Methicillin-Resistant Staphylococcus Aureus (MRSA): Etiology, At-Risk Populations and Treatment

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**Therapeutic options for intracellular MRSA: current views and perspectives**

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**Introduction**

*Staphylococcus aureus* causes a wide variety of diseases, ranging from relatively superficial wound infections (e.g. furunculosis) to deep-seated, life-threatening conditions (cardiovascular infections, severe skin infections, osteomyelitis, or pneumonia). Treatment options are, however, becoming limited due to resistance issues. In particular, Methicillin-Resistant *S. aureus* (MRSA) were first reported in 1961, shortly after introduction of penicillinase-stable β-lactams (such as methicillin). Confined for about 40 years in health care settings, MRSA have also been described in the community (e.g. day care center, schools and sport teams) in various parts of the world. These isolates tend to be highly virulent (1-3), causing serious infections and even death in healthy children and adults (4;5). Moreover, large-scale CDC surveillance studies have now revealed that hospital acquired (HA-) and community-acquired (CA-) MRSA are no more restricted to specific environments, since strains historically associated with CA-MRSA outbreaks (e.g. mainly US300 isolates) are now recovered in nosocomial infections as well (6).

Beyond this high capacity to adapt to environment and to develop resistance, the ability of *S. aureus* to survive within host cells can also contribute to the difficulty of eradicating infection with antibiotics. Although primarily known as extracellular, *S. aureus* is now recognized as an opportunistic intracellular pathogen that easily
adheres to and invades mammalian cells (7-10). This intracellular niche may play an important role in the pathogenesis of several staphylococcal infections, by protecting the bacterium from the lethal action of antibiotics (intracellular forms tend to be poorly susceptible to most available antibiotics) and immune defenses. It may probably explain (i) the recurrent or persistent character of several staphylococcal infections (namely endocarditis, osteomyelitis, complicated skin and soft-tissue infections, or bovine chronic mastitis) even after apparently successful therapy; (ii) the difficulty to eradicate nasal carriage since \textit{S. aureus} can persist in nasal epithelial cells (11); and (iii) the selection or spreading of resistant mutants due to the poor efficacy of most available antimicrobials. This stresses, therefore, the importance of understanding (i) the intracellular lifestyle and fate of this bacterium, and (ii) whether and to what extent antibiotics may or may not act against intracellular \textit{S. aureus}, with a particular emphasis on multi-drug resistant MRSA. We know, indeed, that cellular accumulation and activity in broth are not, \textit{per se}, predictive factors of intracellular efficacy (12;13). This chapter summarized our current knowledge of the intracellular fate of MRSA and its intracellular susceptibility to antibiotics.

1. Intracellular survival of \textit{S. aureus}

1.1. Intracellular life-style

Over the past decades, it has been shown that \textit{S. aureus} invades both phagocytic (macrophages ([14-16]), polymorphonuclear neutrophils [PMN, (7;16)]) and non phagocytic cells (endothelial cells (17), osteoblasts (18;19), skin keratinocytes (20), fibroblasts (21), and bovine mammary epithelial cells (22)) (the intracellular fate of \textit{S. aureus} is illustrated in Fig.1). In non phagocytic cells, host cell invasion depends on the fibronectin-mediated bridging between staphylococcal fibronectin-binding proteins (FnBPs, a family of adhesins expressed at the cell surface of the bacteria) and host cell integrins (23), which induces actin cytoskeleton
rearrangements though zipper-type mechanism (24). In PMN and macrophages, which constitute a first line of defense in the clearance of microorganisms, internalization process seems to involve both phagocytosis and FnBPs-mediated internalization since fibronectin-bound \textit{S. aureus} are more efficiently ingested than non opsonized \textit{S. aureus} (25;26).

After internalization, the fate of the bacterium varies depending on cell type or on the strain. In macrophages and PMN (7;27;28), electron microscopy studies have shown that \textit{S. aureus} remains confined in membrane-limited vacuoles, suggesting that this organism can resist to bactericidal mechanisms within phagolysosomes. In contrast, a few organisms are found entirely free in the cytosol of infected endothelial cells (29) or epithelial cells (30).

1.2. Post-phagocytosis changes in response to the intracellular environment

In order to survive intracellularly, bacteria have to cope with the eukaryotic environment. Using a microarray procedure, Garzoni and colleagues have demonstrated that, following internalization by human lung epithelial cells (31), \textit{S. aureus} extensively reprograms its transcriptome to maintain a metabolically dormant state (instead of the active bacterial replication observed in broth [see figure 2]) until the intracellular environment becomes suitable for escape. Hence, the expression of genes involved in bacterial metabolism, nutrient transport and cell-wall synthesis is markedly down-regulated. This is noticeably different from what is observed with intracellular \textit{Salmonella} (32) or \textit{Shigella} (33), which show up-regulation of genes involved in division processes. In contrast, there is an increased expression of genes involved in host cell invasion (e.g. gene coding for FnBPs proteins), neutralization of free radicals and iron scavenging. Of particular importance also, the
expression of virulence factors that damage the host (such as \textit{hla} [haemolysin alpha] (31)) is reduced in intracellular \textit{S. aureus} (to preserve host cell integrity (27)) while expression of genes involved in resistance to host defense appears critical. In particular, it has been shown recently that bacteria expressing high levels of staphyloxanthin (a carotenoid pigment blocking attack by host reactive oxygen species (34;35)) shows increased tolerance to H$_2$O$_2$, which is reflected intracellularly by an increased bacterial growth (36).

1.3. Intracellular persistence of small colony variants of \textit{S. aureus}

First described in the earlier 1900’s, Small Colony Variants (SCVs) represent naturally occurring subpopulations of \textit{S. aureus} that are often associated with persistent and recurrent illnesses, namely pulmonary infections associated with cystic fibrosis, chronic osteomyelitis or keratosis follicularis [Darier-White disease](37;38). These variants are characterized by a slow growth due to auxotrophism by defects in hemin/menadione (39) or thymidine biosynthesis (40). These metabolic alterations make also them respectively more resistant to aminoglycosides (decreased antibiotic uptake) or sulfonamides (bypass of the pathway inhibited by trimethoprim-sulfamethoxazole due to the thymidine dependence of this process). Being relatively unstable, SCVs can also revert to a highly virulent phenotype. Combined with their increased ability to invade eukaryotic cells (due to enhanced expression of FnBPs (41)), all these characteristics make SCV-related infections difficult to diagnose and to treat.

1.4. Clinical relevance of \textit{S. aureus} intracellular persistence

Despite clear evidences of \textit{in vitro} persistence, the ability of \textit{S. aureus} to reside and multiply intracellularly \textit{in vivo} is still an ongoing debate. Yet, intracellular
foci were documented in patients suffering from recurrent rhinosinusitis (11;42), and seem to constitute a significant risk factor for antibiotic failure. Likewise, intracellular bacteria were evidenced in fibroblasts of biopsy specimens from periprosthetic tissues (43), or surrounded by glycocalyx in osteoblasts from surgical bone specimens (44). The ability of S. aureus to secrete glycocalyx further contributes to difficulty in eradication, since this extracellular polymeric material offers significant advantage for the bacteria (increased bacterial adherence to bone matrix, tissues and biomaterials (45;46); protection from the lethal action of antibiotics (47)). Similar evidences of in vivo intracellular persistence are, however, still lacking for life-threatening cardiovascular infections, severe skin and soft-tissues diseases and pulmonary infections associated with cystic fibrosis, probably due to technical difficulties in localizing such reservoirs.

2. Intracellular activity of antibiotics

Over the past decades, cellular accumulation of antibiotics has been the subject of a large number of studies (see (12;13;48) for detailed description of mechanisms of antibiotics uptake, distribution and efflux in eukaryotic cells), but its importance for activity against intracellular bacteria remains largely controversial. Studies examining a series of antibiotics belonging to the main pharmacological classes have convincingly shown that accumulation per se is not a predictive factor of antibiotic efficacy. Interestingly, antibiotics belonging to pharmacological classes known for their low cellular accumulation (such as β-lactams) are not necessarily inactive against intracellular forms of susceptible bacteria (as demonstrated for S. aureus or L. monocytogenes). Conversely, and contrary to most original assumptions, macrolides failed to show activity towards intraphagocytic S. aureus despite their massive cellular accumulation. In order to be effective intracellularly, it is therefore critical to select antibiotics reaching the infected subcellular compartment
(phagolysosomes and cytosol) in some cell types at concentration levels far above the minimal inhibitory concentration (MIC) for a reasonable period of time, but also being able to express activity therein.

Routine evaluation of antibiotic activity is performed on extracellular bacteria, ignoring cellular pharmacokinetics (PK) and the modulation of pharmacodynamics (PD) by host cell environment. In this context, a complete evaluation of antistaphylococcal antibiotics should include an assessment of their ability to control intracellular infections. While animal models are currently being developed (49), models of cultured cells remain valuable tools because they offer the possibility to explore in details the pharmacological parameters governing the response of intracellular bacteria to the drug in the absence of host factors. Figure 3 shows a summarized view of the intracellular activity of antibiotics representative of the main pharmacological classes compared at their respective human Cmax (see Table 1, with a full description of their intrinsic activity at both neutral and acidic pH) against a MSSA and a MRSA (MLSb). As a whole, activity is grossly similar against both strains for all antibiotics tested except for azithromycin to which the MRSA is resistant. Interestingly, activity of cloxacillin (as a representative of β-lactams) is not affected, suggesting that the MRSA phenotype does not express it-self intracellularly (see 2.5 for more details). Figure 4 shows the effect reached in the same conditions against a series of MRSA with variable MICs to these agents. With most antibiotics, activity decreases gradually when MICs increases, corroborating a concentration-dependent profile of activity. The β-lactam cloxacillin makes exception, with a similar effect obtained whatever the MIC of the strain. This is in accordance with the non-concentration pharmacodynamic profile of this class of drug. Linezolid, also known as a poorly concentration-dependent antibiotic, becomes inactive as soon as the bacteria show elevated MICs.
2.1. Trimethoprim-sulfamethoxazole

Along with clindamycin, trimethoprim-sulfamethoxazole is the most commonly used antimicrobial agents for outpatient treatment of CA-MRSA infections. Despite its marked activity towards both extracellular HA-MRSA and CA-MRSA, this combination of antibiotics is not effective for the treatment of experimental intracellular \textit{S. aureus}, even when using high extracellular concentrations (up to 100 mg/L).

2.2. Azithromycin

A large activity of azitromycin towards intracellular \textit{S. aureus} is expected based on its massive cellular accumulation \((\text{50})\) and its co-localization with the bacteria in acidic vacuoles \((\text{51})\). However, azithromycin never yields a truly intracellular effect towards intraphagocytic \textit{S. aureus} \((\text{28;52})\), probably in relation with the deleterious effect of acidic pH on its antibacterial efficacy, and its bacteriostatic character. Improved potency (lower static concentrations) can, however, be obtained in the presence of verapamil \((\text{53})\), which blocks azithromycin efflux by P-glycoprotein \((\text{54;55})\).

2.3. Tetracyclines

For a long time, tetracyclines were considered as the drugs of choice for the treatment of several intracellular infections. Despite their apparent cellular accumulation \((10- to 60\text{-fold} [(\text{56;57})])\), these antibiotics reduces only modestly the intracellular inoculum of \textit{S. aureus} \((\text{28})\), again probably in relation with their bacteriostatic character.

2.4. Vancomycin

Vancomycin often remains the treatment of choice against MRSA infections. Unfortunately, it only shows a modest effect on intracellular \textit{S. aureus} \((\text{28;58})\).
Moreover, a further loss of intracellular efficacy ($E_{\text{max}}$) is obtained when cells are infected by less susceptible strains, such as heteroresistant subpopulations of vancomycin-intermediate $S.\ aureus$ [hVISA] or VISA isolates (59). Poorer outcome in patients treated with vancomycin for staphylococcal infections implicating less susceptible isolates may thus result from insufficient eradication of both extracellular and intracellular bacteria. An illustrative case was reported by Julian and colleagues (60), who described a chronic infective endocarditis that did not respond to vancomycin. Bacteriemia was documented over a 33-day period with isolates showing progressive elevated MIC values to vancomycin. Resolution was finally obtained by surgical procedures, indicating that persistent foci were the cause of the therapeutic failure.

2.5. Glycylcyclines

Tigecycline achieves quickly high intracellular accumulation within PMN but shows only a modest effect towards intracellular $S.\ aureus$, probably again in relation with its bacteriostatic character (61;62).

2.6. β-lactam antibiotics

For a long time, it has been stated that β-lactams would not be active against intracellular organisms because they do not accumulate within eukaryotic cells. Clinical data tend to prove the contrary, since β-lactams are highly effective in the treatment of listeriosis (63), an infection caused by the intracellular organism $Listeria\ monocytogenes$. Cellular models have now rationalized this observation by showing that appropriate doses (i.e. high concentrations such as serum levels) and prolonged time of exposure (imposed by the time-dependent activity of these agents in extracellular models of infection) allow to obtain sustained cellular concentrations of
the same order of magnitude as those reached in the serum. This explains a certain degree of intracellular activity, including against *S. aureus* (28;64).

A still more unanticipated observation is that MRSA regain almost full susceptibility to cloxacillin or meropenem after phagocytosis by human THP-1 macrophages (Fig. 5) or upon internalization by keratinocytes (65;66). This is clearly due to the acidic pH (67) prevailing in the phagolysosomes, since neutralization of these organelles (using lysosomotropic agent such as ammonium chloride) renders intraphagocytic MRSA again insensitive to the action of β-lactams. These findings have been rationalized by the observation that acidic pH caused a conformational change of the Penicillin-Binding Protein 2a (PBP2a, a unique transpeptidase poorly inhibited by β-lactams and responsible for the MRSA phenotype (68-70)), improving the access of penicillins to the active site and the subsequent acylation of the protein required for antibiotic activity (71). Lack of effective inhibition of PBP2a at neutral pH has indeed been ascribed to the closed conformation of its active site (69;72).

### 2.7. Oxazolidinones

It has been shown previously that linezolid kills more efficiently MRSA compared to vancomycin (73). In the intracellular environment, however, this bacteriostatic agent (28;59) kills only modestly intracellular *S. aureus* with a maximal effect similar to that of vancomycin.

### 2.8. Ansamycins

Rifampicin accumulates from 2- to 5-fold in cells (74), and demonstrates potent intracellular killing of rifampicin-susceptible *S. aureus* (28;59), yielding even truly intracellular bactericidal effect towards SCVs (< 3 log CFU decrease, as described by the CLSI criteria) for prolonged time exposure (72 h) (62).
2.9. Daptomycin

This last resort drug for MRSA infections is characterized by (i) extremely fast killing effects towards MRSA at all growth phases (which may be particularly useful for the treatment of indolent, deep-seated infections such as endocarditis or osteomyelitis in which bacteria reach stationary phase (75)); and (ii) marked intraphagocytic killing of both MSSA (76) and MRSA (77). As demonstrated for vancomycin, daptomycin is however less efficient against intracellular VISA isolates due to reduced intrinsic activity (59;77). This intracellular effect is obtained in spite of a low cellular accumulation (Cc/Ce < 1 [(76)]) and is further increased in the presence of P-glycoprotein inhibitors (verapamil or elacridar) since this antibiotic is substrate of these transporters, as demonstrated in THP-1 macrophages and MDCK (Madin-Darby Canine Kidney) cells (76).

2.10. Fluoroquinolones

These antibiotics accumulate to variable levels in eukaryotic cells (5- to 20-fold (78)) and are suspected to easily diffuse in all subcellular compartments, as they show activity against cytosolic (L. monocytogenes [(79-81)]), phagosomal (L. pneumophila [(82;83)]) or phagolysosomal (S. aureus [(28;52;84)]) bacteria. Within this class, molecules with higher level of accumulation but also higher intrinsic activity like moxifloxacin are more active intracellularly than derivatives with lower cellular accumulation and intrinsic activities (such as ciprofloxacin)(28). These properties, combined with a bactericidal character, make of moxifloxacin one of the most effective antibiotics against intracellular S. aureus, yielding potent intracellular killing in a concentration- and time-dependent fashion (-2 and -3 log cfu after 24 h and 48 h, respectively) against both MSSA (28;52;85) and CA-MRSA isolates (86;87).
Cellular accumulation of fluoroquinolones is highly modulable but surprisingly, changes in accumulation do not necessarily translate in improved efficacy. In J774 macrophages, ciprofloxacin, but not moxifloxacin, is substrate of the multidrug transporter MRP4 (88). Inhibition of this transporter increases ciprofloxacin cellular content to values close to those reached by moxifloxacin (53;89;90). However, this is not accompanied by an improved activity against intracellular S. aureus but well on L. monocytogenes (53;89), because the additional amount of drug accumulates in the cytosol. In human THP-1 macrophages, differentiation by PMA specifically increases the accumulation of moxifloxacin but not of levofloxacin by a still unknown mechanism (84). Again, this specifically increases the intracellular activity against L. monocytogenes but not on S. aureus, presumably for the same reason.

2.11. Quinupristin-dalfopristin

This combination of streptogramins accumulates within cells (91), and demonstrate potent intraphagocytic killing of S. aureus causing typically a 2 – 2.5 log decrease in intracellular inoculum (91). This effect is also observed for MRSA isolates of current clinical and epidemiological interest, including VISA and Vancomycin Resistant S. aureus (VRSA) strains (59;92).

2.11. Novel investigational antibiotics

2.11.1. Novel glycopeptides

Loss of susceptibility to vancomycin in MRSA stimulated the search for alternative therapy. In this context, novel glycopeptides have been successfully designed to keep activity against Vancomycin-resistant S. aureus (VRSA) isolates and Vancomycin-intermediate S. aureus (VISA) to a lower extent (see for review: (93-95), because of a dual mode of action implying both inhibition of cell-wall synthesis and membrane destabilization (96;97). Telavancin (98;99) and oritavancin (100;101) accumulate to high levels (~ 50- and 200-fold after 24 hours, respectively)
in the lysosomes of murine macrophages \((102;103)\), show superior \textit{in vitro} activity in broth, and are highly bactericidal towards both extracellular and intracellular forms of vancomycin-susceptible and vancomycin-resistant \textit{S. aureus} \((\text{Fig.2, Fig.4})\) \((58;59;104)\) compared to vancomycin. Oritavancin is also highly efficient against intracellular SCV \((62)\), and this activity is still reinforced when combined with other bactericidal agents such as rifampicin or moxifloxacin \((105)\).

\subsection{2.11.2. Anti-MRSA cephalosporins}

Ceftobiprole and ceftaroline are two cephalosporins under development that show similar activity against both MSSA and MRSA \((106-108)\). This is due to the fact that they strongly bind to PBP2a, being able to induce the conformational change of the protein required for antibiotic activity \((109)\), as did acidic pH for conventional \(\beta\)-lactams. Ceftobiprole shows similar intrinsic activities against MSSA and MRSA (HA- or CA-MRSA) at neutral and acidic pH, as well as a similar potency against intracellular MSSA or MRSA in models of human macrophages or keratinocytes \((110)\).

\subsection{2.11.3. Macrolide / ketolide}

CEM-101 is a novel ketolide that, in contrast to azithromycin, keeps activity under acidic conditions and is not substrate for P-glycoprotein. Accordingly, it is considerably more active against intracellular forms of \textit{S. aureus} \((111)\), as well against intraphagocytic \textit{Listeria monocytogenes} and \textit{Legionella pneumophila}.

\subsection{2.11.4. Oxazolidinones}

Novel investigational oxazolidinones (such as radezolid [RX-1741] \((112;113)\) or torezolid [TR-700] \((114;115)\)) have been designed to keep activity against linezolid-resistant \textit{S. aureus} strains. As compared to linezolid, they show increased intrinsic activities against linezolid-resistant \textit{S. aureus} (lower MIC values), and higher
intracellular accumulation accompanied by an increased potency towards both linezolid-susceptible and linezolid-resistant *S. aureus* (116-118).

### 2.11.5. Iclaprim

Iclaprim is a diaminopyrimidine dihydrofolate reductase inhibitor that has completed clinical development for the treatment of skin and skin structure infections (cSSSI). This compound demonstrates potent intraphagocytic activity against different intracellular organisms, including *L. monocytogenes* (119) and *L. pneumophila* (120), compared to trimethoprim-sulfamethoxazole. Data are however still lacking for intracellular *S. aureus*.

### 2.11.6. Linopristin-flopristin

Linopristin-flopristin (NXL103, formerly XRP2868) is a novel semi-synthetic combination of streptogramins (vol/vol, 30/70). Compared to quinupristin-dalfopristin, it demonstrates slightly lower MICs values against MRSA (121), but similar activity against intracellular *S. aureus* (approx. -2 log CFU (122)).
Conclusions

Gaining an intracellular niche, even briefly, may afford several opportunities to \textit{S. aureus} to survive and promote disease. Because antibiotics are considerably less effective intracellularly than extracellularly, treatment of relapsing and persistent \textit{S. aureus} infections is particularly challenging. In this context, the possibility that conventional anti-MRSA antibiotics (vancomycin, tetracyclines, linezolid, azithromycin, trimethoprim-sulfamethoxazole) may have limited intracellular activity should be borne in mind when selecting appropriate treatment for infections in which intracellular foci are suspected. In this respect, cellular models may help in selecting for further development molecules with low MICs values and high efficacy (\textit{Emax}) against both extracellular and intracellular MRSA.
BOX.1. Intraphagocytic model of infection

Gentamicin protection assays are widely used for the determination of antibiotic intracellular activities. Briefly, phagocytes are incubated in the presence of pre-opsonized *S. aureus* (e.g. multiplicity of infection [MOI] equivalent to 4 bacteria per cells) during 1-2 h (28;52) before extensive washing in pre-warmed phosphate buffered saline (PBS) and incubation in the presence of gentamicin during 45 min-1 h. Cells are then incubated in the presence of increasing concentrations of antibiotics (28;64;123) and, after different time intervals, cells are washed free from antibiotics, lysed by resuspension in water, and the corresponding samples then processed for CFU counting and protein content determination. In order to prevent the development of extracellular bacteria, control cells systematically include exposition to gentamicin (1/2- to 1-fold the MIC, avoiding marked acidification and subsequent cell death)(28;52). Complete sterilization of the extracellular medium has been also reported in lysostaphin protection assay, but a significant amount of lysostaphin is able to enter the cells, which may interfere with the expression of antibiotic activity or CFU counting (52).
Table 1. Comparative susceptibilities of antibiotics at neutral and acidic pH

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Human Cmax (mg/L)</th>
<th>MSSA (ATCC 25923)</th>
<th>MRSA (ATCC 33591)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH 7.4</td>
<td>pH 5.5</td>
</tr>
<tr>
<td>Cell-wall/membrane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin (OXA)</td>
<td>8</td>
<td>0.25</td>
<td>0.03</td>
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<tr>
<td>Cloxacillin (CLX)</td>
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<td>0.125</td>
<td>0.06</td>
</tr>
<tr>
<td>Meropenem (MEM)</td>
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<td>Daptomycin (DAP)</td>
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<td>0.25</td>
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<td>Vancomycin (VAN)</td>
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<td>1</td>
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<tr>
<td>Telavancin (TLV)</td>
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<td>1</td>
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<td>Oritavancin (ORI)</td>
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<tr>
<td>Nucleic acid synthesis</td>
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<td>Ciprofloxacin (CIP)</td>
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<td>0.5</td>
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<td>Moxifloxacin (MXF)</td>
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<td>0.125-0.25</td>
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<td>Metabolism</td>
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<td>Trimethoprim-sulfamethoxazole (STX)</td>
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<td>Protein synthesis</td>
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<td>1-2</td>
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<td>Azithromycin (AZI)</td>
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</tr>
<tr>
<td>Clindamycin (CLI)</td>
<td>20</td>
<td>0.125</td>
<td>8-16</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin (Q-D)</td>
<td>10</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Linezolid (LZD)</td>
<td>20</td>
<td>1-2</td>
<td>2</td>
</tr>
</tbody>
</table>
**Figure 1.** Pictorial description of the infectious cycle of *S. aureus* within eukaryotic cells, illustrating adhesion to host cells, residence and multiplication within phagolysosomal apparatus, and the possible escape into the cytosolic compartment in endothelial and epithelial cells.
Figure 2. Kinetics of MRSA (strain ATCC 33591) bacterial growth in Mueller Hinton broth (extracellular bacteria) or THP-1 cells (intracellular bacteria).
Figure 3. Comparison of the intracellular activities of antibiotics against MSSA and MRSA. The graphs show the activity of a series of antibiotics against MSSA strain ATCC 25923 (fully susceptible) and MRSA strain ATCC 33591 (resistance in broth to β-lactams, azithromycin and clindamycin) phagocytized by human THP-1 macrophages. Activity is expressed as the change in bacterial counts following 24 h of exposure to each of the selected antibiotics using an extracellular concentration corresponding to their respective human Cmax. The limit of detection is set at – 5 log₁₀ cfu. The dotted lines represent the extracellular activities of each antibiotic (broth), while the gray zone represents the bacterial growth.

AZI, azithromycin; CIP, ciprofloxacin; CLI, clindamycin; CLX, cloxacillin; DAP, daptomycin; LZD, linezolid; MXF, moxifloxacin; N.D., Not determined; ORI, oritavancin; Q-D, quinupristin-dalfopristin (vol/vol, 30/70); RIF, rifampicin; TET, tetracycline; TGC, tigecycline; TLV, telavancin; STX, trimethoprim-sulfamethoxazole; VAN, vancomycin.
**Figure 4A.** Correlation between intrinsic and intracellular activities of a series of antibiotics (vancomycin, cloxacillin, rifampicin and linezolid) against *S. aureus* isolates (including MRSA strains of clinical epidemiological interest). The abscissas show antibacterial intrinsic activities, as determined by standard MIC assays. The ordinates show intraphagocytic activities (human THP-1 macrophages) expressed as the change in bacterial counts following 24 h of exposure to each of the selected antibiotics (using an extracellular concentration corresponding to their respective human Cmax [as represented by the vertical dotted line]). The gray zone corresponds to the bacterial growth.
Figure 4B. Correlation between intrinsic and intracellular activities of a series of antibiotics (moxifloxacin, daptomycin, quinupristin-dalfopristin, and novel glycopeptides antibiotics [namely oritavancin and telavancin]) against S. aureus isolates (including MRSA strains of clinical epidemiological interest). The abscissas show antibacterial intrinsic activities, as determined by standard MIC assays. The ordinates show intraphagocytic activities (human THP-1 macrophages) expressed as the change in bacterial counts following 24 h of exposure to each of the selected antibiotics (using an extracellular concentration corresponding to their respective human Cmax [as represented by the vertical dotted line]). The gray zone corresponds to the bacterial growth.
Figure 5. Influence of concentration on the extracellular (Mueller Hinton Broth adjusted to pH 7.4 and pH 5.5) and intracellular activities of cloxacillin against MSSA strain ATCC 25923 and MRSA strain ATCC 33591. The graph shows antibiotic activity over a 24 h period against bacteria in broth (pH 7.4 vs. pH 5.5) or post-phagocytized by human THP-1 macrophages. Activity is expressed as the change in bacterial counts from time 0 h. The gray zone corresponds to the bacterial growth. Adapted from data presented in reference (65).
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