Bactericidal activity of orally available agents against methicillin-resistant Staphylococcus aureus

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Background: The recent proliferation of community-acquired (CA) methicillin-resistant Staphylococcus aureus (MRSA) has led to a marked increase in the need for outpatient treatment of MRSA infections. Many oral agents active against MRSA have been available for years, and a paucity of literature compares them, leaving physicians with little guidance for choosing among them. The purpose of the present study was to compare the bactericidal effects of orally available antibiotics against MRSA and to determine whether there were differences in antimicrobial killing activity against CA-MRSA and hospital-acquired (HA) MRSA isolates.

Methods: A total of 12 unique patient MRSA isolates were studied. Six strains were CA, carrying the staphylococcal chromosomal cassette (SCCmec) type IVa, while six were HA and carried SCCmec type II. Time–kill methods were used to study the bactericidal activity of the orally available antimicrobials linezolid, rifampicin, trimethoprim/sulfamethoxazole, clindamycin, minocycline, and moxifloxacin alone and in combination in vitro.

Results: Trimethoprim/sulfamethoxazole was rapidly bactericidal resulting in >2 log10 cfu/mL decrease at 8 h and >3 log10 cfu/mL decrease at 24 h in vitro. No antibiotic combination exhibited better killing than trimethoprim/sulfamethoxazole alone. Adding rifampicin to trimethoprim/sulfamethoxazole showed a trend towards antagonism in vitro. There were no differences in the bactericidal activity of any antimicrobial or antimicrobial combination against MRSA isolates carrying SCCmec type IVa versus those carrying SCCmec type II.

Conclusion: Trimethoprim/sulfamethoxazole is rapidly bactericidal against MRSA in vitro when compared with most other orally available antimicrobials. No differences in bactericidal activity were detected when activities against CA-MRSA and HA-MRSA were compared.

Keywords: trimethoprim/sulfamethoxazole, linezolid, time–kill, bactrim

Introduction

Once mainly confined to the healthcare setting, methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a prominent cause of skin and soft-tissue infections in the community.1 Community-acquired MRSA (CA-MRSA) infections may be highly aggressive perhaps related to the nearly uniform presence of genes encoding Panton-Valentine leucocidin.1 Hence, rapidly acting oral antibiotics for the treatment of MRSA infections in the outpatient setting are needed.

There is a dearth of published data regarding the relative efficacy of the currently available oral antimicrobials against MRSA. In this investigation, we studied the in vitro bactericidal activity of six orally available antistaphylococcal antimicrobials...
alone and in combination against MRSA. We hypothesized that antimicrobials that have been available for decades would have comparable in vitro activity to linezolid. Based on previous investigations with MRSA that revealed in vitro antagonism between the penicillins and rifampicin, we also hypothesized that the addition of rifampicin to various other antimicrobials would not be synergistic against MRSA.

Materials and methods
A total of 12 well-characterized strains of MRSA isolated from unique patients were selected for the study. Strains were classified epidemiologically as CA or hospital-acquired (HA) according to CDC guidelines.

MICs of oxacillin, trimethoprim/sulfamethoxazole and clindamycin were assayed with the VITEK system (BioMerieux). Clindamycin susceptibility was confirmed using the double-disc diffusion test. In vitro susceptibilities of linezolid, minocycline and rifampicin were determined by tube dilution according to CLSI standards.3 Susceptibilities to moxifloxacin were determined using the moxifloxacin Etest.

Bactericidal activity was studied using antimicrobial agents singly and in combination at the following concentrations: linezolid, 8 mg/L; rifampicin, 1 mg/L; trimethoprim/sulfamethoxazole, 1/20 mg/L; amoxicillin/clavulanate, 8/2 mg/L; clindamycin, 2 mg/L; minocycline, 1 mg/L; moxifloxacin, 2 mg/L. The concentration for each agent was chosen carefully to reflect its serum level mid-way between the peak and trough concentration based on conventional oral dosing and pharmacokinetics. We felt that for in vitro studies with concentrations-dependent antibiotics with no post-antibiotic effect, these represent the most clinically relevant concentrations. Except for amoxicillin/clavulanate, we tested antibacterial efficacy of antibiotics against susceptible strains only. Time–kill studies were performed in Mueller–Hinton broth containing calcium (25 mg/L) and magnesium (12.5 mg/L) at 37°C with a starting inoculum of ~2 x 10⁶ cfu/mL. At times 0, 4, 8, 24 and 48 h, aliquots were removed, serially diluted in PBS and plated onto Mueller–Hinton agar to quantify cfu/mL. These time–kill experiments were performed in duplicate for all isolates at all time points. For the purposes of this study, bactericidal activity was defined as a ≥3 log₁₀/mL decrease in bacterial counts at 24 h. Synergy was defined as ≥2 log₁₀/mL reduction in cfu with the combination as compared with the single more active agent at 24 h. Antagonism was said to be present if the cfu was ≥2 log₁₀/mL higher after incubation with the combination than with the single more active agent at 24 h.

Table 1. Time–kill responses of orally available antimicrobials alone and in combination against MRSA

<table>
<thead>
<tr>
<th>Drug (number of isolates tested)</th>
<th>4 h log₁₀ cfu/mL decrease (SD)</th>
<th>8 h log₁₀ cfu/mL decrease (SD)</th>
<th>24 h log₁₀ cfu/mL decrease (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT* (11)</td>
<td>1.31 (0.38)</td>
<td>2.30 (0.51)</td>
<td>3.26 (0.46)</td>
</tr>
<tr>
<td>Linezolid (12)</td>
<td>0.21 (0.16)</td>
<td>0.27 (0.16)</td>
<td>0.81 (0.23)</td>
</tr>
<tr>
<td>Rifampicin (12)</td>
<td>0.64 (0.21)</td>
<td>0.85 (0.25)</td>
<td>1.47 (0.41)</td>
</tr>
<tr>
<td>AMC† (12)</td>
<td>0.03 (0.75)</td>
<td>−0.25 (1.41)</td>
<td>−1.87 (1.52)</td>
</tr>
<tr>
<td>Minocycline (11)</td>
<td>0.26 (0.12)</td>
<td>0.41 (0.20)</td>
<td>0.82 (0.23)</td>
</tr>
<tr>
<td>Clindamycin (6)</td>
<td>−0.69 (0.15)</td>
<td>−0.63 (0.11)</td>
<td>−0.11 (0.18)</td>
</tr>
<tr>
<td>Moxifloxacin (3)</td>
<td>3.08 (0.40)</td>
<td>3.09 (0.52)</td>
<td>3.33 (0.11)</td>
</tr>
<tr>
<td>SXT + rifampicin (11)</td>
<td>0.69 (0.21)</td>
<td>0.94 (0.31)</td>
<td>1.63 (0.35)</td>
</tr>
<tr>
<td>SXT + linezolid (11)</td>
<td>0.14 (0.10)</td>
<td>0.34 (0.20)</td>
<td>0.81 (0.26)</td>
</tr>
<tr>
<td>Linezolid + rifampicin (12)</td>
<td>0.63 (0.19)</td>
<td>0.84 (0.30)</td>
<td>1.54 (0.37)</td>
</tr>
<tr>
<td>Minocycline + rifampicin (11)</td>
<td>0.41 (0.16)</td>
<td>0.88 (0.26)</td>
<td>1.39 (0.41)</td>
</tr>
<tr>
<td>Clindamycin + rifampicin (6)</td>
<td>−0.64 (0.11)</td>
<td>−0.40 (0.15)</td>
<td>0.48 (0.07)</td>
</tr>
<tr>
<td>Moxifloxacin + rifampicin (3)</td>
<td>1.04 (0.15)</td>
<td>1.39 (0.03)</td>
<td>2.72 (0.38)</td>
</tr>
</tbody>
</table>

* SXT, trimethoprim/sulfamethoxazole.
† AMC, amoxicillin/clavulanate.
We found that the in vitro bactericidal activity of trimethoprim/sulfamethoxazole against MRSA was significantly better than that of linezolid, rifampicin, clindamycin or minocycline, which are the other major oral antibiotics active against MRSA. Moxifloxacin was more rapidly bactericidal than trimethoprim/sulfamethoxazole at the 4 and 8 h time-point. However, at 24 h there was no difference in the bactericidal activity of moxifloxacin when compared with trimethoprim/sulfamethoxazole ($P = 0.8$). The use of moxifloxacin alone for MRSA infections is limited by the low rate of susceptibility and, perhaps even more importantly, by the rapid one-step emergence of resistance among putatively susceptible isolates, as shown at 48 h in one of the three isolates we studied. Our findings are consistent with other time–kill studies of trimethoprim/sulfamethoxazole against resistant S. aureus.5 Combinations of the various antibiotics with rifampicin did not increase in vitro activity. Specifically, adding rifampicin to trimethoprim/sulfamethoxazole, a common clinical practice, showed a trend towards antagonism. A limitation of our study is the clonal relatedness within both CA- and HA-MRSA as revealed by PFGE. However, variability in the antimicrobial susceptibility pattern and the epidemiological unrelatedness of the isolates suggest that the strains were not identical. As expected, the CA and HA clones were unrelated.

We were unable to find prospective studies of trimethoprim/sulfamethoxazole in treating patients with MRSA infections of skin and soft tissue. Extensive study of S. aureus early in the antibiotic era showed a good correlation between in vitro bactericidal effect and successful treatment in vivo.6 More recent results, however, have cast doubt on the relationship between in vitro killing and in vivo efficacy for the treatment of S. aureus infections. For example, despite having less killing in vitro, vancomycin was shown to be superior to trimethoprim/sulfamethoxazole for the treatment of endocarditis and septic thrombophlebitis in intravenous drug users.7 However, for unclear reasons all treatment failures in the study were in patients with methicillin-susceptible S. aureus infection. Similarly, while linezolid has only a modest killing effect on S. aureus in vitro, clinical studies suggest that linezolid may be as effective as vancomycin in treating MRSA skin and soft-tissue infections.8 Finally, while combination therapy with rifampicin has consistently shown indifference or antagonism in vitro, limited clinical data suggest treatment efficacy for S. aureus infections when rifampicin is added.9 This is most convincingly demonstrated in the case of fluoroquinolones and rifampicin in combination, which successfully eradicate S. aureus in the presence of a foreign body.10 Such conflict between in vitro and in vivo data underscores the need for clinical trials in the treatment of S. aureus infections now that resistance to β-lactam antibiotics is widespread. A recent survey of CA-MRSA infections in three US states found that 97% of isolates were still susceptible to trimethoprim/sulfamethoxazole.10

There is marked financial impact of widespread oral treatment of MRSA with drugs such as linezolid. The average wholesale cost of a 10 day course of linezolid is US$1352, as compared with US$18 for trimethoprim/sulfamethoxazole (average wholesale price McKesson; Prime Vendor). In light of the ongoing pressures on physicians to deliver effective but economic care, the tremendous cost disparities between oral treatment options makes the need for clinical data even more compelling. At the present time, the high rate of susceptibility of MRSA to trimethoprim/sulfamethoxazole, its bactericidal activity and its

![Figure 1. Time–kill curves of orally available antimicrobials against MRSA.](https://academic.oup.com/jac/article-abstract/58/3/680/747730/682)
excellent bioavailability along with low cost make trimethoprim/sulfamethoxazole a good option for the oral treatment of susceptible MRSA infections.

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Transparency declarations

None to declare.

References


