remained elevated at pH 5.5 but were remarkably low after preincubation with H_2O_2. Intracellular potency was much higher (275-1400 fold lower Cs) against the *menD* mutant than the COL strain, but this difference was not seen with NAC. Vancomycin MICs were unaltered in all conditions, with no marked effect of NAC on intracellular potency.

CONCLUSION. A cooperation between acidic and oxidant species confers high potency to β-lactam against intracellular forms of *menD* SCVs of MRSA.

Antibiotic	Strain	MIC at pH 7.4		MIC at pH 5.5		Intracellular Cs [a]	
		control	+ H_2O_2 [b]	control	+ H_2O_2 [b]	control	+ NAC [c]
Cloxacillin	WT	128	128	1	1	11	27
	menD	128	128	128	0.125	0.04	35
Doripenem	WT	16	16	0.5	0.125	18	12
	menD	16	8	8	0.016	0.02	25
Meropenem	WT	32	16	1	0.25	1	16
	menD	32	16	4	0.03	0.001	15
Vancomycin	WT	1	1	1	1	5.3	4
	menD	1	1	1	1	0.3	2

[a] Static concentration (i.e. extracellular conc. resulting in no apparent intracellular bacterial growth after 24 h of incubation of infected cells with the antibiotic); determined by graphical interpolation using sigmoidal regressions of data from conc-effects studies (extracellular concentration: 0.001-150 mg/L)
[b] Bacteria preincubated during 30 min with 10 mM H_2O_2 before addition of the antibiotic
[c] Infected cells co-incubated with the antibiotic and 25 mM N-acetylcysteine

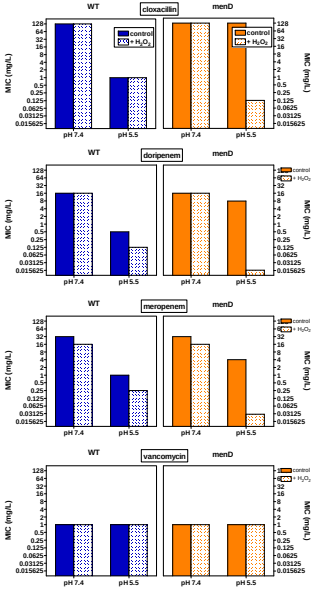
INTRODUCTION & OBJECTIVES

- Small Colony Variants (SCVs) of *Staphylococcus aureus* are associated with persistent infections. They have a particular tropism for the intracellular environment, which is often assumed to protect them from the action of most antibiotics [1].
- Surprisingly, β-lactams regain activity against MRSA phagocytosed by THP-1 macrophages [2]. This has been ascribed to the acidic pH prevailing in the phagolysosomes where the bacteria sojourn, which causes penicillin-binding protein (PBP) 2a to undergo a conformational change from a closed to an open state allowing its acylation by β-lactams [3].
- The aim of this study was to examine whether this mechanism also applies to a menadione-dependent (*menD*) SCV of the COL MRSA strain, for which we previously demonstrated a reduced growth rate intracellularly, associated with a modest increase in antibiotic intracellular potency (lower static concentration [C_s]) and no change in maximal relative efficacy (E_{max}) [4].

RESULTS

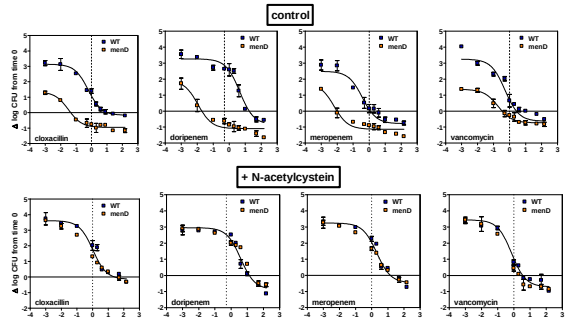
Comparative MICs against the parental strain (WT) with normal phenotype and its menadione-dependent mutant (menD)

- at pH 7.4 and 5.5
- in control conditions or after 30 min preincubation with 10 mM H_2O_2



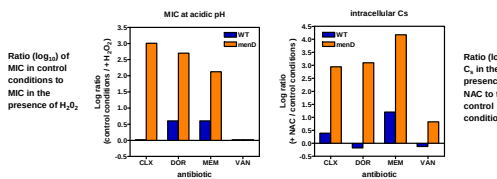
⇩ Low MIC for the *menD* mutant observed only at acidic pH after pre-exposure to H_2O_2

Intracellular activity (concentration-effect) against the parental strain (WT) with normal phenotype and its menadione-dependent mutant (menD)
Top: control conditions; bottom: cells co-incubated with 25 mM NAC



↓ β-lactams are highly potent towards the *menD* intracellularly. This effect is abolished by scavenging oxidant species.

Comparison of the combined effect of acid pH and oxidant species on the extracellular potency (MIC; left) or the intracellular potency (C_s; right) of antibiotics against the parental strain (WT) with normal phenotype and its menadione-dependent mutant (menD)



↓ The combined effect of acid pH and oxidant stress markedly and specifically increases both the extracellular and intracellular potencies of β-lactams towards the *menD* mutant.

METHODS

- **Bacterial strains:** strain COL (wild-type, HA-MRSA) and its menadione-depedent mutant constructed by allelic replacement with an *ermC* cassette inactivating the *menD* gene [5].
- **Susceptibility testing in broth:** MICs determined following CLSI recommendations in CA-MHB adjusted at pH 7.4 or pH 5.5, in control conditions or for bacteria pre-exposed for 30 min to 10 mM H_2O_2.
- **Intracellular activity:** infection of THP-1 human monocytic cells and assessment of antibiotic activities after 24 h incubation using a broad range of extracellular concentrations [4], in control conditions or in the presence of 25 mM N-acetylcysteine (scavenger of oxidant species produced by phagocytic cells [6]). Curve fitting to determine pharmacodynamic parameters (relative maximal efficacy [E_{max}] and static concentration [C_s]).

CONCLUSION

- **Extracellularly,** acid pH is unable to fully restore susceptibility of SCV to β-lactams, in contrast to what is observed for normal phenotype MRSA.

  Combination of acid pH AND oxydant species, however, reduces MIC to values even lower than those observed for the parental strain.
- **Intracellularly,** a menadione-dependent SCV is much more susceptible to β-lactams than its parental strain, probably because of the combination of local acid pH and exposure to oxidative burst
- These effects seem specific to β-lactams, as they are not observed for vancomycin, another antibiotic acting on cell wall synthesis.

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