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Urosepsis with *Actinobaculum schaalii* and Aerococcus urinae

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*Actinobaculum* was isolated from urine only after prolonged incubation in 5% CO2 after discrepency between urine Gram stain and initial culture results was observed. Additional patients were diagnosed using this method. The prevalence of *Actinobaculum* species in urinary tract infections is underestimated since it is not isolated by routine urine culture procedures.

CASE REPORT

A 78-year-old man was admitted to the neurology ward with a subdural hematoma and contusio cerebri following a fall. An indwelling bladder catheter was placed because of urinary incontinence from a coexisting delirium. After 3 weeks of admission, the patient developed an episode of fever with a temperature of 39°C, nausea, vomiting, tachycardia, and hypertension. C-reactive protein was 155 mg/liter, and leukocytes were 14.0 × 109/liter (90% neutrophils). Urine microscopy of sedimented urine showed more than 50 leukocytes and 25 to 50 erythrocytes per field (×400), hyaline casts, and bacteria. A urosepsis was assumed, and the patient was empirically treated with ceftriaxone (1,000 mg every 24 h [q24h] intravenously) and gentamicin (5 mg/kg q24h intravenously) for the first 48 h. From both urine and blood collected on the same day, *Actinobaculum schaalii* and *Aerococcus urinae* were cultured.

Gram stain of uncentrifuged urine showed gram-positive rods and gram-positive cocci in clusters in the presence of many leukocytes (>20 leukocytes per field [×100]) (Fig. 1). Poor growth of gram-positive cocci was observed on blood agar and MacConkey agar after overnight culture at 37°C in ambient air. The isolate was identified as *Aerococcus* species by phenotypic tests (colony morphology, Gram stain morphology, catalase, growth in 6.5% NaCl, pyrrolidonyl-arylamidase, leucine-aminopeptidase, β-glucuronidase, hippurate hydrolysis, and vancomycin susceptibility) and was confirmed to be *Aerococcus urinae* by sequence analysis of 16S rRNA DNA. Growth of gram-positive rods on blood agar and not MacConkey was present only after 48 h of reincubation at 37°C in 5% CO2. After subculture, growth was better on blood agar than on chocolate agar. Growth was best in 5% CO2 and equal under anaerobic conditions and in ambient air. After 24 h, colonies were pinpoint. After 48 h, colonies were nonhemolytic, dome-shaped, and glistening gray, with entire edges ranging in size from 0.5 to 1 mm appearing as two separate isolates. Catalase and oxidase reactions were negative. Motility by hanging drop was negative. Gram stain of a 24-h liquid culture (Brewer thioglycolate broth) showed irregular staining gram-positive rods with a diphteroid appearance, including Chinese-character configuration. Bacteria were nonspore-forming and not acid fast with a modified Kinyoun stain or Ziehl-Neelsen stain. The biochemical test results obtained with API Coryne system (API Laboratory Products, bioMerieux, France) were as follows: negative results for nitrate reduction; positive results for pyrazaminidase, pyrrolidonyl arylamidase, and α-glucosidase; negative results for alkaline phosphatase, β-glucuronidase, β-galactosidase, N-acetyl-β-glucosaminidase, urease, gelatin hydrolysis, and esculine hydrolysis; and fermentation of ribose, xylose, maltose, and sucrose. As in a previous report, the API Coryne system resulted in profile code 6010621, associated with poor identification of *Gardnerella vaginalis* (6). The absence of beta-hemolysis on agar containing human blood and resistance to metronidazole were inconsistent with this identification. 16S rRNA gene sequence analysis showed 99% sequence similarity with *A. schaalii*. *A. urinae* and *A. schaalii* were also isolated from blood, each separately, from the anaerobic bottle of two blood culture sets after 24 and 48 h incubation, respectively, using the BACTEC 9240 (Becton Dickinson, Cockeysville, Md.). In vitro susceptibility was performed by the Kirby-Bauer disk diffusion method on Columbia blood agar in 5% CO2 using 0.5 McFarland inoculum. According to the Clinical and Laboratory Standards Institute criteria for *Streptococcus* spp., the isolate was susceptible to penicillin, amoxicillin, ceftriaxone, tetracycline, clarithromycin, clindamycin, nitrofurantoin, and co-trimoxazole. No zone was present around ciprofloxacin.

The patient responded well after 7 days of therapy with ceftriaxone. The urinary catheter was removed, and treatment was continued with 500 mg amoxicillin q6h orally for another 14 days, guided by the in vitro susceptibility results. A urologic investigation that was performed because of persistent macroscopic hematuria 1 week after completion of treatment revealed no signs of urinary tract obstruction or malignancy. Cystoscopy showed a diffuse cystitis, and empirical treatment was started with ciprofloxacin. This time, a urine culture showed *A. schaalii* only. Blood cultures were not collected. The patient’s symptoms improved after treatment with ciprofloxacin. However, since the isolate showed in vitro resistance to ciprofloxacin and urine cultures remained positive, ciprofloxacin was switched to 960 mg co-trimoxazole q12h orally for another 4 weeks under the presumptive diagnosis of chronic
Urinary tract infections are predominantly caused by members of the family Enterobacteriaceae. However, gram-positive bacteria and polymicrobial infections are not uncommon in patients with complicated and nosocomial urinary tract infections (7).

The current case describes a dual infection of *A. schaalii* and *A. urinae* in the urinary tract with bacteremia in an elderly man with an indwelling urinary catheter. In recent years, *A. urinae* has become an established albeit rare pathogen in urinary tract infections predominantly in elderly patients and has been reported to be a cause of sepsis and endocarditis as well (1, 9). In contrast, cases of human infection with *Actinobaculum* species are limited to a few reports (2–6, 8).

*Actinobaculum* is a gram-positive rod closely related to the genus *Actinomyces* and was first described as a cause of human infection in 1997 (5). Currently, four species within the genus have been described, *Actinobaculum suis*, *A. schaalii*, *Actinobaculum massiliae*, and *A. urinae*, and the latter three have been described in cases of human infection for six patients. In one patient, the organism was isolated from a skin abscess (8).

It was cultured from urine in two patients with cystitis and one with pyelonephritis (3, 4, 6). *Actinobaculum* was isolated from blood in a patient with chronic pyelonephritis and a patient with chronic renal failure (2, 5). In the latter two patients, the urinary tract was identified as a possible source of bacteremia but was not confirmed microbiologically.

In the current case, *A. schaalii* was cultured from both urine and blood, which shows it to be a uropathogen with invasive potential. As in the previous reports, *Actinobaculum* was isolated from urine only after prolonged incubation in 5% CO₂, which was done because of the discrepancy between urine Gram stain and initial culture results. As in most laboratories, routine urine cultures are incubated only overnight at 37°C in ambient air using Columbia blood agar and MacConkey agar. Thus, the dual infection of the urinary tract and the source of the bacteremia with *A. schaalii* would have been missed without routine Gram stain of the urine, which was recently introduced in our laboratory. Although routine urine Gram stain may not be cost-effective in most laboratory settings, it may be an important diagnostic adjunct in selected patients with a high suspicion of urinary tract infection and a negative routine urine culture. Over a 6-month period, prolonged incubation in 5% CO₂ of urine that showed significant leucocyturia and gram-positive rods on microscopy in the absence of contamination resulted in four additional cases of urinary tract infection with *A. schaalii* (n = 2), *A. urinae*, and *A. massiliae*. All four patients were males between 38 and 90 years of age with underlying urological conditions, i.e., longstanding lower urinary tract symptoms of unknown origin, neurogenic bladder due to paralysis for which intermittent catheterization was needed (n = 2), and urethra stricture due to lichen sclerosus et atrophicus.

The delayed growth of gram-positive rods on blood agar after incubation in 5% CO₂ or under anaerobic conditions from urine with significant pyuria and of gram-positive rods on urine Gram stain is suggestive of infection with *Actinobaculum* spp. Although the growth of the isolate described here was stimulated by CO₂, others have reported *Actinobaculum* to be an aerotolerant anaerobic bacterium (2, 8). The API Coryne system can be used to further characterize such isolates. The test results obtained with API Coryne were in keeping with the biochemical characteristics of *A. schaalii* that have been summarized in previous reports, including the negative urease test which distinguishes *A. schaalii* from *A. urinae* (2, 3, 6).

Besides the importance of making a clinical and microbiological diagnosis in a patient, it is important to be informed on the prevalence of the various etiologies of urinary tract infection in specific patient groups and their susceptibility to the antimicrobials used. In this case, the *A. schaalii* isolate, which was susceptible in vitro to most of the drugs commonly used for urinary tract infections, was resistant to ciprofloxacin, which is a first-line antibiotic in the treatment of prostatitis. All four additional isolates were susceptible to the beta-lactam antibiotics, tetracycline and nitrofurantoin; two were resistant to co-trimoxazole; and one was intermediate susceptible to clarithromycin and resistant to clindamycin. The inhibition zones around ciprofloxacin were 40 mm, 18 mm (two isolates), and 6 mm (no zone).

In conclusion, this report adds five additional patients to the six published cases of *Actinobaculum* infection since its first description as a cause of human infection in 1997 (5). *Actinobaculum* infections appear to occur in patients with underlying urological pathology. Since there are only a few reports, the invasive potential of *Actinobaculum* is likely to be limited. However, the prevalence of *Actinobaculum* species in urinary tract infections is underestimated since it is not isolated by routine urine culture procedures, and routine urine Gram stain

![FIG. 1. A Gram stain of uncentrifuged urine shows gram-positive rods and gram-positive cocci in clusters in the presence of many leukocytes (magnification, ×1,000).](http://jcm.asm.org/)
is not performed in many laboratories. Urine Gram stain is an important diagnostic tool for patients with a high suspicion of urinary tract infection but negative routine urinary culture, especially in the presence of structural abnormalities of the urinary tract.

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