Antimicrobial Susceptibility Profiles of *Staphylococcus aureus* Isolates Recovered from Humans, Environmental Surfaces, and Companion Animals in Households of Children with Community-Onset Methicillin-Resistant *S. aureus* Infections

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Running Title: *S. aureus* Antimicrobial Susceptibility in Households

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Abstract

Our objective was to determine antibiotic susceptibility profiles of Staphylococcus aureus isolates recovered from 110 households of children with community-onset methicillin-resistant S. aureus (MRSA) infections. Cultures were obtained from household members, household objects, and dogs and cats, yielding 1,633 S. aureus isolates. The S. aureus isolates were heterogeneous, although more than half were methicillin-resistant. The highest proportion of MRSA was found in bathrooms. The majority of isolates were susceptible to antibiotics prescribed in outpatient settings.
Antimicrobial resistant bacterial infections are a global problem (1-3). Few studies have described the antimicrobial resistance profiles of *Staphylococcus aureus* strains in the community, specifically in household environments (4, 5). Household vectors, including humans, environmental fomites, and companion animals, may serve as reservoirs for MRSA transmission (5-7). We describe the antibiotic susceptibility patterns of *S. aureus* isolates recovered from these household vectors.

Understanding the antibacterial resistance profiles of *S. aureus* strains in the environment may inform empiric antibiotic selection in clinical settings.

Following approval from the Washington University Human and Animal Institutional Review Boards, pediatric patients (n=110) with community-onset MRSA infections and their household contacts (n=388) were enrolled through St. Louis Children’s Hospital (SLCH) and community pediatric practices from January 2013 to May 2014, as previously described (4). Study visits were conducted in participant’s homes, occurring up to 10 times over 24 months in 3 month intervals. During each visit, cultures were obtained from the axillae, anterior nares, and inguinal folds (Eswabs, Becton Dickinson [BD], Franklin Lakes, NJ) from all consenting household members, up to 21 frequently touched standardized household objects (*Table 1*, Eswabs and Baird Parker Agar contact plates, Hardy, Santa Maria, CA) (4, 8), and the anterior nares and dorsal fur of indoor dogs and cats (BBL CultureSwab Liquid Amies, Regular Aluminum Wire, BD).

In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines for creating a cumulative antibiogram report (9), the first *S. aureus* isolate recovered from each pet, household object, or body site of each household member was included in the analysis. Antibiotic susceptibility testing (*Table 1*) of *S. aureus* isolates was performed by Kirby-Bauer disk diffusion (10, 11). High-level mupirocin resistance was confirmed by detection of *mupA* (12). Isolates with intermediate susceptibilities were
categorized as “resistant” (11). MRSA isolates resistant to β-lactams plus three additional systemic antimicrobial classes (i.e., excluding mupirocin) were classified as multi-drug resistant (MDR4) (13, 14).

Statistical analysis was conducted with SPSS 22 for Windows (IBM SPSS, Chicago, IL). Isolate susceptibilities were compared between human, pet, and environmental isolates using the Fisher’s exact or chi-square test. P-values <0.05 were considered significant.

As summarized in Table 1, 1,633 unique S. aureus isolates were characterized, including 770 human isolates (47%) (110 from sites of infection, 660 from sites of colonization), 815 environmental isolates (50%), and 48 companion animal isolates (3%) (39 from dogs, 9 from cats). Overall, 52% of the S. aureus isolates recovered from household environmental surfaces were methicillin resistant, as were 52% of human colonization isolates (index patients and household contacts), and 63% of pet colonization isolates. All isolates were susceptible to trimethoprim-sulfamethoxazole, linezolid, ceftaroline, and rifampin, while the majority of isolates were susceptible to clindamycin, tetracycline, and mupirocin (Table 1). Overall, multidrug resistance (MDR4) was low, comprising 4% of all recovered S. aureus isolates.

S. aureus isolates recovered from index patient infection cultures had a higher prevalence of erythromycin (83%) and ciprofloxacin (58%) resistance when compared to isolates recovered from index patient colonization sites (54%, p<0.001 and 33%, p<0.001, respectively). Index patient infecting isolates trended to possess a higher prevalence of MDR4 than their colonizing isolates (9% vs. 3%, p=0.07). There were no significant differences in antibiotic susceptibilities between colonizing isolates recovered from index patients and those from household contacts.

Household environmental surfaces with the highest prevalence of MRSA isolates were the soap bar/dish in the bathtub/shower (71%), bathroom hand towel (68%), and telephone (64%). MDR4 isolates were most commonly recovered from the soap bar/dish in the bathtub/shower (6%),
refrigerator door handle (6%), computer keyboard/mouse (5%), and bathroom light switch (5%). There were no significant differences in antibiotic susceptibilities when comparing isolates recovered from different areas of the home, e.g. living room, bathroom, kitchen, and bedroom.

The overall number of *S. aureus* isolates recovered from pets was relatively small (n=48), though these isolates were most frequently methicillin resistant (63%) compared to isolates recovered from human (58%) or environmental sources (52%; p=0.03). Companion animal isolates also had the highest prevalence of MDR4 (8%) when compared to human (4%) or environmental isolates (3%; p=0.04). Resistance to erythromycin and mupirocin was higher in isolates recovered from cats compared to dogs (89% vs. 44%, p=0.02; 22% vs. 0%, p=0.03, respectively).

In this study of antimicrobial susceptibility patterns of *S. aureus* isolated from household vectors, more than half of the recovered isolates were MRSA; of note, the majority of isolates were susceptible to systemic antibiotics commonly prescribed for *S. aureus* infections in outpatient settings (15), and were universally susceptible to the newer antimicrobials linezolid and ceftaroline (16, 17). Interestingly, 5% of isolates recovered from index patients were mupirocin resistant, which is higher than findings of a prior study by our group conducted from 2007-2009, in which 2% (50 of 2,425) of *S. aureus* isolates collected from a similar patient population were mupirocin resistant (12).

Environmental surfaces may serve as reservoirs for MRSA transmission within households. In this study, the soap bar/dish, bathroom hand towel, and telephone possessed the highest prevalence of MRSA. Additionally, MDR4 strains were commonly recovered from the refrigerator door handle, computer keyboard/mouse, and bathroom light switch. Similar to other studies, these findings may reflect the high frequency of contact with these surfaces by a variety of household members, compared to surfaces likely to be unique to the index patient (e.g., bed linens or bath towel) (5, 18). In our population, a high proportion of strains recovered from pet dogs and cats were MRSA, consistent with a
notable increase in the prevalence of antibiotic resistant staphylococcal strains in companion animals over the past decade (19, 20).

The present study analyzed a broad range of antimicrobial susceptibility profiles of *S. aureus* isolates from households of children with MRSA infections. Strengths of this study include the large number of isolates collected and the breadth, standardization, and longitudinal sampling of humans, household environments, and companion animals. Though the isolates are from a single metropolitan area, the households represent a diverse geographic (121-mile diameter) and socio-demographic catchment. A limitation is that several sampled sites (cats and various environmental surfaces) did not provide the minimum number - 30 - of isolates necessary to audit an antibiogram profile (11), which may provide a limited picture of antimicrobial trends.

In conclusion, we observed a heterogeneous population of *S. aureus* isolates in households of children with MRSA infections. As personal *S. aureus* colonization, as well as colonization of household contacts and environmental surfaces, are putative reservoirs for subsequent infection, we are encouraged by the fact that the majority of isolates were susceptible to commonly prescribed antibiotics for community-onset *S. aureus* infection.
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Table 1. Antimicrobial susceptibility profiles of *S. aureus* isolates recovered from households of children with community-onset MRSA infection

<table>
<thead>
<tr>
<th>Location Isolate Recovered</th>
<th>Number <em>S. aureus</em> isolates tested</th>
<th>% Susceptible</th>
<th>% MDR&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1633</td>
<td>45 90 47 100 100 98 68 100 100 96 4</td>
<td></td>
</tr>
<tr>
<td>People</td>
<td>770</td>
<td>42 90 44 100 100 98 65 100 100 96 4</td>
<td></td>
</tr>
<tr>
<td>Index Patient</td>
<td>263</td>
<td>26 91 34 100 100 99 58 100 100 95 5</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>110&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 94 17 100 100 99 42 100 100 98 9</td>
<td></td>
</tr>
<tr>
<td>Colonization</td>
<td>153</td>
<td>44 89 46 100 100 99 67 100 100 94 3</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>61</td>
<td>48 89 53 100 100 100 67 100 100 97 3</td>
<td></td>
</tr>
<tr>
<td>Axillae</td>
<td>31</td>
<td>42 90 48 100 100 100 61 100 100 87 0</td>
<td></td>
</tr>
<tr>
<td>Inguinal folds</td>
<td>61</td>
<td>41 89 39 100 100 98 69 100 100 95 3</td>
<td></td>
</tr>
<tr>
<td>Household Contact Colonization&lt;sup&gt;f&lt;/sup&gt;</td>
<td>507</td>
<td>50 89 49 100 100 98 68 100 100 96 4</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>228</td>
<td>55 90 52 100 100 99 69 100 100 97 4</td>
<td></td>
</tr>
<tr>
<td>Axillae</td>
<td>110</td>
<td>44 90 46 100 100 99 69 100 100 95 5</td>
<td></td>
</tr>
<tr>
<td>Inguinal folds</td>
<td>169</td>
<td>47 88 47 100 100 96 67 100 100 95 4</td>
<td></td>
</tr>
<tr>
<td>Adult Household Contact Colonization</td>
<td>278</td>
<td>49 88 45 100 100 97 67 100 100 95 4</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>120</td>
<td>56 88 48 100 100 98 69 100 100 96 3</td>
<td></td>
</tr>
<tr>
<td>Axillae</td>
<td>62</td>
<td>42 90 40 100 100 98 69 100 100 95 3</td>
<td></td>
</tr>
<tr>
<td>Inguinal folds</td>
<td>96</td>
<td>46 85 44 100 100 95 63 100 100 93 5</td>
<td></td>
</tr>
<tr>
<td>Children Household Contact Colonization</td>
<td>229</td>
<td>51 91 55 100 100 99 69 100 100 97 4</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>108</td>
<td>55 91 57 100 100 99 69 100 100 99 5</td>
<td></td>
</tr>
<tr>
<td>Axillae</td>
<td>48</td>
<td>46 90 52 100 100 100 67 100 100 94 6</td>
<td></td>
</tr>
<tr>
<td>Inguinal folds</td>
<td>73</td>
<td>48 91 52 100 100 99 73 100 100 97 3</td>
<td></td>
</tr>
<tr>
<td>Pets</td>
<td>48</td>
<td>38 85 48 100 100 100 69 100 100 96 8</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>39</td>
<td>41 87 56 100 100 100 74 100 100 100 8</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>16</td>
<td>38 88 69 100 100 100 81 100 100 100 6</td>
<td></td>
</tr>
<tr>
<td>Dorsal Fur</td>
<td>23</td>
<td>44 87 48 100 100 100 70 100 100 100 9</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>9</td>
<td>22 78 11 100 100 100 44 100 100 78 11</td>
<td></td>
</tr>
<tr>
<td>Anterior nares</td>
<td>4</td>
<td>25 75 25 100 100 100 25 100 100 75 25</td>
<td></td>
</tr>
<tr>
<td>Dorsal Fur</td>
<td>5</td>
<td>20 80 0 100 100 100 60 100 100 80 0</td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>815</td>
<td>48 90 50 100 100 99 71 100 100 97 3</td>
<td></td>
</tr>
<tr>
<td>Living room</td>
<td>166</td>
<td>44 88 52 100 100 99 69 100 100 97 4</td>
<td></td>
</tr>
<tr>
<td>TV remote control</td>
<td>52</td>
<td>42 90 44 100 100 98 67 100 100 96 4</td>
<td></td>
</tr>
<tr>
<td>Telephone</td>
<td>36</td>
<td>36 92 53 100 100 100 71 100 100 97 0</td>
<td></td>
</tr>
</tbody>
</table>
Computer keyboard and mouse | 37  | 51  | 87  | 62  | 100 | 100 | 100 | 73  | 100 | 100 | 97  | 5 |
Videogame controller | 41  | 46  | 81  | 54  | 100 | 100 | 100 | 98  | 66  | 100 | 100 | 98  | 5 |
Bathroom | 404 | 50  | 90  | 49  | 100 | 100 | 100 | 98  | 72  | 100 | 100 | 98  | 2 |
Sink faucet handle | 43  | 49  | 86  | 49  | 100 | 100 | 98  | 67  | 100 | 100 | 98  | 5 |
Hand towel | 22  | 32  | 86  | 41  | 100 | 100 | 100 | 62  | 100 | 100 | 96  | 5 |
Index bath towel | 27  | 63  | 93  | 59  | 100 | 100 | 100 | 78  | 100 | 100 | 100 | 0 |
Toilet handle | 33  | 58  | 88  | 55  | 100 | 100 | 94  | 79  | 100 | 100 | 100 | 0 |
Door handle | 32  | 47  | 91  | 41  | 100 | 100 | 100 | 63  | 100 | 100 | 97  | 0 |
Light switch | 39  | 49  | 92  | 49  | 100 | 100 | 100 | 64  | 100 | 100 | 100 | 5 |
Sink | 52  | 48  | 94  | 54  | 100 | 100 | 100 | 75  | 100 | 100 | 98  | 0 |
Bathtub or shower | 44  | 50  | 89  | 46  | 100 | 100 | 96  | 82  | 100 | 100 | 98  | 2 |
Soap bar or dish in bathtub or shower | 17  | 29  | 82  | 29  | 100 | 100 | 88  | 65  | 100 | 100 | 100 | 6 |
Toilet seat | 47  | 60  | 89  | 55  | 100 | 100 | 98  | 81  | 100 | 100 | 100 | 0 |
Countertop | 48  | 48  | 92  | 46  | 100 | 100 | 96  | 70  | 100 | 100 | 96  | 2 |
Kitchen | 190 | 49  | 91  | 52  | 100 | 100 | 99  | 71  | 100 | 100 | 95  | 3 |
Hand towel | 22  | 55  | 91  | 50  | 100 | 100 | 100 | 68  | 100 | 100 | 96  | 0 |
Sink faucet handle | 35  | 46  | 97  | 60  | 100 | 100 | 100 | 66  | 100 | 100 | 94  | 3 |
Sponge/cloth | 32  | 47  | 94  | 59  | 100 | 100 | 100 | 75  | 100 | 100 | 94  | 3 |
Refrigerator door handle | 56  | 47  | 86  | 49  | 100 | 100 | 100 | 71  | 100 | 100 | 95  | 6 |
Kitchen table | 46  | 52  | 91  | 44  | 100 | 100 | 98  | 74  | 100 | 100 | 96  | 0 |
Bedroom | 55  | 40  | 89  | 46  | 100 | 100 | 98  | 64  | 100 | 100 | 95  | 4 |
Bed sheets and pillowcase | 55  | 40  | 89  | 46  | 100 | 100 | 98  | 64  | 100 | 100 | 95  | 4 |

Abbreviations: MET, methicillin; CLI, clindamycin; ERY, erythromycin; SXT, trimethoprim-sulfamethoxazole; RIF, rifampin; TET, tetracycline; CIP, ciprofloxacin; LZD, linezolid; CPT, ceftaroline; MUP, mupirocin; MDR4, multi-drug resistance.

* As predicted by cefoxitin testing.
* Clindamycin susceptible isolates exhibiting inducible clindamycin resistance (n=144) were considered clindamycin resistant.
* Multi-drug resistance: β-lactam resistance plus resistance to three additional systemic antimicrobial drug classes (i.e. excluding mupirocin).
* Some infection isolates were unable to be obtained by the study team and are thus missing various susceptibility data; therefore % susceptible is out of less than 110: RIF (N=84), CIP (N=85), LZD (N=76), CPT (N=64), MUP (N=64).
* Study entry criteria specified an MRSA infection.
* Does not include the isolates recovered from index patients.