High Frequencies of Clindamycin and Tetracycline Resistance in Methicillin-Resistant
Staphylococcus aureus Pulsed-Field Type USA300 in a Boston Ambulatory Health Center

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Running Title: ANTIMICROBIAL RESISTANCE IN MRSA USA300
Individual and multiple resistance to clindamycin, tetracycline, erythromycin, levofloxacin and mupirocin were detected in a large proportion of methicillin-resistant Staphylococcus aureus pulsed-field type USA300 isolates collected at an ambulatory health center in Boston. The clindamycin, tetracycline, and mupirocin resistance genes identified in these isolates are commonly associated with plasmids.

Methicillin-resistant Staphylococcus aureus (MRSA) has long been recognized as an important cause of nosocomial morbidity and mortality, and more recently, as a cause of disease arising in the community among previously healthy persons without traditional risk factors for infection (referred to as community-associated MRSA). One particular strain of MRSA, designated USA300-0114 based on pulsed-field gel electrophoresis (PFGE) typing, has emerged as a predominant and widely disseminated strain linked to transmission in community settings nationwide (12). Most USA300 isolates are resistant only to β-lactam and macrolide antimicrobial agents; however, isolates resistant to tetracycline, clindamycin, fluoroquinolones, and mupirocin have been reported (3, 4, 12). This report describes: (1) high prevalences of clindamycin and tetracycline resistance among USA300 isolates collected at a single urban ambulatory health center in Boston; and (2) the identification of multiple resistance to erythromycin, clindamycin, levofloxacin, tetracycline, and mupirocin in a MRSA strain closely related to USA300-0114 (USA300-0247), and in one isolate of USA300-0114.

All MRSA isolates collected at the health center during the 19-month period between May 2004 and November 2005 were forwarded to the Massachusetts Department of Public Health State Laboratory Institute for PFGE typing. Over 50% of patients attending this health center are men who report that they have sex with men (MSM), and many health center patients
are HIV-infected (7, 9). PFGE was performed with Smal enzyme digestion as previously described (8). Gel analyses were performed with BioNumerics software, version 4.0, Applied Maths, Kortrijk, Belgium. Antimicrobial susceptibility testing (AST) was performed with the Dade MicroScan WalkAway instrument (West Sacramento, CA). Isolates resistant to erythromycin and susceptible to clindamycin were subjected to disk diffusion testing for detection of inducible clindamycin resistance (D-zone test) (2). AST was also performed on a second set of USA300 isolates, comprised of all isolates collected during a similar time period from outpatients tested at a Boston-area community health network serving adult and pediatric patients at 3 hospitals and 20 primary care practices. A subset of isolates resistant to erythromycin, clindamycin, levofloxacin, and tetracycline was forwarded to the Centers for Disease Control and Prevention (CDC), Atlanta, for broth microdilution susceptibility and PCR testing. Susceptibilities to minocycline, doxycycline, and mupirocin were determined by the reference broth microdilution method described by the Clinical and Laboratory Standards Institute using cation-adjusted Mueller-Hinton broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) (2). Quality control strains included S. aureus ATCC 29213, Enterococcus faecalis ATCC 25922, and S. aureus ATCC 43300. PCR testing was performed to identify genes conferring tetracycline resistance (tetK, tetM), inducible or constitutive clindamycin resistance (ermA, ermC), and mupirocin resistance (mupA) (10, 11, 12).

Between May 2004 and November 2005, culture specimens yielding MRSA were obtained from 123 health center patients. Only the first MRSA isolate collected from each patient was included in this analysis. Among 115 isolates with a known source, 103 (90%) were collected from skin and soft tissue sites, 11 (10%) from nares or nasopharynx, and 1 (1%) from urine. Among 123 total isolates, 102 (83%) had PFGE patterns corresponding to either MRSA
strain type USA300-0114 (73 isolates, 59% of total) or USA300-0247 (29 isolates, 24% of total)(Figure). AST data are summarized in the Table. All 12 multi-resistant isolates (11 USA300-0247 and 1 USA300-0114) tested at CDC contained \textit{tetK} and \textit{ermC} genes; none contained \textit{tetM} or \textit{ermA} genes. All were susceptible to minocycline and doxycycline, and all contained the \textit{mupA} resistance gene and had mupirocin MICs of $\geq 128$ µg/ml. Among 26 USA300-0114 and 4 USA300-0247 isolates collected from the nearby health network, only 2 were clindamycin-resistant and none were tetracycline-resistant.

In a recent report of MRSA skin infections among adult emergency department patients in 11 U.S. cities, 97% of MRSA isolates were USA300, 5% were resistant to clindamycin, 8% were resistant to tetracycline (9), and 1% were resistant to both clindamycin and tetracycline (Gregory E. Fosheim, unpublished data). The prevalence of resistance to these agents among USA300 isolates in our investigation, particularly USA300-0247 isolates, was considerably higher, and was also higher than the prevalence of resistance among isolates collected at the nearby health network. Furthermore, we identified multiple resistance to erythromycin, clindamycin, levofloxacin, and tetracycline in 55% of USA300-0247 isolates collected; and elevated mupirocin MICs ($\geq 128$ µg/ml) were detected in all 12 isolates for which mupirocin MICs were determined. USA300 isolates with multiple resistance to the same agents have previously been reported among patients of a San Francisco community health network (4). Multiple resistance has also been identified in non-USA300 MRSA strains isolated from children in community settings in Taiwan (1). In a study involving HIV-infected MSM with skin infections in Los Angeles, 3.2% and 35.5% of MRSA isolates were resistant to clindamycin and tetracycline, respectively, and multiple resistance was not reported (6). Examination of clinical and demographic features of our case-patients, including antibiotic exposure and underlying
disease conditions, may prove useful in identifying risk factors and elucidating mechanisms associated with resistance acquisition in this community. On a molecular level, an important mechanism of resistance acquisition in USA300 MRSA appears to be the transfer of plasmids from bacterial reservoirs; in the isolates studied in this report, resistance to clindamycin, tetracycline, and mupirocin are all mediated by resistance genes that are typically located on plasmids (4, 11, 12).

Among USA300 isolates resistant to tetracycline, we consistently found \textit{in vitro} susceptibility to minocycline, doxycycline, and trimethoprim-sulfamethoxazole. These findings suggest several inexpensive oral treatment options for skin infections with multi-drug resistant USA300 isolates. However, the clinical efficacy of these drugs in the treatment of MRSA infections has not been extensively documented, and incision and drainage should still be considered the primary therapy when skin abscesses are present (5). The high prevalence of elevated mupirocin MICs described in this report raises concerns about the \textit{in vivo} efficacy of mupirocin for eradication of MRSA nasal colonization in certain populations. The appearance of multi-drug resistant MRSA in the community setting emphasizes the importance of routine collection of specimens for culture and AST, not only for individual patient management, but also for development of community-wide treatment and prevention strategies.

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References


Figure: Comparison of PFGE SmaI macrorestriction patterns of the two major USA300 strain types found at the health center.
TABLE: Prevalence of resistance to antimicrobial agents among predominant USA300 MRSA strain types at a Boston ambulatory health center, 2004-2005

<table>
<thead>
<tr>
<th>Resistance to:</th>
<th>USA300-0114</th>
<th>USA300-0247</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>72 / 73</td>
<td>26 / 29</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>58 / 73</td>
<td>29 / 29</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>36 / 73</td>
<td>22 / 29</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10 / 73</td>
<td>21 / 29</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 / 73</td>
<td>0 / 29</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0 / 73</td>
<td>0 / 29</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0 / 73</td>
<td>0 / 29</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0 / 73</td>
<td>0 / 29</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0 / 73</td>
<td>0 / 29</td>
</tr>
<tr>
<td>Erythromycin, levofloxacin, clindamycin, and tetracycline</td>
<td>2 / 73</td>
<td>16 / 29</td>
</tr>
<tr>
<td>Inducible clindamycin resistance on D-zone test</td>
<td>0 / 33</td>
<td>0 / 6</td>
</tr>
<tr>
<td>Mupirocin MIC ≥128 µg/ml*</td>
<td>1 / 1</td>
<td>11 / 11</td>
</tr>
</tbody>
</table>

* Mupirocin MICs were determined for a subset of isolates found to be resistant to erythromycin, levofloxacin, clindamycin, and tetracycline.