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Aerococcus urinae and Trimethoprim-Sulfamethoxazole▽

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

Aerococcus urinae is a fastidious Gram-positive coccus most frequently associated with urinary tract infections (UTIs) but also with bacteremia and endocarditis (3). *A. urinae* is 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 we tested them in cation-adjusted Mueller-Hinton (MH) broth (CAMHB) supplemented with 2.5% lysed horse blood (LHB). The medium 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 medium that contains LHB. Previous studies that reported SXT resistance in *A. urinae* either utilized MH base without a supplement (14) or MH base plus 5% sheep's blood (19) or did not name the medium used for testing (6). In the first study of *Aerococcus*-like organisms (4), horse blood was used as a supplement to the medium, 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 nonallergic patients (7), reports of inherent resistance to SXT among *A. urinae* isolates may significantly impact treatment decisions. In this study, we investigated the effect of the biological test medium used on SXT MICs for 27 characterized clinical *A. urinae* isolates.

A. urinae was 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 their identification as *A. urinae*.

SXT susceptibility testing was performed using a Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution MIC testing method (1) on in-house-prepared panels. MIC tests were incubated at 35°C in the presence of 5% CO_2 for 48 h before reading. MICs were read at 80% growth inhibition compared to a growth control well, and CLSI staphylococcal criteria were used to interpret 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 to 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 we tested them using CAMHB supplemented with LHB (1). A modal SXT MIC of ≤0.25/4.75 μg/ml (range, ≤0.25/4.75 to 2.0/38 μg/ml; Table 1) was obtained. However, when testing was performed in CAMHB plus 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 1). As sheep blood is rich in thymidine (13), we sought to determine if the thymidine was responsible for this observation. TP (Sigma, St. Louis, MO) at 0.1 U/ml was added to CAMHB plus 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 to 2/38 μg/ml). At a concentration of 0.1 U/ml, TP has been shown to restore the activity of SXT in medium that contains thymidine concentrations of up to 0.6 μg/ml, whereas supplementing biological test medium 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 of the addition of TP on the MICs obtained in CAMHB-LSB (Table 1) but very low MICs when the isolates where tested in the presence of LHB (Table 1). It is interesting that TP does not reduce SXT MICs for the enterococci, as thymine can be used by these organisms as a trimethoprim inhibitor (16). It appears from these studies that for *A. urinae*, like other bacteria (5), thymine, in contrast to thymidine, is only a poor SXT activity-blocking agent.

The interference of thymidine in test medium with SXT activity *in vitro* raises the question of whether thymidine could affect the drug's *in vivo* activity. Thymidine, however, is degraded rapidly *in vivo* in animal models (9) and its concentra-

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TABLE 1. Summary of SXT susceptibility test results obtained for 27 isolates with a variety of test media[a]

Medium	Modal MIC[b] (range)	% Susceptible
CAMHB-LHB	≤0.25/4.75 (≤0.25/4.75–1/19)	100
CAMHB-LSB	>4/76 (>4/76)	0
CAMHB-LSB-TP	1/19 (1/19–2/38)	100
Human urine	1/19 (0.25/4.75–2/38)	100
Human urine-TP	0.5/9.5 (0.25/4.75–1/19)	100

[a] SXT MICs were interpreted using the CLSI staphylococcal breakpoint for susceptibility of 1 μg/ml.
[b] MICs are in micrograms per milliliter.

tions in human serum and urine are reportedly below 0.03 μg/ml (13). We thus tested our 27 *A. urinae* isolates in 0.2-μm-filter-sterilized (Millipore, Billerica, MA) human urine and observed MICs that were significantly ($P < 0.0001$, Student *t* test) higher than those observed in CAMHB-LHB (modal MIC of 1/19 μg/ml versus ≤0.25/4.75 μg/ml; Table 1). 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 to 1.0/19 μg/ml), but this difference did not achieve statistical significance ($P = 0.8$, Student *t* test). All MICs obtained in urine were considerably lower than the urine trimethoprim concentrations reportedly achievable by dosing at 100 mg once daily, which range from 30 to 160 mg/liter (11). In contrast, when *Enterococcus* species are tested against SXT in human urine, MIC increases of more than 60- to 360-fold have been noted (18). This is likely due to the fact that the enterococci can additionally circumvent trimethoprim-blocked production of tetrahydrofolic 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 of the use of SXT for treatment of UTIs due to *A. urinae*, and in this limited data set, only two patients were treated with SXT; they did not have follow up cultures or visit their health care providers.

In summary, while it is suggested that SXT resistance is a key feature of the identification of *A. urinae* (4, 6), we urge laboratories to be cognizant of the medium 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 study and our previous study (10). CAMHB-LHB is the medium recommended by CLSI for antimicrobial susceptibility testing of *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 appears from the present work that *in vitro* resistance of *A. urinae* is related to exogenous thymidine present in the biological test medium used 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.

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