

***In vitro* activity of clinically implemented β -lactams against *Aerococcus urinae*: presence of non-susceptible isolates in Switzerland**

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SUMMARY

We analyzed the *in vitro* susceptibility to several β -lactams and vancomycin of 80 *Aerococcus urinae* isolates collected during 2011-2012 in Switzerland. MICs were determined by Etest (bioMérieux) on Müller-Hinton agar with 5% sheep blood and interpreted according to the CLSI and EUCAST criteria set for viridans streptococci. MIC_{50/90} for penicillin, amoxicillin, ceftriaxone and vancomycin were 0.016/0.064 mg/l, 0.032/0.064 mg/l, 0.125/0.5 mg/l and 0.38/0.5 mg/l, respectively. Three (3.8%) isolates were resistant to ceftriaxone regardless of the criteria used (MICs ≥ 2 mg/l); one of them was also non-susceptible to penicillin (MIC of 0.25 mg/l) according to CLSI. β -lactam resistance in *A. urinae* is a concern and suggests that more studies are needed to determine the molecular mechanisms of such resistance.

KEY WORDS: *Aerococcus*, Penicillin, Amoxicillin, Ceftriaxone, PBPs, MIC.

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Aerococcus urinae is an α -hemolytic, catalase-negative, Gram-positive coccus usually detected in urine samples (Rasmussen, 2013). Particular conditions required for *Aerococcus* spp. growth and difficulties in its identification have contributed to the underestimation of the clinical impact of this pathogen. Recently, we observed that with a prevalence of 4% this organism ranked sixth as a urinary tract infection (UTI) pathogen, indicating that its occurrence is probably greater than previously estimated (i.e., 0.2-0.8%) (Guilarte, 2014). Moreover, *A. urinae* can also cause more severe infections, such as spondylodiscitis, peritonitis, cellulitis,

bacteremia and endocarditis which are associated with high morbidity and mortality. Therefore, resistance to β -lactams is of particular concern because this class of antibiotics represents the typical first-line therapeutic option and is recommended in combination with aminoglycosides for the treatment of infective endocarditis (Rasmussen, 2013).

It is assumed that *A. urinae* is widely susceptible to antibiotics and that β -lactams resistance is improbable (Rasmussen, 2013). However, this general assumption is based on the results of only three investigations. In 2001, Skov *et al.* analyzed the *in vitro* activity of penicillin, amoxicillin, and ceftriaxone for 56 *A. urinae* isolates concluding that resistance was sporadic (Skov, 2001). Shelton-Dodge *et al.* reported similar results investigating the susceptibility of 30 isolates collected during 2007-2008 (Shelton-Dodge, 2011). Finally, Senneby *et al.* reported the penicillin and cefotaxime MIC val-

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ues but tested only 14 isolates (Senneby, 2012). Overall, none of these studies could exhaustively be informative on the current susceptibility of clinically implemented β -lactams against *A. urinae* isolates. We note that this constitutes an important gap in clinical knowledge because it is evident that *A. urinae* is more important as an aetiological agent of human infections than previously believed (Guilarte, 2014). Even more significant is the fact that, as we recently observed for quinolones, antibiotic resistance is also likely to emerge in this species (Cattoir, 2011, Guilarte, 2014).

The present analysis investigated the *in vitro* activity of β -lactams and vancomycin in a collection of *A. urinae* strains previously analyzed for quinolone susceptibility and corresponding mechanisms of resistance. In particular, 80 isolates (of which 71 were from urine samples) collected during 2011-2012 in the Laborato-

ry of Clinical Microbiology of the Institute of Infectious Diseases, University of Bern (Bern, Switzerland) were tested (Guilarte, 2014). Species identification was achieved implementing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics) and confirmed by sequencing of the 16S rDNA (MicroSeq 500; Applied Biosystem) (Senneby, 2013). Production of β -lactamases was ruled out implementing the cefinase disk assay (Oxoid). MICs for penicillin, amoxicillin, ceftriaxone, and vancomycin were determined by Etest (bioMérieux) on Müller-Hinton agar supplemented with 5% sheep blood and incubating overnight at 35°C in a 5% CO₂ atmosphere. Since no interpretative criteria for *Aerococcus* spp. are available, MIC results for penicillin, amoxicillin, ceftriaxone, and vancomycin were interpreted according to the 2013 Clinical Laboratory Standard

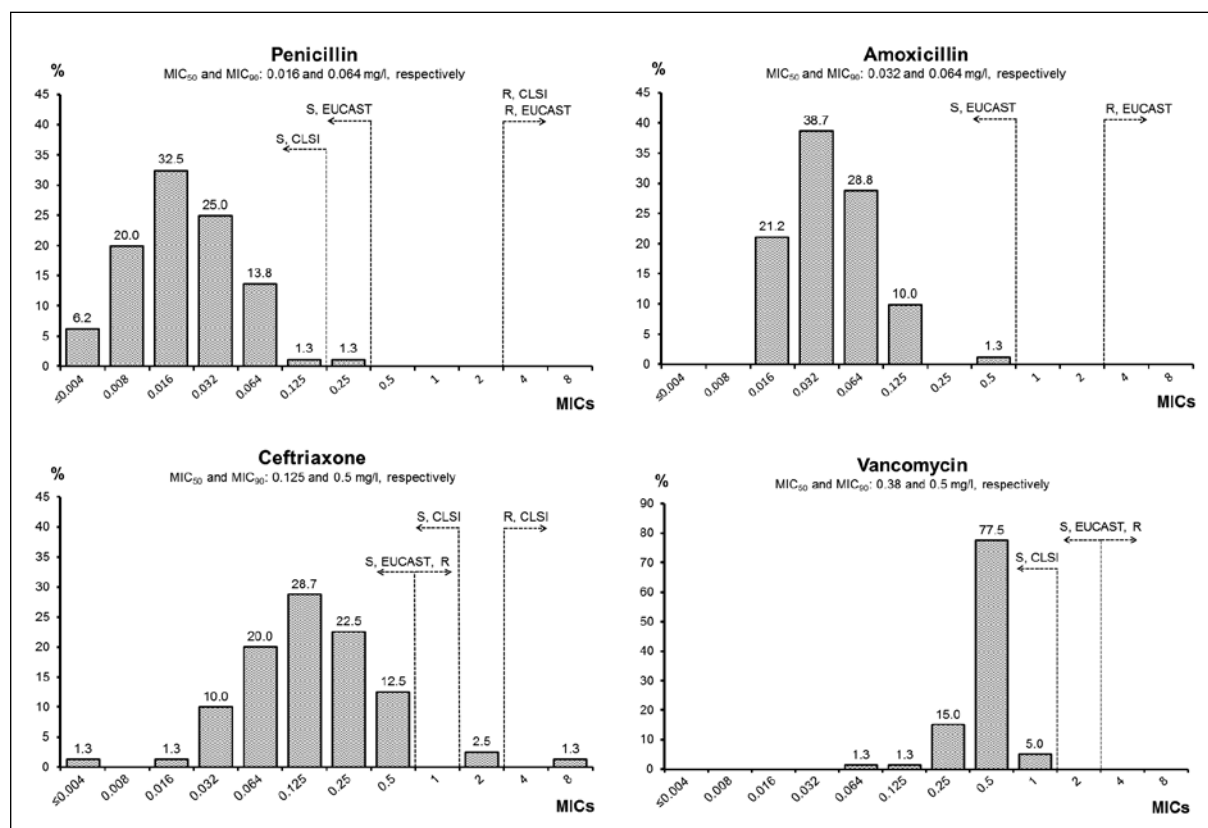


FIGURE 1 - MIC distributions of penicillin, amoxicillin, ceftriaxone and vancomycin tested against 80 *A. urinae* isolates collected during 2011-2012 in Switzerland. MIC values obtained with the Etest were adjusted up to the next highest doubling concentration (e.g., from 0.38 to 0.5 mg/l). Results were interpreted according to the 2013 CLSI and EUCAST criteria (CLSI, 2013, EUCAST, 2013). Three isolates were non-susceptible to β -lactams.

TABLE 1 - Sources and susceptibility profile of three *A. urinae* isolates non-susceptible to β -lactams.

Isolate	Patient		Specimen	MICs (mg/l), interpretation according to CLSI/EUCAST ^a			
	Sex	Age		Penicillin	Amoxicillin	Ceftriaxone	Cefixime
AE-83022	F	67	Urine	0.125, S/S	0.125, NA/S	2, I/R	8, NA/NA
AE-35475	M	86	Urine	0.25, I/S	0.125, NA/S	8, R/R	64, NA/NA
AE-76981	F	83	Perirenal fluid	0.032, S/S	0.125, NA/S	2, I/R	32, NA/NA

S, susceptible; I, intermediate; R, resistant, NA, not available. ^aSusceptibility results were interpreted according to the 2013 CLSI and EUCAST criteria set for viridans streptococci: penicillin (CLSI: S \leq 0.125 mg/l; R \geq 4 mg/l - EUCAST: S \leq 0.25 mg/l; R \geq 4 mg/l); amoxicillin (CLSI: NA - EUCAST: S \leq 0.5 mg/l; R \geq 4 mg/l); ceftriaxone (CLSI: S \leq 1 mg/l; R \geq 4 mg/l - EUCAST: S \leq 0.5 mg/l; R \geq 1 mg/l); cefixime (CLSI: NA - EUCAST: NA).

Institutes (CLSI) and/or European Committee on Antimicrobial Susceptibility Testing (EUCAST) cut-offs set for *Streptococcus* spp. viridans group (CLSI, 2013, EUCAST, 2013).

As shown in Figure 1, the MIC values for the four antibiotics tested had a typical Gaussian distribution. In particular, the MIC_{50/90} for penicillin, amoxicillin, ceftriaxone and vancomycin was 0.016/0.064 mg/l, 0.032/0.064 mg/l, 0.125/0.5 mg/l and 0.38/05 mg/l, respectively. None of the *A. urinae* isolates showed β -lactamase activity. However, as shown in Table 1, three isolates resulted resistant to ceftriaxone regardless of the criteria used; one of them was also non-susceptible to penicillin according to CLSI (CLSI, 2013, EUCAST, 2013). For these three *A. urinae* isolates, cefixime (Etest) was also tested indicating that the strains possessed MICs \geq 8 mg/l. Two of the isolates were responsible for UTIs, whereas the remaining one was isolated from a perirenal collection. Unfortunately, clinical data (e.g., previous use of antibiotics) on the three patients were not available because the first two were outpatients and the other one was hospitalized only one day with incomplete clinical chart information.

Investigations conducted on other Gram-positive species have highlighted that the establishment of resistance to β -lactams is based on the modification of penicillin-binding proteins (PBPs) or on the acquisition of PBPs with low affinity for the drugs (Ohsaki, 2008). For instance, in *Streptococcus pneumoniae* alterations of PBP2b confer penicillin resistance, whereas alteration of PBP2x is more likely to confer cephalosporins resistance (Fani *et al.*, 2013).

The aim of this study was to fill the gap in information on β -lactams susceptibility for contemporary *A. urinae* isolates. To our knowledge, this is the second largest survey analyzing the

phenotypic characteristics of *A. urinae* isolates performed so far. In fact, during the preparation of this work, Humphries and Hindler published the results of an analysis of 128 *A. urinae* isolates. In particular, broth microdilution tests indicated that no penicillin-resistant isolates were present but 4% were non-susceptible to ceftriaxone (Humphries and Hindler, 2014).

The penicillin/cephalosporins non-susceptibility exhibited by several *A. urinae* isolates (e.g., 3.8% of our collection) highlights that resistance to this important class of antibiotics can be present in *A. urinae*. This may explain the poor clinical outcome observed for the aerococcal cases of infective endocarditis when therapy with penicillin or ceftriaxone plus gentamicin was implemented (Ebnother *et al.*, 2002, Kass *et al.*, 2008). Furthermore, our figure emphasizes the importance of routinely performing standard antimicrobial susceptibility tests for *Aerococcus* spp. and not *a priori* assuming that this pathogen is fully susceptible to standard antibiotics. We also believe that obtaining the MIC values for penicillin, amoxicillin, ceftriaxone, and at a last resort vancomycin, should be mandatory for the isolates causing serious infection. Larger and international studies should be planned to carefully monitor the antibiotic susceptibility pattern of *A. urinae* clinical isolates. Moreover, dedicated studies unveiling the molecular basis underlying the β -lactam resistance mechanism are called for.

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