An initially unidentified case of urinary tract infection due to *Aerococcus urinae*

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**INTRODUCTION**

*Aerococcus urinae* is a rarely reported Gram positive cocccus responsible for urinary tract infections (UTI) and blood stream infections (BSI) (Rasmussen 2013), recognized as a separate species in 1992 (Aggurire and Collins, 1992). Interestingly, it has been proposed that the infrequency of its isolation may be attributable to identification difficulties (Shelton-Dodge et al., 2011). Indeed, *A. urinae* bears characteristics of both staphylococci and streptococci, while automated systems and phenotypic methods are often not of assistance (Cattoir et al., 2010). Moreover, the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have not yet established breakpoints for *A. urinae*. Large scale studies aiming to assess the frequency of *A. urinae* in urinary samples of hospital laboratories in the Netherlands (Schuur et al., 1997), Norway (Grude et al., 2003), USA (Sierra-Hoffman et al., 2005) and Sweden (Sennenby et al., 2015) reported 0.2%, 0.3%, 0.25% and 0.27% of all cultures, respectively. We hereby describe a case that highlights the difficulties of *A. urinae* identification.

**CASE REPORT**

On 15 December 2014, an 84-year-old male with a history of prostate cancer in remission and coronary artery disease visited a private clinical laboratory directly, complaining of dysuria. Urinalysis showed 14 to 16 white blood cells (WBCs) per optical field, 1-3 red blood cells (RBCs) per optical field, positive results for leukocyte esterase, trace amounts of hemoglobin and negative results for protein, ketones nitrites and bilirubin. Two days later, the patient received the results of the urine culture with the sample being characterized as casually contaminated. He was told that an environmental bacterium was probably isolated and was asked to undergo a second urine culture. However, the patient’s condition worsened, and he then visited a different laboratory which submitted the sample to our reference laboratory. Our urinalysis showed WBCs too numerous to count, 6-8 RBCs per optical field, many bacteria and positive test results for leukocyte esterase, blood hemoglobin and protein. The urine culture revealed a microorganism that did not grow on MacConkey agar but was alpha-hemolytic on blood agar, where it grew at a concentration of ≥10⁵ CFU/ml. The isolated bacteria were Gram positive forming pairs, tetrads and clusters, catalase negative, bile esculin negative and pyrrolidonyl aminopeptidase (PYR) negative. We performed a disc diffusion test with various antibiotics active against Gram positive microorganisms using the EUCAST interpretive criteria for viridians group Streptococci (Humphries & Hindler 2014), where available, and *Staphylococcus* spp. (Zhang et al., 2000) for the remaining compounds. The microorganism was found susceptible to ampicillin, amoxicillin, amoxicillin-clavulanate, cefaclor, clarithromycin, erythromycin, ciprofloxacin and vancomycin, while being resistant to trimethoprim-sulfamethoxazole and gentamycin. Based on the preliminary microbiological data and the antibiotic susceptibility testing results, we reported a probable *A. urinae* and proceeded to the determination of the 16S rRNA nucleotide sequences. The patient received amoxicillin (1g twice a day for seven days) and was completely free of symptoms after the treatment. A new urinalysis and a follow-up negative urine culture confirmed the treatment’s success.

**SUMMARY**

*Aerococcus urinae* is a microorganism responsible for urinary tract and blood stream infections which are rarely reported in clinical practice. However, it has been proposed that the infrequency of such reports may be partially due to difficulties related to pathogen identification. We present here a case of an elderly male patient with urinary tract infection where *A. urinae* was initially not identified by a private microbiology laboratory. Our report highlights the need to consider *A. urinae* as a causative agent of urinary tract infections because if not identified and properly treated it may lead to endocarditis or septicemia.

**Key words:** *Aerococcus urinae*, Urinary tract infection, Trimethoprim-sulfamethoxazole.
The isolate was molecularly characterized using polymerase chain reaction (PCR) amplification, cloning and sequencing of the 16S rRNA gene. 4 μl of the supernatant of lysed bacteria (isolated from a single colony and boiled in 50 μl of ddH2O), were amplified using Platinum Taq DNA polymerase (Invitrogen, Life Technologies) according to the manufacturer’s instructions. The oligonucleotide primers used were: (Forward) fD1-16S: 5’ AGAGTTTGATCCTGGCTCAG 3’ and (Reverse) rP2-16S: 5’ ACGGCTACCTTGTTACGACTT 3’ (Weisburg et al., 1991).

The cycling conditions used were: 94°C (5 min) (1X); 94°C (30 s) 58°C (30 s) and 72°C (2 min) (35X); and 72°C (10 min) (1X). The PCR product was then cloned on PCRII-TOPO vector (Invitrogen, Life Technologies) and sequenced using T7 and SP6 primers. The comparison of the 16S rRNA nucleotide sequence obtained using the BLASTN algorithm (Altschul et al., 1997), revealed 99.75% sequence identity with A. urinae.

**DISCUSSION**

A. urinae is commonly susceptible to various classes of antimicrobial agents even though in vitro resistance to penicillin, ceftriaxone (Lupo et al., 2014) and fluoroquinolones (Humphries et al., 2011) has been reported. Thus, the main clinical significance of A. urinae isolation relies on its in vitro resistance to sulfonamides, given that trimethoprim-sulfamethoxazole (SXT) is frequently used empirically for the treatment of UTIs. It has been shown that this in vitro resistance is medium-dependent (Humphries et al., 2011), even though the same authors recently proposed that lacking more evidence, A. urinae should be reported as co-trimoxazole resistant (Humphries & Hindler 2014). In our case, no inhibition halo was observed around the SXT disc using a horse blood agar plate.

If not appropriately treated by the clinician or if not identified by the microbiologist, A. urinae can cause serious complications such as endocarditis and septicemia, both originating from a simple UTI (Zhang et al., 2000). Recent reports also support the possible association between A. urinae bacteriuria and other severe complications, such as necrotic urethritis (Babaer et al., 2015), vertebral osteomyelitis (Jerome et al., 2015) and chorioamnionitis with probable early-onset neonatal infection in a pregnant woman (Jost et al., 2014). Therefore, the difficulties related to its identification by the clinical laboratory must be emphasized. In Gram stain, the microorganism forms pairs and clusters similarly to staphylococci, but is catalase negative and alpha-hemolytic corresponding to alpha-hemolytic streptococci, which could be considered contaminants. Though after 48 hours A. urinae colonies may be confused with those of enterococci, the microorganism is negative for pyrrolidonyl aminopeptidase (PYR) and bile esculin. A. urinae also presents similar characteristics to those of pediococci which, however, are bile esculin positive and vancomycin-resistant. 16S rRNA gene sequencing is considered the gold standard for its identification (Lee et al., 2016). Nevertheless, it should be stressed that the introduction of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) in clinical laboratories has lately led to increased isolation rates of A. urinae (Rasmussen, 2016).

The present case report emphasizes the importance of carrying out a thorough microbiological investigation in clinical practice, and supports the assumption that UTI incidents due to A. urinae may be more frequent than those actually reported.

**References**


