Actinotignum schaalii is an emerging uropathogen; however, routine culture protocols and usual phenotypic methods do not allow for easy detection and identification. Herein, we report the first Korean case of urinary tract infection caused by *A. schaalii* in a 79-year-old patient with prostate cancer. A gram-positive rod bacterium was isolated from the patient's urine after 2 days of culture and identified as *A. schaalii* using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and DNA target sequencing.

**Key Words:** Actinotignum schaalii, Uropathogen, DNA target sequencing, MALDI-TOF

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**INTRODUCTION**

*Actinotignum schaalii* (formerly *Actinobaculum schaalii*) is a facultative anaerobic, gram-positive, coccoid rod-shaped species phylogenetically related to *Actinomyces*. The genus *Actinotignum* consists of *A. schaalii*, *A. urinale*, and *A. sanguinis* [1]. *A. schaalii* is part of the urinary microbiota and has been reported in human infections, mostly urinary tract infection (UTI) [2–4]. This gram-positive bacterium is frequently considered a contaminant or reported not to grow in culture due to technical issues of the culture protocol or difficulty of identification. *A. schaalii* grows slowly on blood agar–enriched media routinely used for urine culture in most laboratories. Furthermore, routine phenotypic identification tools such as the VITEK-2 or MicroScan system cannot identify this organism. However, recent new methods such as molecular-based methods or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can accurately identify this emerging bacterial species [1].

Herein, we report a case of UTI caused by *A. schaalii* in a 79-year-old patient with prostate cancer. To the best of our knowledge, this is the first case of clinical infection caused by *A. schaalii* in Korea. This organism was identified using both MALDI-TOF and DNA target sequencing.

**CASE REPORT**

A 79-year-old male diagnosed with metastatic prostatic cancer visited the emergency department for drowsiness and hesitant speech. He also complained of dysuria, flank pain, and sense of residual urine for three days. His body temperature was 39.1°C, blood pressure 115/67 mmHg, pulse rate 134 beats per min, and respiratory rate 22 breaths per min.

Blood tests showed a leukocyte count of 8,030/mm<sup>3</sup> with 76.1%
neutrophils, an elevated C-reactive protein level of 18.73 mg/dL (reference interval [RI], 0–0.3 mg/dL), and an erythrocyte sedimentation rate of 49 mm/hr (RI, 0–22 mm/hr). His procalcitonin level was 0.57 ng/mL (RI, 0–0.5 ng/mL). The serum levels of sodium, potassium, and creatinine were within normal ranges. Imaging studies did not show any acute change or increased metastasis of prostate cancer compared with previous ones. He was empirically treated with piperacillin/tazobactam after obtaining urine and pe-

Fig. 1. (A) Pinpoint, whitish to gray colonies on a blood agar plate at two days of culture. (B) Microscopic morphology of Actinotignum schaalii (Gram stain, ×1,000).

Fig. 2. Neighbor-joining phylogenetic tree showing the relationships of A. schaalii and other Actinomyces species. Reference sequences are from species type strains; GenBank accession numbers are given in parentheses.
ripheral blood. The urine sample was inoculated on a blood agar plate (BAP) and MacConkey plate, according to routine bacterial procedures. The leukocyte count and leukocyte esterase were negative. Pinpoint, white to gray colonies were grown to greater than 10⁵ CFU/mL on BAP on the day after incubation, and a gram-positive, rod-shaped bacterium was isolated after 30 hr of incubation (Fig. 1). The microorganism could not be identified by the GP ID card of the VITEK 2 system (bioMérieux, Marcy l’Etoile, France). However, using the VITEK MS (bioMérieux), this organism was identified as *A. schaalii* with 99.9% confidence. For accurate strain identification, we performed 16S ribosomal RNA (rRNA) target sequencing. DNA was extracted from the isolates using the MagNA Pure 96 system (Roche Diagnostics, Mannheim, Germany). The 16S rRNA gene was amplified utilizing standard methods according to the Clinical and Laboratory Standards Institute guidelines [5] using the following primer pairs: forward (4F): 5′-TTG GAG AGT TTG ATC CTG GCT C-3′ and reverse (534R): 5′-TAC CGC GGC TGC TGG CAC-3′, as well as forward (27F): 5′-AGA GTT TGA TCM TGG CTC AG-3′ and reverse (801R): 5′-GGC GTG GAC TTC CAG GGT ATC CTG GCT C-3′. Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI prism 3730 Genetic Analyzer (Applied Biosystems). The amplified sequences were compared with the NCBI Blast sequence database. The isolated 16S rRNA sequence matched that of *A. schaalii* (GenBank accession number CP008802.1) with 99.0% identity (665/672 bp). The most closely related species was *A. sanguinis* (GenBank accession number HG798952.1) with a sequence identity of 97.9% (659/673). Consequently, the gram-positive rods were identified as *A. schaalii*. Over 99% homology was detected with *A. schaalii* type strain sequence Y12329. A phylogenetic tree based on 1,000 bootstrap replicates of the 16S rRNA gene sequences was reconstructed with MEGA7 software (http://www.megasoft ware.net) (Fig. 2).

Antibiotic treatment with piperacillin/tazobactam injection was continued for seven days. The patient’s altered mental state recovered without any further intervention. After treatment with antibiotics, his symptoms improved and fever subsided.

**DISCUSSION**

Bacteria belonging to the genera *Actinobaculum* and *Actinotignum* are small, nonmotile, gram-positive, coccoid to rod in shape, straight to slightly curved, and non-spore forming. These facultative anaerobic bacteria grow slowly (48 hr) and preferentially on enriched BAP [1]. All strains are positive for hippurate hydrolysis and negative for acid production from inulin, lactose, mannitol, raffinose, sorbitol, and trehalose. CAMP, catalase, and oxidase reactions are negative. Acetoin production, esculin hydrolysis, and gelatin liquefaction are negative. Arginine dihydrolase, α-fucosidase, α-galactosidase, β-galactosidase, lipase C14, pyrazinamidase, trypsin, α-chymotrypsin, cystine arylamidase, valine arylamidase, and nitrate reductase activities are negative [6-10]. Pure cultures of *A. schaalii* in the urine tends to be initially dismissed as contamination in healthy patients. In addition, these colonies on BAP resembles coryneform skin commensals frequently isolated as contaminants [11] and grows slowly. For these reasons, *A. schaalii* can be overlooked or dismissed as a probable contaminant. In addition, *A. schaalii* is difficult to distinguish from commensal bacteria both morphologically and phenotypically. However, *A. schaalii* can affect elderly and young people, especially individuals at risk of UTIs such as those with spinal cord injuries and/or catheters, as well as patients with diabetes or multiple sclerosis, acquired immunodeficiency disease syndrome/human immunodeficiency virus, or underlying urologic abnormalities [3, 12].

UTIs are considered the most common bacterial infections, but accurately assessing the incidence of UTIs is difficult because the presence of symptoms and a positive urine culture do not occur simultaneously [13]. However, for subpopulations at increased risk of UTIs, proper UTI diagnosis and treatment are important. In our case, the patient had been treated for metastatic prostate cancer for several years and arrived at the hospital reporting UTI symptoms such as dysuria, flank pain, sense of residual urine, and fever. We observed pinpoint and small whitish to grey colonies on BAP from the second day of urine culture. Without knowledge of the patient’s history of prostate cancer and urinary symptoms, these colonies could have been dismissed as contaminants or commensals. However, we isolated these colonies and identified them using MALDI-TOF MS [14, 15]. MALDI-TOF MS allowed rapid and accurate identification of the bacterial pathogen in our case.

For *A. schaalii*, very little information is available about its *in vitro* susceptibility to antimicrobial agents [16]. However, some studies showed a characteristic antimicrobial susceptibility pattern including susceptibility to penicillin, amoxicillin, cefuroxime, tetracycline, and clindamycin and resistance to ciprofloxacin and
metronidazole [12, 16, 18]. The optimal duration of antibiotic treatment is not clearly defined. Previous cases showed that patients still had positive urine cultures for A. schaalii after initial treatment with β-lactam [12]. Moreover, one week of treatment with amoxicillin was not successful. This suggests that treatment may need to be prolonged to at least 2 weeks [17].

A. schaalii is thought to be a commensal in the genitourinary tract, but could be the cause of UTIs [18, 19]. The function of this organism in healthy people is unclear. In our case, the patient's urinary symptoms and underlying prostate cancer implied that A. schaalii could be the causative pathogen. We expect that MALDI-TOF MS and molecular studies will lead to more accurate diagnosis and increase our understanding of A. schaalii infections.

요 약

Actinotignum schaalii는 신종 요로감염균의 일종이지만, 일반적인 배양법과 표현형 분석법으로는 이를 검출하고 동정하기 어려운 특징이 있다. 저자들은 79세 간질신암 환자에서 발생한 A. schaalii 요로감염증 국내 첫 증례를 보고하고자 한다. 환자의 소변검체에서 배양 이중한 후 그람양성 박테리아로 배양되었고, 이는 MALDI-TOF 절편분석기와 16S rDNA 염기서열분석으로 A. schaalii로 동정되었다.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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