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Ten Cases of *Actinobaculum schaalii* Infection: Clinical Relevance, Bacterial Identification, and Antibiotic Susceptibility

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Nine of 10 strains of *Actinobaculum schaalii* caused urinary tract infections in predisposed individuals. Identification included 16S rRNA gene sequence analysis and use of the API Coryne and Rapid ID32A test systems. *A. schaalii* is easily overlooked due to its slow growth in ambient air and its resemblance to the normal bacterial flora on skin and mucosa.

The genus *Actinobaculum*, first described in 1997, includes the *Actinobaculum suis* and *Actinobaculum schaalii* species. *A. suis* is an important cause of urinary tract infections (UTIs) and abortions in sows and was formerly assigned to a variety of genera, including *Corynebacterium*, *Eubacterium*, and *Actinomyces* (9, 17). *A. schaalii* is a new species recovered from human blood and urine and is suspected to cause UTIs (9, 12). Two newly described species, *Actinobaculum massiliae* and *Actinobaculum urinale*, were recovered from the urine of elderly women with chronic cystitis (6, 7). *A. massiliae* is also described as a new cause of superficial skin infections (16). Problems with identifying *Actinobaculum* spp. with traditional phenotypic tests have obscured their pathological role for many years. We describe 10 cases of *A. schaalii* infections.

Nine strains of *A. schaalii* were available for growth, biochemical, and susceptibility tests. The strains were cultured on nine different culture media at 35°C in ambient air, in air with 5% CO_2, and anaerobically. The media were 5% Columbia sheep blood agar (Becton Dickinson [BD], Heidelberg, Germany), 5% and 10% horse blood agar (Statens Serum Institut [SSI], Copenhagen, Denmark), chocolate agar (SSI), *Brucella* blood agar with hemin and vitamin K1 (BD), anaerobic plates (SSI), nutrient agar plates (SSI), semisolid agar containing pepsin blood and thioglycolate (SSI), and serum broth (SSI). The CAMP reaction was performed on CAMP plates containing sheep erythrocytes (SSI) with a streak of a beta-hemolytic strain of *Staphylococcus aureus*. The strains were characterized by using the API Coryne and Rapid ID32A systems in accordance with the manufacturer's instructions (API bioMérieux, Marcy l'Etoile, France). Carbohydrate fermentation reactions were read after 24 and 48 h of incubation.

A Quantitect SYBR green kit (QIAGEN) was used with real-time PCR mixtures (50-μl total volume) containing 1× PCR buffer and a 200 μM concentration of each primer. The primers used for amplification of the 16S rRNA gene, BSF-8 (5′-AGAGTTTGATCCTGGCTCAG-3′) and BSR-534 (5′-ATTACCGCGGCTGCTGGC-3′), produced a 526-bp fragment of the 16S rRNA gene. Samples of 1 and 5 μl were tested by PCR. PCRs were performed by using an Opticon DNA engine (MJ Research). The amplification profile included incubation at 95°C for 15 min followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. PCR samples were spin column purified using Microcon YM-100 filter units (Millipore) for DNA sequencing. DNA strands of the amplicons were sequenced on an ABI PRISM 3100 Avant genetic analyzer (Applied Biosystems) using BSF-8 and BSR-534 as sequencing primers and a BigDye v.3.1 kit (Applied Biosystems). Sequencing data were edited using SeqScape software (Applied Biosystems), and the data were then compared to deposited sequences in the NCBI database using the BLAST search engine.

MICs for benzylpenicillin, cefuroxime, amdinocillin, nitrofurantoin, ciprofloxacin, tetracycline, gentamicin, and clindamycin were determined with the E-test (AB Biodisk, Solna, Sweden). An inoculum suspension of 1 McFarland standard in 0.9% NaCl was applied to *Brucella* blood agar containing hemin and vitamin K1 (BD). The MICs were read after 48 h of anaerobic incubation at 35°C. *Bacteroides fragilis* ATCC 25285 was used as a quality control strain.

Clinical data and predisposing conditions are summarized in Table 1. Patients 1, 3, 5, 6, and 8 had a history of recurrent symptoms of UTI and unexplained pyuria for months or years before *A. schaalii* was identified.

Our nine isolates were nonmotile, non-acid-fast, non-spore-forming, gram-positive coccoid rods. The isolates grew on all nine media after anaerobic incubation for 3 days, with the largest colonies seen on agar plates containing horse and sheep blood. On 5% Columbia sheep blood agar in an anaerobic atmosphere at 35°C for 48 h, *A. schaalii* cells grew as gray colonies of <1 mm in diameter. The colonies showed weak β-hemolysis on agar plates containing horse and sheep blood after 2 to 5 days. The CAMP reaction was absent. The isolates grew either well or poorly in air with 5% CO_2 and either poorly or not at all in ambient air. All strains were catalase and oxidase negative. Our isolates were compared with related species, and general information about the isolates is given in

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TABLE 1. Clinical data on 10 Danish patients infected with *Actinobaculum schaalii*[c]

Case no.	Age (yrs)	Sex	Clinical presentation	Specimens with *A. schaalii*	Concomitant flora	Predisposing conditions	Treatment
1	70	M	Urosepsis	Blood, urine	None	Prostatic hyperplasia	Cefuroxime and gentamicin
2	63	M	Urosepsis	Blood	Coagulase-negative staphylococci	Carcinoma vesicae	Meropenem, gentamicin, and nephrostomy catheter
3	63	M	Pyelonephritis	Pus from kidney cysts[a]	None	Prostatic hyperplasia, congenital cystic kidney malformation	Ampicillin, gentamicin-ciprofloxacin, and surgical drainage
4	9 mo	F	Cauda equine syndrome	Intradural abscess with fistulation to the skin	Nonhemolytic streptococci	Syringomyelia	Penicillin, metronidazole, and surgical drainage
5	70	F	Urosepsis	Blood, urine	None	Diabetes mellitus, multiinfarct dementia	Cefuroxime and gentamicin
6	77	F	Cystitis	Urine	None	Apoplexia, ischemic heart disease	Nitrofurantoin followed by ampicillin
7	84	M	Cystitis	Urine	None	Fracture of collum femoris, Alzheimer's disease	Cefuroxime
8	65	F	Cystitis	Urine (cultured twice before initiation of treatment)	*Aerococcus urinae*	Nephrectomia and pyelonephrose of remaining kidney	Amoxicillin followed by amdinocillin
9	84	F	Cystitis	Urine (cultured twice before initiation of treatment)	*Staphylococcus aureus*	Urinary catheter, colon carcinoma, rheumatoid arthritis	Sulfamethizole and a change of catheter
10	65	M	Urosepsis, bleeding, and shock[b]	Blood	None	Paraplegia, diabetes mellitus, kidney stones	Cefuroxime, ciprofloxacin, metronidazole, and nephrectomia

[a] Identified exclusively with universal 16S rRNA gene amplification/sequencing, as the bacteria were seen in a Gram stain, but the cultures were negative, probably because the patient was treated with intravenous antibiotics for 2 weeks before sampling.
[b] After endoscopic removal of kidney stones, the patient developed shock, probably resulting from urosepsis (leukocyte count, 28.5×10^9 cells/liter; C-reactive protein, 297) and bleeding from the kidney. After 2 days, the kidney had to be removed. Pathological examination of the kidney revealed hematoma, microabscesses, tubular necrosis, and chronic pyelonephritis.
[c] All patients recovered from the infection.

Table 2, while the results with the API Coryne system are given in Table 3. In the manufacturer's database, the API Coryne numerical profiles for the isolates were identified as doubtful or unacceptable profiles for *Arcanobacterium bernardiae*, *Arcanobacterium hemolyticum*, *Arcanobacterium pyogenes*, or *Gardenerella vaginalis*. Automatic reading of the Rapid ID32A test strip yielded the numerical codes 0400077705 (five strains), 0420077705 (three strains), and 0430077705 (one strain). According to the manufacturer's database, these were identified as very good (0400077705) or unacceptable (0420077705 and 0430077705) profiles for *Actinomyces meyeri*.

Sequence similarities of 96 to 100% were found by 16S rRNA gene sequencing for all strains matching closely to *A. schaalii*. The best taxon match and second best taxon match had a major identity score difference. *Actinomyces* sp. ($n = 4$), *Arcanobacterium* sp. ($n = 3$), *Mycobacterium* sp. ($n = 2$), and *Myceligeneris* sp. ($n = 1$) were recognized as the phylogenetically closest taxa.

The strains showed only small interisolate susceptibility variations and were susceptible to penicillin, cefuroxime, amdinocillin, nitrofurantoin, tetracycline, and clindamycin, with low MICs (Table 4). Reduced activities were seen with ciprofloxacin and gentamicin. Preliminary E-tests showed in vitro resistance to trimethoprim and sulfamethoxazole.

Our observations support, as recently reported (9, 12), the hypothesis that *A. schaalii* can cause UTIs in predisposed individuals. The difficulties in isolating and identifying *Actinobaculum* spp. are known (6, 7, 12). *A. schaalii* can be overlooked or interpreted as a contaminant due to its slow growth under aerobic conditions and its resemblance to the normal bacterial flora on skin and mucosa.

During 2004, seven strains of *A. schaalii* were identified in Viborg, Denmark, which has a population of 230,000, by the

TABLE 2. Comparison of *A. schaalii* with related species[a]

Organism	Reference(s)	Main source(s)
Actinobaculum schaalii	Our isolates	Urine, blood, abscess
Actinobaculum massiliae	6, 16	Urine, abscess
Actinobaculum urinale	7	Urine
Gardnerella vaginalis	2	Genital tract, blood, urine
Arcanobacterium bernardiae	3, 10, 15	Abscesses, blood, urine
Arcanobacterium haemolyticum	1, 11, 15	Throat, wounds
Arcanobacterium pyogenes	5, 13, 15	Wounds, abscesses, blood
Actinomyces turicensis	1, 14	Genital/skin infections, urine
Varibaculum cambriensis	8	Abscesses, IUCDs

[a] See Table 3 for characterization of these pathogens. IUCDs, intrauterine contraceptive devices.

TABLE 3. Characterization of *Actinobaculum* spp. and related human pathogens by the API Coryne system[a]

Characteristic	Reaction of[b]:								
	Actinobaculum schaalii	*Actinobaculum massiliae*	*Actinobaculum urinale*	*Gardnerella vaginalis*	*Arcanobacterium bernardiae*	*Arcanobacterium haemolyticum*	*Arcanobacterium pyogenes*	*Actinomyces turicensis*	*Varibaculum cambriensis*
β-Hemolysis on sheep blood agar	w[c]	−	w	−	V	+	+	w	−
Nitrate reduction	−	−	−	−	−	−	−	−	V
Pyrazinamidase	−[d]	+	−	V (89)	+	+	−	−	−
Pyrrolidonyl arylamidase	+	−	−	−	V (71)	V (59)	+	−	−
Alkaline phosphatase	V (11)[e]	−	−	−	−	+	V (71)	−	−
β-Glucuronidase	−	−	+	−	−	V (18)	+	−	−
β-Galactosidase	−	−	−	V (43)	−	V (85)	+	−	V
α-Glucosidase	+	+	−	V (65)	+	V (87)	+	+	+
N-Acetyl-β-glucosaminidase	−	−	−	V (18)	−	V (83)	V (47)	−	−
Esculin hydrolysis	V (11)[e]	−	−	−	−	−	−	−	−
Urease activity	−	−	+	−	−	−	−	−	−
Gelatin hydrolysis	−	−	−	−	−	−	+	−	−
Acid from:									
Glucose	V (56)[f]	+	+	+	V (50)	+	+	+	+
Ribose	+	+	+	+	+	V (83)	+	+	V
Xylose	V (44)[f]	+	−	−	−	−	+	+	V
Mannitol	−	−	−	−	−	−	−	−	−
Maltose	+	+	+	+	+	+	+	V	+
Lactose	−	−	−	−	−	+	+	−	−
Sucrose	V (44)	−	+	V (13)	−	V (50)	V (56)	+	+
Glycogen	−	+	−	V (53)	+	−	V (19)	−	−

[a] See Table 2 for additional information on the organisms.

[b] All species are catalase negative, facultative, anaerobic gram-positive rods. API Coryne system profiles for our nine isolates were compared with those in the manufacturer's database for *G. vaginalis* and the *Arcanobacterium* spp. and those described in the reference concerned for *A. massiliae* (1 strain) (6), *A. urinale* (1 strain) (7), *A. turicensis* (43 strains) (14), and *V. cambriensis* (15 strains) (8). In the API Coryne system, the numerical code for *A. turicensis* is 0010000. This difference can be explained by the fact that Sabbe et al. read the carbohydrate fermentation reactions after 72 h, as acid production from glucose, ribose, and xylose is reported not to be recognized within 24 h for *A. turicensis* (4). Abbreviations and symbols: +, ≥90% of strains positive; −, ≤10% of strains negative; V, variable; w, weak. Values in parentheses are percentages of strains with a positive reaction.

[c] The isolates showed weak β-hemolysis, but only after 2 to 5 days. *A. schaalii* is described as nonhemolytic in the literature ($n = 6$) (9, 12).

[d] Reported as variable in the literature ($n = 6$) (9, 12).

[e] Reported as negative in the literature ($n = 6$) (9, 12).

[f] Reported as positive in the literature ($n = 6$) (9, 12).

TABLE 4. Antimicrobial susceptibilities of nine strains of *Actinobaculum schaalii*

Antimicrobial agent	MIC range (mg/liter)	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)
Penicillin	0.003–0.032	0.008	0.023
Cefuroxime	<0.016	<0.016	<0.016
Amdinocillin	0.25–1.5	0.5	1
Nitrofurantoin	0.38–2	0.5	1
Clindamycin	0.016–0.064	0.023	0.047
Tetracycline	0.125–0.5	0.125	0.25
Gentamycin	1–4	1.5	2
Ciprofloxacin	2–4	3	4

Department of Clinical Microbiology at Viborg Hospital. In this department, urine cultures are routinely incubated in a CO_2-enriched atmosphere. This practice probably facilitates the identification of *A. schaalii*, and this finding strongly suggests that *A. schaalii* infections are more common than was previously recognized.

It is recommended that the identification of *A. schaalii* be done by performing both the API Coryne and Rapid ID32A test systems, at least until the manufacturers' databases have been updated. In doubtful cases, the strains should be referred to a reference laboratory for definite confirmation by 16S rRNA gene sequencing.

In the case of patient 8, treatment failure was observed after therapy with amoxicillin (500 mg three times daily) for 1 week. Since treatment failure with amoxicillin was also reported for a patient with a chronic UTI due to *A. massiliae* (6), treatment with β-lactam antibiotics for a prolonged period may be required.

Microbiologists and clinicians should be aware of *A. schaalii* and related species in cases of unexplained chronic pyuria, especially if the microscopic findings differ from the growth results under aerobic conditions. In these cases, urine samples should be cultured on appropriate media and incubated in an anaerobic atmosphere.

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