support of this, we found that expression of the pan-NK marker CD56 on malaria-responsive γδ-T cells correlated with the magnitude of IFN-γ responses in semi-immune Papua New Guinean children [1]. Similarly, in malaria-naive individuals, expression of the NK complex-encoded receptor CD94 on γδ-T cells correlated with IFN-γ output [8]. Thus, NK-receptor expression on NK-like γδ-T cells (and possibly NK cells) may partially control IFN-γ responsiveness to P. falciparum, as does NK-receptor expression in Plasmodium berghei murine malaria [14, 15]. Parasite factors, such as P. falciparum erythrocyte membrane-1, which negatively regulates early IFN-γ production [16], may also be involved, as well as other factors, as age and previous exposure to P. falciparum.

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Doxycycline, Not Minocycline, Induces Its Own Resistance in Multidrug-Resistant, Community-Associated Methicillin-Resistant Staphylococcus aureus Clone USA300

To the Editor—Recently, a multidrug-resistant (MDR) strain of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA)—genotype USA300, which harbors plasmids that confer resistance to tetracycline, macrolides, and clindamycin—was identified [1]. Some experts propose that doxycycline or minocycline may be effective antimicrobials for the treatment of infections due to MDR strains of CA-MRSA genotype USA300 [2]. This recommendation was based on a study that examined 31 tetracycline-resistant CA-MRSA isolates that carried the tetracycline resistance gene, tet(K); none had tet(M). These isolates were all found to be susceptible to doxycycline and minocycline on the basis of broth microdilution testing [3]. In a study by Trzcinski et al. [4], for MRSA isolates that tested positive for tet(K) and negative for tet(M), minumum inhibitory concentrations (MICs) of doxycycline, but not minocycline, were increased after exposure to subinhibitory concentrations of tetracycline. We speculate that doxycycline, but not minocycline, would induce its own resistance in CA-MRSA isolates that tested positive for tet(K) and negative for tet(M). We evaluated the susceptibility of tetracycline-resistant CA-MRSA strains, including MDR strains of genotype USA300, to doxycycline and minocycline and the effect of incubation in subinhibitory concentrations of tetracyclines on these MICs.

We collected 60 clinical MRSA isolates during 2004–2006 that were reported to be tetracycline resistant on the basis of broth microdilution testing. MICs of tetracycline, doxycycline, and minocycline—which were determined by use of the Etest (AB Biodisk)—confirmed that 55 (92%) of the 60 isolates were tetracycline resistant. Genotyping was performed by pulsed-field gel electrophoresis [1] and revealed that 53 (96%) of the 55 tetracycline-resistant isolates were CA-MRSA genotype USA300; of those 53 isolates, 16 (30%) were identified as containing the pUSA03 plasmid by use of whole-plasmid
Table 1. Mean minimum inhibitory concentrations (MICs) of multidrug-resistant strains of community-associated methicillin-resistant *Staphylococcus aureus* genotype USA300 before and after antibiotic exposure.

<table>
<thead>
<tr>
<th>Antibiotic exposure of isolates</th>
<th>Tetracycline</th>
<th>Doxycycline</th>
<th>Minocycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24.4 (12–96)</td>
<td>1.6 (1.5–2)</td>
<td>0.13 (0.094–0.19)</td>
</tr>
<tr>
<td>0.1 μg/mL of tetracycline</td>
<td>45.8 (32–64)</td>
<td>2.4 (2–3)</td>
<td>0.13 (0.125–0.19)</td>
</tr>
<tr>
<td>0.1 μg/mL of doxycycline</td>
<td>114.1 (64–192)</td>
<td>5.1 (4–6)</td>
<td>0.15 (0.125–0.19)</td>
</tr>
<tr>
<td>0.5 μg/mL of doxycycline</td>
<td>69.4 (64–96)</td>
<td>8.7 (8–12)</td>
<td>0.15 (0.125–0.19)</td>
</tr>
<tr>
<td>0.01 μg/mL of minocycline</td>
<td>105.2 (64–256)</td>
<td>6.7 (6–8)</td>
<td>0.15 (0.125–0.19)</td>
</tr>
</tbody>
</table>

The difference in the geometric mean MIC of doxycycline between the noninduced strains and the induced strains at 0.1 and 0.5 μg/mL was P<.001 for each comparison.

DNA sequencing [1]. The tetracycline resistance genes *tet(K)* and *tet(M)* were identified by use of polymerase chain reaction, as previously described elsewhere [5], with the FPR3757 strain as the positive control for *tet(K)* and the negative control for *tet(M)* and with the NRS1 strain as the positive control for *tet(M)* and the negative control for *tet(K)*.

Of the 55 tetracycline-resistant isolates identified by use of the Etest, 52 (95%) tested positive for the *tet(K)* gene and negative for the *tet(M)* gene; 2 (4%) tested negative for *tet(K)* and positive for *tet(M)*; and 1 (2%) tested positive for *tet(K)* and positive for *tet(M)*. Of the 52 isolates that tested positive for *tet(K)* and negative for *tet(M)*, 5 (10%) were selected for further study. Strains grown overnight in tryptic soy broth were diluted to 10^6 colonies/mL in tryptic soy broth that contained subinhibitory concentrations of tetracycline (0.1 μg/mL), doxycycline (0.1 and 0.5 μg/mL), or minocycline (0.01 μg/mL) and were grown until a cell density of 10^8 colonies/mL was achieved. Susceptibility testing was repeated using the Etest (table 1).

These isolates demonstrated an increase in the mean MIC of doxycycline above the Clinical and Laboratory Standards Institute (CLSI) breakpoint for susceptibility (i.e., 4 μg/mL) after incubation in broth containing doxycycline MICs of 0.1 and 0.5 μg/mL; incubation in broth containing tetracycline and minocycline caused similar increases. Minocycline MICs were unchanged after incubation in subinhibitory concentrations of minocycline.

Our data corroborate the data from previous smaller studies that reported tetracycline-resistant CA-MRSA strains, including MDR strains of clone USA300, were predominantly resistant as a result of the *tet(K)* gene. Concentrations of doxycycline and minocycline that were within the therapeutic range for doxycycline in serum [6] induced *tet(K)*-mediated resistance, raising the MIC of doxycycline above the CLSI susceptibility breakpoint of 4 μg/mL. Minocycline susceptibility was not affected by *tet(K)*, even after incubation in subinhibitory concentrations of minocycline. This difference in susceptibility indicates that doxycycline, but not minocycline, is a substrate for the drug-inducible efflux pump encoded by *tet(K)*.

As alternative antimicrobials are examined for the treatment of infections due to MDR strains of CA-MRSA genotype USA300, caution is recommended in the use of doxycycline, given its ability to induce resistance in CA-MRSA isolates that tested positive for *tet(K)* gene and negative for *tet(M)*. Minocycline may be an efficacious option, because we did not identify this type of inducible resistance to minocycline.

References


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