correspondence

conventional Mueller–Hinton medium and resembled a nutritionally variant S. aureus, although a specific nutritional requirement was not identified. Modified Kirby–Bauer susceptibility testing was performed on Mueller–Hinton blood agar (with 5% sheep blood) and Columbia blood agar. No zone of inhibition was evident around a 1 μg oxacillin disc. MIC determinations with Etest were 8 and 16 mg/L with Mueller–Hinton and Columbia blood agar, respectively. Large zones of inhibition were found with modified Kirby–Bauer testing of amoxycillin/clavulanic acid (29–32 mm diameter; 20/10 μg disc) and of penicillin (30–32 mm diameter; 10 U disc). Etest penicillin MICs were 0.75–2 mg/L. β-Lactamase determinations with the chromogenic cephalosporin nitrocefin were repeatedly negative even after attempts at induction by oxacillin. Determinations of mecA gene presence by polymerase chain reaction were negative. By modified Kirby–Bauer assessment, the isolate was susceptible to clindamycin, erythromycin, fusidic acid, gentamicin, and vancomycin.

The youth remained an out-patient and was not initially treated since the S. aureus was present in only small amounts among the respiratory flora. A significant clinical deterioration was not apparent. Subsequently, the patient was followed as an out-patient and the resistant S. aureus could not be recovered despite multiple samplings of sputum. Eight months later, however, the patient was admitted to hospital with an acute pulmonary deterioration, and the resistant S. aureus was recovered again. A clinical response to intravenous vancomycin was apparent. We have not been able to recover such a bacterium from any other patient from the cystic fibrosis clinic, and isolation precautions for our patient have been maintained. The bacterial isolate described here poses a problem to both laboratory and infection control services. Without the assessment of β-lactamase status, the organism may have been deemed a hyper-β-lactamase producer. In addition, the lack of the mecA gene would have furthered this supposition. The inhibitory effect of the amoxycillin/clavulanic acid combination was probably a function of the greater susceptibility of the bacterium to penicillin than oxacillin as evident from the MIC determinations. It is crucial therefore that MIC and mecA gene determinations should not be used as the sole criteria for determining oxacillin resistance. deLencastre et al. have proposed that an alternative β-lactamase-independent mechanism, which is distinct from the mecA gene-mediated mechanism, could be responsible for low-level intrinsic resistance.

β-Lactamase-negative MRSA are relatively uncommon. Indeed, both old and recent reviews of β-lactam resistance in S. aureus find that the β-lactamase negative frequency among MRSA is less than 1%. Richardson et al. have previously described a nursery outbreak of β-lactamase negative MRSA. Unlike our own isolate, the epidemic strain from the latter study showed significant penicillin resistance. Both circumstances, however, highlight the unusual nature of β-lactamase negative MRSA and the laboratory diligence which may be required to elucidate them.

References


Effect of minor pH changes on the in-vitro activity of azithromycin and clarithromycin

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Sir,
The in-vitro antibacterial activity of the macrolide antibiotics is known to be affected by pH changes: their activities increase in alkaline conditions and decrease in acidic media. We have previously reported a two-
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four-fold increase in azithromycin MICs and a decrease in zone diameters around azithromycin discs due to acidic conditions produced by incubation in CO₂ compared with ambient air. The standardized method for broth dilution susceptibility testing recommended by the National Committee for Clinical Laboratory Standards (NCCLS) calls for the use of cation adjusted Mueller–Hinton broth (CAMHB) with a pH of 7.3 ± 0.1. When testing susceptibility of bacteria to most macrolides, the pH of the CAMHB can vary from 7.2 to 7.4 without adversely affecting MICs with the standard quality control (QC) strains Staphylococcus aureus ATCC 29213 and Enterococcus faecalis ATCC 29212.

This report documents the effect of minor variations in pH when standard QC strains were tested against a macrolide, clarithromycin, and an azalide, azithromycin. Thirty-five replicate tests were performed with S. aureus ATCC 29213 and 33 tests with E. faecalis ATCC 29212. Broth microdilution tests followed the NCCLS procedures, except that the broth was adjusted to yield a pH of 7.0, 7.2, 7.4 or 7.6 after autoclaving. Drug concentrations included half dilution intervals (each step representing half the usual two-fold serial dilutions) and ranged from 96 mg/L to 0.03 mg/L.

The Table provides the geometric mean MICs of both drugs at each pH against the two QC strains. Whereas a two-fold difference in clarithromycin MICs was observed at pH 7.0 versus 7.6 with both QC strains, MICs of azithromycin showed a four-fold difference with the S. aureus strain and a five-fold difference for the E. faecalis strain. Even within the extremes of the acceptable pH range of 7.2–7.4, there was a nearly two-fold difference in azithromycin MICs. Within that narrow pH range, MICs of clarithromycin differed minimally. Despite the marked susceptibility of azithromycin MICs to minor pH changes, 100% of the MIC results with tests performed at pH 7.2 and 7.4 fell within the recently revised QC range for S. aureus ATCC 29213, and the proposed QC range for E. faecalis ATCC 29212. Outside this pH range, though, the number of MICs within the control limits decreased significantly (see Table). In order to obtain consistent results when testing macrolide and azalide compounds, careful control of the pH of the test medium is essential. Extrapolation of these in-vitro observations to the conditions encountered in vivo is strictly a matter of conjecture. We can only conclude that absolute MIC values of azithromycin are elusive since they can be altered by very minor changes in the pH of the medium. For reproducible susceptibility test results, the pH of the test medium must be strictly controlled.

References


Table. Effect of pH on azithromycin and clarithromycin MICs with two quality control strains

<table>
<thead>
<tr>
<th>QC strain</th>
<th>pH</th>
<th>Azithromycin geometric mean % in control</th>
<th>Clarithromycin geometric mean % in control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>geometric mean MIC (mg/L)</td>
<td>geometric mean MIC (mg/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% in control</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>7.0</td>
<td>3.00</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>1.47</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>0.89</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>0.79</td>
<td>100</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>7.0</td>
<td>17.27</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>12.14</td>
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</tr>
<tr>
<td></td>
<td>7.6</td>
<td>3.44</td>
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</tr>
</tbody>
</table>

a Geometric mean MIC of 35 replicate tests with S. aureus ATCC 29213 and 33 tests with E. faecalis ATCC 29212.
b Percentage of tests results within control limits.