Comparative intracellular activity of 10 antistaphylococcal antibiotics (AABs) against a stable small colony variant (SCV) of *S. aureus* in a model of human THP-1 macrophages

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ABSTRACT

Background: SCVs of *S. aureus* are frequently isolated from cystic fibrosis patients (CF). Bacteria with this phenotype persist intracellularly, contributing to the chronic and recurrent character of the infection. We have examined the activity of commonly-recommended (and 1 experimental) antistaphylococcal antibiotics (AABs) against SCVs in human macrophages.

Methods: We used a CF's clinical thymidine-auxotrophic mecA negative SCV isolate with a stable phenotype. MICs were measured by microdilution in MHB (reading at 48 h). Intracellular activities were determined after phagocytosis by human THP-1 macrophages (changes in post-phagocytosis inoculum [delta log CFU] after 24 and 72 h in cells incubated with AABs at an extracellular concentration corresponding to their respective most commonly reported Cmax).

Results:

AAB (C_{max} [mg/L])	MIC (mg/L)	Intracellular activity (delta log CFU from time 0) 24 h	72 h
none		0.22 ± 0.07 [a]	[c]
vancomycin [VAN] (50)	0.5	-0.17 ± 0.05	-1.35 ± 0.08
SYNERCID [Q-D] (11)	0.5	-0.22 ± 0.03	-2.06 ± 0.16
oxacillin [OXA] (64)	0.125	-0.23 ± 0.04	-1.31 ± 0.06
daptomycin [DAP] (57)	0.125	-0.39 ± 0.02	-1.38 ± 0.01
linezolid [LNZ] (16)	2	-0.39 ± 0.01	-1.52 ± 0.08
gentamicin [GEN] (18)	0.125	-0.44 ± 0.09	-1.54 ± 0.09
tigecycline [TGC] (1)	0.125	-0.96 ± 0.12	-1.14 ± 0.02
moxifloxacin [MXF] (4)	0.125	-1.20 ± 0.06	-2.53 ± 0.04
rifampin [RIF] (18)	0.0005	-1.24 ± 0.03	-3.39 ± 0.21
oritavancin [ORI] (25)	0.25 [a]	-2.53 ± 0.10	-2.98 ± 0.11

[a] with GEN (1xMIC) to prevent extracellular growth
[b] extracellular growth could not be prevented (but was not seen with AABs)
[c] determined in the absence of polysorbate 80

At 24 h, intracellular growth was minimal and most AABs showed only a bacteriostatic effect, with TGC, MXF and RIF causing a 1 log decrease, and only ORI approaching a bactericidal effect (defined as 3 log CFU decrease). The activity of all drugs (except TGC) markedly progressed over time, with Q-D, MXF, RIF and ORI approaching or exceeding the limit of bactericidal effect at 72 h. Addition of thymidine (100 µg/mL) did not enhance activity except for Q-D (-1.55 ± 0.12 at 24h).

Conclusion: Most registered AABs act only poorly on intracellular SCVs unless cells are exposed for prolonged times to them, which may explain the difficulty of eradicating these organisms in CF patients. Yet, RIF and MXF proved the most efficient among commonly recommended AABs. Because of its fast eradicating activity, ORI warrants being further studied in this and other related models of difficult-to-treat intracellular staphylococcal infections.

INTRODUCTION

Small Colony Variants (SCVs) of *S. aureus* have been associated with persistence of infection in cystic fibrosis (CF) patients, osteomyelitis, and device-related infections (1). They are characterized by an altered metabolic state, with most conspicuous changes consisting of a slower growth rate, a reduced production of α-hemolysin, and auxotrophy towards thymidine (thymidine-dependent SCVs) or hemin (hemin-dependent SCVs). Compared with their wild-type counterparts, SCVs persist more easily within host cells, where they are protected from host defenses and antibiotics (2).

Being relatively unstable, SCVs can revert to normally virulent, rapidly growing phenotype, and cause host cell lysis, contributing therefore to the recurrence of infection.

It seems, therefore, important to evaluate antibiotic activity against intracellular forms of SCVs in order to select appropriate therapy.

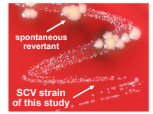
AIM OF THE STUDY

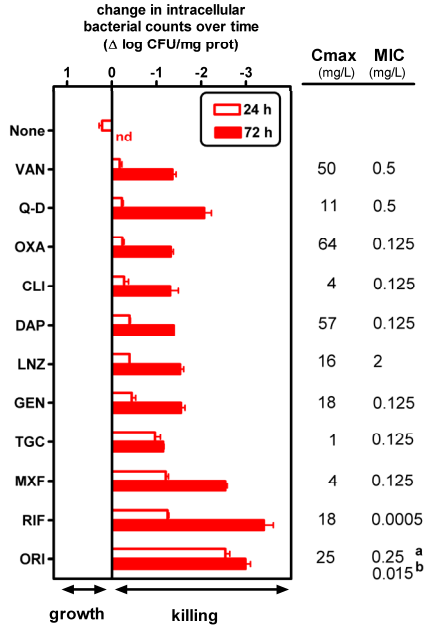
To compare the intracellular activity of a series of antibiotics against a stable, thymidine-dependent SCV variant of a CF *mecA*-negative isolate of *S. aureus*. To this effect, we used a model of infection in THP-1 human macrophages and antibiotic concentrations mimicking the C_{max} reached in patients undergoing therapy with conventional dosages.

RESULTS

Intracellular activity of antibiotics against *S. aureus*

(24 h and 72 h incubation with antibiotics at a concentration corresponding to their C_{max} in humans)





	Cmax (mg/L)	MIC (mg/L)
None		
VAN	50	0.5
Q-D	11	0.5
OXA	64	0.125
CLI	4	0.125
DAP	57	0.125
LNZ	16	2
GEN	18	0.125
TGC	1	0.125
MXF	4	0.125
RIF	18	0.0005
ORI	25	0.25 [a] 0.015 [b]

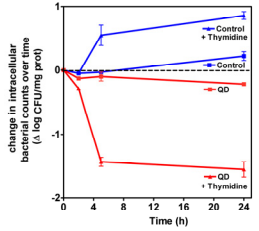
Change in intracellular counts of *S. aureus* SCV, after 24 h (open bars) or 72 h (closed bars) of incubation in the presence of the indicated antibiotics at their human Cmax.
Abbreviations: VAN, vancomycin; Q-D, quinupristin-dalfopristin (synercid); OXA, oxacillin; CLI, clindamycin; DAP, daptomycin; LNZ, linezolid; GEN, gentamicin;TGC, tigecycline; MXF, moxifloxacin; RIF, rifampin; ORI, oritavancin.
Controls : Gentamicin at its MIC was maintained during the whole incubation period; il allows to control extracellular contamination during 24 h but not for more prolonged incubations (72 h). Intracellular growth of the control can therefore not be measured at 72 h.
ORI MIC: determined in the absence (a) or in the presence (b) of 0.002 % of polysorbate.

- **At 24 h,**
  - bacterial growth was minimal
  - the majority of antibiotics caused only a modest decrease in bacterial counts (< 1 log decrease for vancomycin, quinupristin-dalfopristin, oxacillin, clindamycin, daptomycin, linezolid, and gentamicin)
  - tigecycline, moxifloxacin, and rifampin achieved ≈ 1 log decrease
  - only oritavancin achieved ≥ 2 log decrease
- **At 72 h,**
  - tigecycline showed no further change in intracellular bacterial counts
  - most of the other antibiotics showed some further activity (≈ 1 log; 2-3log for quinupristin-dalfopristin, moxifloxacin and rifampin)
  - oritavancin produced an additional 0.5 log decrease in intracellular bacterial counts

Influence of thymidine on intracellular growth and intracellular activity of antibiotics.

Because the SCV used in this study is thymidine-dependent, we repeated the same experiments in medium supplemented with 100 µg/ml of thymidine and limiting the observation period to 24 h.

In this condition, the intracellular growth of the bacteria was restored, but the activity of antibiotics at 24 h was not significantly modified, except for quinupristin-dalfopristin (see figure).



Change in intracellular counts of *S. aureus* SCV upon 24 h incubation.
control: culture medium added by 0.125 mg/L GEN
control + Thymidine: idem + 100 mg/L thymidine
QD: culture medium + synercid at 11 mg/L
QD + Thymidine: idem + 100 mg/L thymidine

METHODS

- **Bacteria:** we used a CF clinical isolate with stable thymidine auxotrophic SCV phenotype (3), growing as tiny, non-pigmented and non-hemolytic colonies on sheep blood agar. This strain was grown aerobically on Muller Hinton II medium (Becton Dickinson) with low and controlled content of thymidine.
- **Susceptibility testing:** MICs were determined by microdilution method in Muller Hinton broth and MICs were read after 48 h of incubation.
- **Intracellular activity:** infection of THP-1 macrophages was performed as previously described (4), with one hour phagocytosis (4 bacteria/cell), followed by a washing with 50 mg/L gentamicin (to eliminate extracellular bacteria) and reincubation in fresh medium containing either the tested antibiotic or gentamicin at its MIC (0.125 mg/L; control). Intracellular activity was measured after 24 h and 72 h exposure to antibiotics at a concentration corresponding to their respective human C_{max}. Results are expressed as changes in post-infection inoculum (Δ log CFU/mg cell protein; CFU counting determined after 48 h incubation of cell lysates plated on brain heart infusion agar.)

CONCLUSIONS

- Most currently available anti-staphylococcal agents act only poorly on intracellular SCVs, unless upon prolonged exposure.
- None of the tested antibiotics is able to sterilize the infected macrophages (within the 72 h time frame of these experiments), which may explain the difficulty of eradicating these organisms in CF patients.
- Reversion to normal phenotype of *S. aureus* by addition of thymidine to the culture medium allows restoration of intracellular growth of bacteria but does not enhance the antimicrobial activity, except for Q-D.
- Due to its rapid and significant bactericidal activity, oritavancin warrants further *in vitro* and *in vivo* studies in models of difficult-to-treat staphylococcal infections with SCVs.

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