Aerococcus urinae may cause urinary tract infections, bacteremia, and endocarditis. No standardized susceptibility test methods or interpretive criteria have been proposed for this organism. This study reports the MIC results for 128 A. urinae isolates tested by broth microdilution. The isolates had low MICs to amoxicillin, cefotaxime, ceftriaxone, doxycycline, linezolid, meropenem, penicillin, rifampin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. However, 55% of the isolates had MICs to clindamycin of >0.25 μg/ml, 44% had MICs to erythromycin of >0.25 μg/ml, and 16% had MICs to levofloxacin of >2 μg/ml.

In the laboratory, A. urinae, particularly when isolated from urine, may be misidentified as an alpha-hemolytic Streptococcus strain due to shared phenotypic properties, including colony morphology and negative catalase reactions (13, 20). However, improved diagnostic technologies, such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (21), are allowing clinical laboratories to correctly identify A. urinae with increasing frequency.

The lack of standardized susceptibility test methods and interpretive criteria for Aerococcus spp. are problematic for clinical laboratories and clinicians. There are a limited number of published studies that address susceptibility testing of A. urinae, and these usually include a small number of isolates. A variety of test methods have been reported, and interpretive criteria for streptococci (2), staphylococci (22), and even enterococci (R.M.H., personal observation) have been applied. The Clinical and Laboratory Standards Institute (CLSI) has described a broth microdilution MIC test for Streptococcus pneumoniae and Streptococcus spp. that utilizes Mueller-Hinton broth supplemented with 2.5 to 5% lysed horse blood (23). In this study, we report the results of antimicrobial susceptibility testing for a collection of 128 unique A. urinae isolates, performed using this method, against 14 antimicrobial agents. Based on information in the literature and the MIC distributions obtained in our study, we propose the use of CLSI viridans group streptococcal interpretive criteria, all isolates demonstrated good growth, and the MIC results obtained for the 128 isolates are shown in the Table 1. Using the CLSI viridans group streptococcal interpretive criteria, all isolates were susceptible to penicillin (MIC ≤ 0.12 μg/ml), which is similar to a previous report in which 54/56 A. urinae isolates had MICs to penicillin of ≤0.12 μg/ml using agar dilution and Mueller-Hinton agar supplemented with 5% lysed horse blood (18). Four isolates had MICs to amoxicillin of >0.12 μg/ml (Table 1), although the modal MIC for amoxicillin was 1 dilution lower than that of penicillin (Table 1). No interpretive criteria have been set for viridans group streptococci for amoxicillin, and so at this time, we are not proposing interpretive criteria for A. urinae (Table 1). Interestingly, the modal MIC for cefotaxime and ceftriaxone was 0.25 μg/ml, which was significantly higher than the modal MICs of penicillin (0.03 μg/ml) and amoxicillin (0.015 μg/ml) (P = 0.014, Student’s t test). The modal ceftriaxone MIC obtained for the isolates tested in our study was significantly lower than that obtained by Skov and colleagues (18), in which a modal MIC of 2 μg/ml was noted when using agar dilution; this difference may be due in part to the different test methods used. In a second study, Sierra-Hoffman and colleagues (2) noted only 87.7% susceptibility (MIC ≤ 1 μg/ml) to ceftriaxone using the viridans group streptococci interpretive criteria and disk diffusion or Etest (bioMérieux, Marcy l’Etoile, France) on sheep blood agar, among 49 A. urinae isolates, although MIC distributions were not reported in that study (2). Using the CLSI viridans group strepto-
TABLE 1 MICs of 14 antimicrobials for *A. urinae* (n = 128), tested by broth microdilution in Mueller-Hinton broth supplemented with 2.5% lysed horse blood and incubated in 5% CO₂, and proposed interpretive criteria, based on CLSI viridans group streptococci and *Staphylococcus sp.* interpretive criteria

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of isolates with MIC (µg/ml) of:</th>
<th>Modal MIC (µg/ml)</th>
<th>% susceptible</th>
<th>Proposed breakpoint (µg/ml) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.015</td>
<td>0.03</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Penicillin</td>
<td>39</td>
<td>69</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>42</td>
<td>32</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>5</td>
<td>12</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>4</td>
<td>6</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Meropenem</td>
<td>59</td>
<td>33</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>39</td>
<td>45</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>32</td>
<td>26</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>103</td>
<td>14</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Doxycline</td>
<td>35</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>70</td>
<td>32</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>SXT</td>
<td>63</td>
<td>28</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Rifampin</td>
<td>128</td>
<td>80</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Linezolid</td>
<td>32</td>
<td>58</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>5</td>
<td>38</td>
<td>80</td>
<td>5</td>
</tr>
</tbody>
</table>

**a** NP, no proposed breakpoint.

**b** NS, due to the rare occurrence of isolates with MICs outside the susceptible range, no intermediate or resistant categories are suggested.

**c** MIC less than or equal to the value in the column header.

**d** MIC greater than or equal to the value in the column header.

Coecal interpretive criteria, 96% of the isolates in this study were susceptible to ceftriaxone and 99% were susceptible to cefotaxime (Table 1). All but one isolate tested susceptible to meropenem (MIC ≤ 0.5 µg/ml), with a modal MIC of ≤0.015 µg/ml for all isolates. The sole meropenem-non-susceptible isolate was also reproducibly resistant to ceftriaxone (4 µg/ml) and cefotaxime (2 µg/ml) but susceptible to penicillin (0.06 µg/ml), according to the viridans group streptococci interpretive criteria. The identification of this isolate was confirmed by partial 16S rRNA gene sequencing, the method for which was described elsewhere (25).

The combination of penicillin and gentamicin has been shown to be synergistic in *in vitro* for *A. urinae* isolates (17, 18), suggesting that, like for the viridans group streptococcus, combination therapy with an aminoglycoside may be prudent for serious infections caused by *A. urinae*. Indeed, in the literature, the majority of in-vivo susceptibility is prevalent in aerococci, but it warrants further study.

Using the viridans group streptococci interpretive criteria (MICs of ≤0.25 µg/ml are susceptible), clindamycin susceptibility was found in 45% of isolates and erythromycin susceptibility in 66%. However, the CO₂ incubation conditions used by our laboratory likely attributed to the low percentage of susceptibility to these two antimicrobials, as this atmosphere lowers the medium pH and yields elevated MICs (24). Despite this, it was unclear why more isolates tested susceptible to erythromycin versus clindamycin by the viridans group streptococci breakpoint, and this was only resolved by modifying the clindamycin susceptibility breakpoint to ≤1 µg/ml. We opted to not propose breakpoints for these antimicrobials at this time (Table 1). Clindamycin modification, via adenylation by enzymes encoded by the *lin* genes, has been described and yields a clindamycin-resistant erythromycin-resistant phenotype (the “L-phenotype”) in strains of *Staphylococcus*, streptococci, and enterococci (27). It is unknown if this mechanism is prevalent in aerococcci, but it warrants further study.

All isolates tested susceptible to vancomycin (MIC ≤ 1 µg/ml) and rifampin (MIC ≤ 1.0 µg/ml) (*Staphylococcus* interpretive criteria), as has been shown in other studies (2, 18). Ninety-five isolates were tested for linezolid susceptibility, among which two had MICs of 4 and 8 µg/ml (i.e., were nonsusceptible) (Table 1).

All but four isolates tested susceptible to SXT when the CLSI *Staphylococcus* interpretive criteria were applied (MIC ≤ 2/38 µg/ml) (Table 1). *A. urinae* organisms are classically described as re-
sistant to SXT *in vitro* (3, 18, 20, 22, 28); however, in a previous study (25), we noted that the thymidine present in sheep blood, which is used to supplement antimicrobial susceptibility testing media in many studies, inhibits the *in vitro* activity of SXT against *A. urinae*. While the concentration of thymidine in human urine and serum is typically low (25), it may vary depending on a patient’s dietary folate intake. The genome of *A. urinae* ACS-230-V-Col10a contains a gene predicted to encode the high-affinity folate transport binding protein FolT, which is also found in *Enterococcus* organisms. This folate transporter may explain why *A. urinae* tests resistant to SXT in the presence of thymidine and folate. While the urinary concentration of SXT may be high enough to overcome this pathway, a conservative approach for laboratories would be to report *A. urinae* as resistant to SXT.

Because of the low frequency of infections due to *A. urinae*, there are limited clinical outcome data described in the literature. The appropriateness of the interpretive criteria suggested in this study and the antimicrobials chosen for testing were therefore assessed based on the MIC distributions found in the present study and the literature (1, 2, 18), such that the breakpoints did not bisect MIC distributions. Adapting breakpoints from a similar organism group with similar MIC distributions is a strategy consistent with that applied to several organisms included in the CLSI M45–A2 document (29). The only antimicrobial agent for which this strategy was a concern was ceftriaxone. The ceftriaxone MIC distribution observed by Skov and colleagues (18) had a modal MIC of 2 μg/ml, which is intermediate by the viridans group streptococcal interpretive criteria (24). However, our present study and that of Sierra-Hoffman and colleagues (2) found a much lower modal MIC for ceftriaxone; the differences noted in these two studies may be related to the testing methodology, as discussed above.

Laboratories should perform susceptibility testing for *A. urinae* when isolated from normally sterile specimens, such as blood. However, given that *A. urinae* organisms are generally susceptible to agents used to treat uncomplicated urinary tract infections, including β-lactams, susceptibility testing may not be required on a routine basis when *A. urinae* is isolated from the urine. In contrast, 16% of the isolates in this study were not susceptible to levofloxacin, an antimicrobial commonly used for the treatment of urinary tract infections. However, to our knowledge, treatment failures with fluoroquinolones have not been reported. Fluoroquinolones are renally excreted, and as such, it is likely that this resistance was noted for erythromycin, clindamycin, and levofloxacin. The isolates were ≥95% susceptible to all other antimicrobials tested. In addition to providing a recommendation to laboratories for susceptibility testing of *A. urinae*, we provide data against which MICs can be compared for this group of organisms.

**REFERENCES**


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**Antimicrobial Susceptibility of Aerococcus urinae**

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**June 2014 Volume 52 Number 6 jcm.asm.org** 2179


