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In Vitro Antimicrobial Susceptibility of *Aerococcus urinae*

Romney M. Humphries, Janet A. Hindler

Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

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.**

Aerococcus urinae is a Gram-positive coccus that colonizes the human urinary tract and may cause symptomatic urinary tract infections (1–3). Importantly, *A. urinae* is also described as the cause of invasive infections, such as endocarditis and bacteremia (4–17), and has been reported to demonstrate resistance to a variety of commonly used classes of antimicrobial agents, in particular, trimethoprim-sulfamethoxazole (SXT) and fluoroquinolones (18, 19). In the laboratory, *A. urinae*, particularly when isolated from urine, may be misidentified as an alpha-hemolytic *Streptococcus* strain due to several 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 streptococci MIC interpretive criteria for *A. urinae*.

All *A. urinae* strains were isolated from urine specimens at concentrations of $\geq 10^5$ CFU/ml between January 2005 and December 2013 by the UCLA Health System Clinical Microbiology Laboratory. Identification was performed using the API 20 Strep (bioMérieux, Durham, NC). Antimicrobial susceptibility testing was performed at the time of isolation using the CLSI reference broth microdilution (BMD) method in cation-adjusted Mueller-Hinton broth supplemented with 2.5% lysed horse blood (23, 24) on panels prepared in-house. Incubation was conducted at 35°C in the presence of 5% CO_2 for 24 h; our laboratory has noted superior growth of *Aerococcus* spp. in 5% CO_2 versus ambient air, which is why these incubation conditions were used (data not shown). The MICs for amoxicillin, cefotaxime, ceftriaxone, clindamycin, doxycycline (39 isolates tested), erythromycin, levofloxacin, linezolid (95 isolates tested), meropenem, penicillin, rifampin, tetracycline, trimethoprim-sulfamethoxazole (SXT), and vancomycin were tested. The CLSI M100–S24 interpretive criteria for the viridans group streptococci (24) were used, as available. The ampicillin interpretive criteria were applied to amoxicillin, and the CLSI *Staphylococcus* spp. interpretive criteria were used for rifampin and SXT (24).

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 for 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-

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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% CO_2, and proposed interpretive criteria, based on CLSI viridans group streptococci and *Staphylococcus* sp. interpretive criteria

Antibiotic	No. of isolates with MIC (μg/ml) of:											Modal MIC (μg/ml)	% susceptible	Proposed breakpoint (μg/ml) for:		
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8			Susceptible	Intermediate	Resistant
Penicillin	39	69	15	5								0.03	100	≤0.12	0.25–2	≥4
Amoxicillin	42	32	35	15	3	1						≤0.015		NP[a]	NP	NP
Cefotaxime	5	12	16	25	39	22	8	1				0.25	99	≤1	2	≥4
Ceftriaxone	4	6	14	24	33	20	22	4	1			0.25	96	≤1	2	≥4
Meropenem	59	33	25	9	0	1	0	1				≤0.015	99	≤0.5	NS[b]	NS
Erythromycin				39[c]	45	12	2	2		1	27	0.25		NP	NP	NP
Clindamycin				32[c]	26	16	19	35[d]				≥2		NP	NP	NP
Tetracycline				103[c]	14	5			2		4	≤0.12	95	≤2	4	≥8
Doxycycline					35[c]	2			1		1	≤0.25		NP	NP	NP
Levofloxacin						70[c]	32	5	9	9	3	0.5	84	≤2	4	≥8
SXT					63[c]	28	30	3	3	1[d]		≤0.25		NP	NP	NP
Rifampin					128[c]							≤0.25	100	≤1	NS	NS
Linezolid						32[c]	58	3	1	1[d]		1	98	≤2	NS	NS
Vancomycin				5[c]	38	80	5					0.5	100	≤1	NS	NS

[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.

coccal 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-nonsusceptible 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 streptococcal 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 vitro* for *A. urinae* isolates (17, 18), suggesting that, like for the viridans group streptococci, combination therapy with an aminoglycoside may be prudent for serious infections caused by *A. urinae*. Indeed, in the literature, the majority of invasive infections caused by *A. urinae* have been treated with a β-lactam in combination with an aminoglycoside (7, 13).

All but 6 isolates tested susceptible to tetracycline (MIC ≤ 2 μg/ml). Doxycycline data were available for 39 isolates, and all but 4 of these had a doxycycline MIC of ≤0.25 μg/ml. There are presently no CLSI interpretive criteria for doxycycline and the viridans group streptococci, although doxycycline interpretive criteria were recently established for *S. pneumoniae*, with a susceptible breakpoint of ≤0.25 μg/ml (24). Two isolates had MICs of 0.5 μg/ml, and these had equivalent tetracycline MICs (0.5 μg/ml). For the 2 isolates with doxycycline MICs of >0.5 μg/ml, both tested resistant to tetracycline (MIC > 8 μg/ml) in our study.

Again, using the CLSI viridans group streptococcal interpretive criteria, 84% of the isolates were susceptible to levofloxacin (MIC ≤ 2 μg/ml), and the modal MIC was ≤0.5 μg/ml. Cattoir and colleagues (26) investigated *A. urinae* fluoroquinolone resistance and noted mutations to the quinolone resistance-determining region (QRDR) of the *gyrA* or *parC* genes in two isolates. Using Etest (bioMérieux, Durham, NC) on lysed horse blood Mueller-Hinton agar, these isolates had levofloxacin MICs of >32 μg/ml, in contrast to isolates without mutation to the QRDR, which had ciprofloxacin and levofloxacin MICs of ≤1 μg/ml (25). As fluoroquinolones are commonly used for the empirical treatment of urinary tract infections, physicians should be aware that resistance to this class of antimicrobials may occur *in vitro* in *A. urinae*, although no clinical data are available that demonstrate the significance of this phenotype *in vivo* for patients treated with fluoroquinolones. Skov and colleagues (18) found 89% susceptibility (MIC ≤ 1 μg/ml) to ciprofloxacin among 56 *A. urinae* isolates, and Shelton-Dodge et al. found 67% susceptibility (MIC ≤ 2 μg/ml) to levofloxacin among 30 *A. urinae* isolates tested by agar dilution (1).

Using the viridans group streptococcal 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 CO_2 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 adenylylation by enzymes encoded by the *lin* genes, has been described and yields a clindamycin-resistant erythromycin-susceptible phenotype (the "L-phenotype") in strains of staphylococci, streptococci, and enterococci (27). It is unknown if this mechanism is prevalent in aerococci, 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 determined using breakpoints that underestimate the activity of levofloxacin in urine is insignificant for the treatment of cystitis. Most isolates will test susceptible to SXT *in vitro* by the BMD method used in this study; however, laboratories may consider reporting *A. urinae* as SXT resistant given that susceptibility to SXT *in vivo* may be dependent on a patient's urinary folate concentrations, which can vary considerably. A limitation of our study is the absence of results for nitrofurantoin and fosfomycin, two agents also used for the treatment of uncomplicated urinary tract infections. In summary, we present *in vitro* susceptibility results for a large collection of *A. urinae* clinical isolates tested by the CLSI reference BMD method. When the CLSI interpretive criteria for the viridans group streptococci were applied, 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.

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