Aerococcus urinae and trimethoprim-sulfamethoxazole

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Abstract

*Aerococcus urinae* are described as resistant to trimethoprim-sulfamethoxazole (SXT), but the test medium may affect this observation. 27 clinical isolates of *A. urinae* tested susceptible to SXT in cation-adjusted Mueller Hinton broth (CAMHB) + lysed horse blood and resistant in CAMHB + lysed sheep blood.

Text

*Aerococcus urinae* are fastidious gram-positive cocci most frequently associated with urinary tract infections (UTI), but also with bacteremia and endocarditis (3). *A. urinae* are classically described as resistant to trimethoprim-sulfamethoxazole (SXT) (3, 6, 14, 15, 19); however, in a previous study (10), we observed 98.8% susceptibility to SXT among 80 clinical *A. urinae* isolates when tested in cation-adjusted Mueller Hinton Broth supplemented with 2.5% lysed horse blood (CAMHB-LHB). The media effect on SXT susceptibility testing has long been recognized (16), whereby thymidine concentrations above 0.03 µg/mL appreciably inhibit the activity of both sulfonamides and trimethoprim (5). Horse erythrocytes contain endogenous thymidine phosphorylase (TP) (5), an enzyme that converts thymidine to thymine, a molecule that is a poor inhibitor of SXT activity (5). Thus, the activity of SXT is restored when tested in media that contains lysed horse blood (LHB). Previous studies that report SXT resistance in *A. urinae* utilized either Mueller Hinton (MH) base without supplement (14), MH + 5% sheep’s blood (19) or the medium used for testing was not listed (6). In the first study of Aerococcus-like organisms (4), horse blood was used as a supplement to the media, although it was not reported that the horse blood was lysed, in which case the endogenous TP remains inside intact erythrocytes.

As SXT is often the drug of choice for the treatment of UTIs among non-allergic patients (7), reports of inherent resistance to SXT among *A. urinae* isolates may significantly impact treatment decisions. In this study, we investigate the effect of biological test media on SXT MICs for 27 characterized clinical *A. urinae* isolates.

*A. urinae* were isolated from the urine of 25 patients between February 2010 and June 2011, using a standard protocol and stocked in Brucella broth supplemented with 15% glycerol (BD Diagnostics, Sparks MD) at -70°C prior to testing. Isolates were subcultured twice on blood agar plates (BD Diagnostics, Sparks, MD) and partial 16S rRNA gene sequence analysis, using MicroSeq (ABI Biosystems, Foster City, CA) as previously described (12) was used to confirm identification as *A. urinae*.  

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SXT susceptibility testing was performed using a Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution MIC method (1) on in-house prepared panels. MIC tests were incubated at 35°C in the presence of 5% CO2 for 48 hours before reading. MICs were read at 80% growth inhibition as compared to a growth control well, and CLSI staphylococcal criteria were used for interpreting SXT MICs (2). *Streptococcus pneumoniae* ATCC 49619 was used for quality control on all SXT MIC tests, and all MICs were within the acceptable range of 0.12/2.4-1/19 µg/mL (not shown). As was expected from our previous observations (10), we found universal susceptibility to SXT among the 27 isolates when tested using CAMHB supplemented with LHB (1). A modal MIC of ≤0.25/4.75 µg/mL (trimethoprim/sulfamethoxazole; range, ≤0.25/4.75–2.0/38 µg/mL, Table) was obtained. However, when testing was performed in CAMHB + defibrinated sheep blood that was lysed using the CLSI protocol recommended for horse blood (1), we observed an MIC of >4/76 µg/mL for all 27 isolates (Table). As sheep blood is rich in thymidine (13), we sought to determine if the thymidine was responsible for this observation. 0.1 U/mL of thymidine phosphorylase (TP, Sigma, St. Louis, MO) was added to the CAMHB + lysed sheep blood (LSB). The addition of TP caused a significant (p<0.001, Student t-test) decrease in the modal SXT MIC to 1/19 µg/mL (range, 1/19–2/38 µg/mL). At a concentration of 0.1 U/mL, TP has been shown to restore the activity of SXT in media that contains thymidine concentrations of up to 0.6 µg/mL, whereas supplementing biological test media with LHB can overcome thymidine concentrations of up to 10 µg/mL (5). These differences may explain why we observed only a modest, but significant, effect on the MICs obtained in CAMHB-LSB by the addition of TP (Table) but very low MICs when the isolates where tested in the presence of LHB (Table). It is interesting to note that TP does not reduce SXT MICs for the enterococci as thymine can be used by these organisms as a trimethoprim inhibitor (16). It would appear from these studies that for *A. urinae*, like other bacteria (5), thymine in contrast to thymidine is only a poor blocking agent of SXT activity.

The *in vitro* interference of SXT activity by thymidine in test medium raises the question of whether the thymidine could affect the *in vivo* activity. Thymidine, however, is degraded rapidly *in vivo* in animal models (9) and human serum and urine concentrations are reportedly below 0.03 µg/mL (13). We thus tested the 27 *A. urinae* in 0.2 µm filter-sterilized (Millipore, Billerica, MA) human urine and observed MICs that were significantly (p<0.0001, Student’s t-test) higher than those observed in CAMHB-LHB (modal MIC of 1/19 µg/mL vs ≤0.25/4.75 µg/mL, Table). However, these MICs were still categorized as susceptible by the CLSI staphylococcal interpretive criteria (2). The addition of 0.1 U/mL TP to the urine caused a minor decrease in the modal MIC, to 0.5/9.5 µg/mL (range, 0.25/4.75–1.0/19 µg/mL), but this difference did not achieve statistical significance (p=0.8, Student’s t-test). All MICs obtained in urine...
were considerably lower than the urine concentrations of trimethoprim reportedly achievable when
dosed at 100 mg once daily, which range from 30 to 160 mg/L (11). In contrast, when *Enterococcus*
species are tested against SXT in human urine, more than a 60 to 360 fold increase in the MICs have
been noted (18). This is likely due to the fact that the enterococci can additionally circumvent
trimethoprim - blocked production of tetra-hydrofolic acid by incorporating exogenous folates (8, 9).
However, even in the scenario of enterococcal UTIs, treatment with SXT is associated with an overall
microbiological eradication rate of 82% (17). We were unable to find reports in the literature on the use
of SXT for treatment of urinary tract infections due to *A. urinae*, and in this limited data set, only two
patients were treated with SXT; neither had follow up cultures or visits to their health care provider.

In summary, while it is suggested that SXT resistance is a key feature for the identification of *A.
urinae* (4, 6), we urge laboratories to be cognizant of the media effect when considering SXT test results
for this organism, as we have now found universal susceptibility among 107 isolates tested in CAMHB-
LHB in this, and our previous study (10). CAMHB-LHB is the medium recommended by CLSI for
antimicrobial susceptibility testing *Streptococcus* species including *Streptococcus pneumoniae* (1).
Although there are no standard recommendations for antimicrobial susceptibility testing of *A. urinae*,
CAMHB-LHB would be a logical medium choice for *A. urinae* when antimicrobial susceptibility testing is
requested. It would appear from this current work that *in vitro* resistance of *A. urinae* is related to
exogenous thymidine present in biological test media, and not to inherent resistance of the organism to
SXT. As SXT is a drug of choice for the treatment of uncomplicated cystitis (7), clinical studies that
investigate the efficacy of this drug for the treatment of UTIs caused by *A. urinae* are required to
determine its clinical value.
Table. Summary of SXT susceptibility test results (n=27) Performed in a Variety of Test Media. SXT MICs were interpreted using the CLSI staphylococcal breakpoint for susceptibility, 1µg/mL.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Modal MIC (µg/ml)</th>
<th>MIC (µg/ml) Range</th>
<th>%S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAMHB + LHB</td>
<td>≤0.25/4.75</td>
<td>≤0.25/4.75 – 1/19</td>
<td>100</td>
</tr>
<tr>
<td>CAMHB + LSB</td>
<td>&gt;4/76</td>
<td>&gt;4/76</td>
<td>0</td>
</tr>
<tr>
<td>CAMHB + LSB + TP</td>
<td>1/19</td>
<td>1/19 – 2/38</td>
<td>100</td>
</tr>
<tr>
<td>Human urine</td>
<td>1/19</td>
<td>0.25/4.75 – 2/38</td>
<td>100</td>
</tr>
<tr>
<td>Human urine + TP</td>
<td>0.5/9.5</td>
<td>0.25/4.75- 1/19</td>
<td>100</td>
</tr>
</tbody>
</table>
References


