Case Report

Actinobaculum schaalii causing urinary tract infections: report of four cases from Argentina

Claudia Barberis[1], Roxana Cittadini[2], Marcelo del Castillo[2], Pablo Acevedo[2], Cristian García Roig[2], María Soledad Ramírez[3], Susana Pérez[4], Marisa Almuzara[1], Carlos Vay[1,2]

[1] *Instituto de Fisiopatología y Bioquímica Clínica. Hospital de Clínicas José de San Martín. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*
[2] *Sanatorio Mater Dei, Ciudad Autónoma de Buenos Aires, Argentina*
[3] *Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina*
[4] *Departamento de Microbiología. Laboratorios IACA. Bahía Blanca, Argentina*

Abstract

Actinobaculum schaalii may be a more common urinary tract pathogen than previously described. Here we report four cases of *A. schaalii* UTIs and we also propose a simple identification scheme to be used in the conventional microbiology laboratory based on standard biochemical tests.

Key words: *Actinobaculum schaalii;* urinary infection; uropathogen

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Introduction

Actinobaculum schaalii is a Gram-positive rod that grows slowly after 4 to 5 days with 5% CO_2 in air at 35 °C and grows better after 48 hours in an anaerobic atmosphere.

A. schaalii can cause urinary tract infections (UTIs) but due to its slow growth it may be easily overgrown by other pathogens and therefore not be identified and be a more common urinary tract pathogen than previously described [1].

In the last years, several reports have been published since it was first described in 1997 [2]. This pathogen has been reported to be responsible for UTIs mainly in elderly patients with underlying urological predisposing factors [1,2] and also in pediatric patients [3]. Two other species from the genus *Actinobaculum*, *A. massiliae* and *A. urinale*, have also been detected in urine and other clinical specimens such as blood and pus from a superficial skin infection [4,5,6]. Besides UTIs, other infections have been reported with *A. schaalii* as the causative agent, including bacteremia, vertebral osteomyelitis and perineal necrotizing cellulitis [7,8,9], *A. schaalii* appears to be an emerging pathogen, in relation with UTIs in the last decade [1,2,3,10].

Actinobaculum schaalii not only encounters phenotypic identification problems, but also isolation of this pathogen is complicated. We report the use of a polyphasic approach to characterize the four Gram-positive strains obtained from human clinical urine samples with the purpose to improve identification of this species in the routine microbiology laboratories.

In addition we also describe the first four cases of UTIs caused by *A. schaalii* in Argentina.

Case Reports

Four male patients, aged between 17 and 95 developed UTIs symptoms. The main clinical characteristics of each case are summarized in Table 1.

In all the urine samples, Gram stain showed rod-shaped organisms with some degree of branching, non-acid-fast and non-spore forming. In all samples, a pure growth of more than 10^5 CFU/mL on chocolate agar incubated at 35 °C in a 5% CO_2 atmosphere and anaerobically after 72 hours was observed.

Table 1: Clinical and microbiology characterisitics of four patiens with *Actinobaculum schaalii* infection

Case N°	Age (years)	Clinical presentation	Underlying disease	Predisposing conditions	Urine leucocytes/HPF	Treatment
1	17	pyelonephritis	CRF, monorenal, congenital uropathy	haemodyalisis	>30	Pip-taz followed by ampicillin
2	40	pyelonephritis	HTA, CRF, urethral stenosis	haemodyalisis, transplant	15	unknown
3	82	dysuria, incontinence	arterial obstruction of lower member	Age, geriatric	>30	Pip-taz
4	95	dysuria, incontinence	Benign prostatic hypertrophy, Stroke, HTA	Age	20	Amox-clav

pip-taz: piperacillin- tazobactam; amox-clav: amoxicillin-clavulanic; CRF: chronic renal failure; HTA: Arterial hypertension

Table 2: Biochemical characteristics of the four *A.schaalii* isolates

Biochemical tests	Results (N°of test for clinical isolates) (n:4)	*A.schaalii* †
Hemolysis (sheep blood agar)	- (4)	-
Catalase	- (4)	-
SPS disk	R (4) *	nd
MRS broth growth	- (4)	nd
CAMP test	- (4)	+^{w}
Hippurate #	+ (4)	+
API Coryne :		
Nitrate reduction	- (4)	-
Urease activity	- (4)	-
Esculin hydrolisis	- (4)	-
Alkaline phosphatase	- (4)	-
Pyrrolidonilarilamidase	+ (4)	d
β glucuronidase	- (4)	-
β galactosidase	- (4)	-
N-acetyl-β glucosaminidase	- (4)	-
α glucosidase	+ (4)	+
Acid from:		
Glucose	+ (4)	+
Xylose	+ (4)	+
Maltose	+ (4)	+
Mannitol	- (4)	-
Sacarose	- (4)	d
Glycogen	- (4)	-
API Rapid ID 32 A:		
Nitrate reduction	- (4)	-
α glucosidase	+ (4)	+
β glucuronidase	- (4)	-
β galactosidase	- (4)	-
N-acetyl-β glucosaminidase	- (4)	-
α galactosidase	- (4)	-
α fucosidase	- (4)	nd
Alkaline phosphatase	- (4)	-

†Data from Lawson et al. (1997) (8)
-, negative; +,positive; w, weak; d, difference between strains; nd, not determined.
* No inhibition zone was observed around SPS disk
# According to the result of reference 3 (using API rapid ID 32 STREP bioMérieux) no discrepancies were observed in our strains using Diagnostic tablets (Rosco)

The chromogenic agar CPS ID medium cultures (bioMérieux, Marcy l´Etoile, France) incubated aerobically at 35 °C after 48 hours were negative. The colonies were gray, less than 1 mm in diameter and catalase and oxidase negative. No hemolysis was observed on 5% sheep blood agar and none of the strains was lipophilic [11].

CAMP reaction of the four isolates was examined with a β-hemolysin producing strain of *Staphylococcus aureus* ATCC 25923, and the results were negative for all of them.

A 30 µg vancomycin disk (Oxoid, Basingstoke, United Kingdom) and a Sodium polyanethol sulfonate SPS disk (Rosco Diatabs, Taastrup, Denmark) were performed in a Columbia agar base and Mueller Hinton agar base with 5% sheep blood respectively and incubated at 35 °C in a 5% CO_2 atmosphere and read at 24 hours and 48 hours after incubation. For the vancomycin disk, any zone of inhibition was considered susceptible. For the SPS disk, a diameter ≥ 10 mm in all the strains was considered susceptible (Table 2).

The growth in Mann, Rogosa and Sharpe (MRS) broth (Difco, BD, Franklin Lakes, USA) was used to differentiate those genera that grew well (slightly turbid) or very well in MRS broth in comparison to other genera that may not. No turbidity was observed in all strains.

The biochemical tests were performed using API strips, Rapid ID 32A and API Coryne systems according to the manufacturer's specifications (bioMérieux, Marcy l´Etoile, France) [2].

Hippurate hydrolysis was examined using Rosco Diatabs (A/S Rosco, Taastrup, Denmark) according to the manufacturer's specifications.

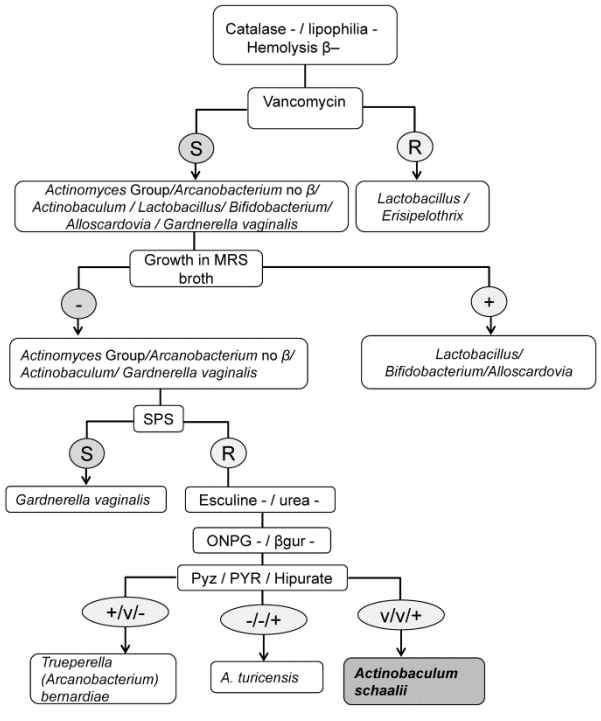
The biochemical characteristics of the four *A. schaalii* isolates are shown in Table 2.

The flowchart for the preliminary identification of *A. schaalii* isolates was performed alongside 85 clinical isolates including *Bifidobacterium scardovii* (n: 2), *Gardnerella vaginalis* (n: 10), *Trueperella bernardiae* (n: 2), *Arcanobacterium haemolyticum* (n: 7), *A. schaalii* (n: 10), *Actinomyces* spp (n: 54). The phenotypic identification was performed by biochemical methods following the algorithm proposed in Figure 1, which shows the phenotypic tests used to differentiate the *A. schaalii* from other related catalase-negative Gram-positive rods.

Sequencing of 16S rRNA was used as the gold standard to identify the isolates and was performed by polymerase chain reaction (PCR) amplification and sequence analysis of the 16S rRNA gene. PCR product. Primers used were those described by Weisburg *et al*. [12] and was carried out with the Taq DNA polymerase based on the manufacturer's specifications (Qiagen, Hilden, Germany). Sequencing of the 1.4 kb PCR product was performed on both DNA strands using an ABIPrism 3100 BioAnalyzer equipment at Macrogen Inc. sequencing facility. The sequences were analyzed with the BLAST V2.0 software available in http://www.ncbi.nlm.nih.gov/BLAST/. Sequenced analysis revealed a 99% identity with the sequences corresponding to the 16s RNA ribosomal gene of *A. schaalii* (FJ 7111719.1).

All *A. schaalii* isolates were tested for antimicrobial susceptibility to penicillin, ciprofloxacin and trimethoprim-sulfamethoxazole by E-test (bioMerieux, Marcy l´Etoile, France) in Brucella agar supplemented with hemin (5 µg/mL), vitamin K (1 µg/mL), and 5% sheep blood. The inoculum size was equivalent to a No.1 McFarland standard [13] and incubated in an anaerobic atmosphere (anaerocult A system, Merck, Darmstadt, Germany) at 35°C for 48 hours. All isolates were highly susceptible to penicillin (MICs ≤0,032 µg/mL) and trimethoprim-sulfamethoxazole (MICs ranges 0,094 -0,125 µg/mL), whereas reduced activities were seen with ciprofloxacin with MICs ranges from 8-32 µg/ml.

Figure 1: Flowchart for preliminary identification of Actinobaculum schaalii



MRS, MannRogosaSharpe ; ONPG, β-galactosidase; β-gur, β glucuronidase; Pyz, pyrazinamidase; PYR, pyrrolidonilarilamidase activity.

Discussion

A. schaalii was first described in 1997 and since then many reports have been published indicating *A. schaalii* as a causative agent for urinary tract infections [1,3]. The common risk factors are prostatic hyperplasia, urinary catheter, and urethral stenosis [3,14].

In the literature we found UTIs report cases not only in elderly patients with underlying genitourinary tract diseases [2], but also in children less than 17 years old [3]. In our study, the included patients were from different ages (Table 1) with only one without predisposing conditions for UTI. Even though this uropathogen may cause septic complications such as urosepsis, bacteremia and endocarditis [7,15,16,17] in this case *A. schaalii* was not isolated in blood.

In cases of unexplained pyuria, the performance of Gram stain should be evaluated and the methodology of culturing samples on appropriate media and incubated in an anaerobic atmosphere for a prolonged period of time should be practiced. This can help to identify the presence of this fastidious uropathogen and consequently avoid starting unnecessary empiric treatment if this infection is suspected. Gram stain morphology could contribute to a presumptive identification of the *Actinomyces-Actinobaculum* group-like organisms. For initial grouping of non-spore-forming-Gram-positive rods, hemolysis and production of catalase were considered the key characteristics to differentiate from catalase-producing *Actinomyces* species. The flowchart was based on few simple and unexpensive tests.

The differentiation of *Arcanobacterium*, *Actinomyces*, *Actinobaculum*, *Bifidobacterium/ Alloscardovia* from other non-spore forming bacilli such as most of *Lactobacillus* species, and *Erysipelothrix* could be approached using a vancomycin disk (30 µg). Following the identification, the lack of growth in MRS broth in all cases, excludes the genera *Lactobacillus* spp, *Bifidobacterium / Alloscardovia* from the *Arcanobacterium / Actinomyces* and *Actinobaculum* species. In addition, the SPS disk was important to discriminate these genera from *Gardnerella vaginalis* [18]. Finally, the 16S r RNA greatly helped in the confirmation of our preliminary identification.

Three out of four patients received a β-lactam antibiotic treatment with a favorable outcome. As previously published, *A. schaalii* shows diminished susceptibility to the antibiotics commonly used in UTIs, particularly, ciprofloxacin or trimethoprim-sulfamethoxazole, but it is highly susceptible to β-lactam antibiotics [3,11,19]. Even though the length of the antimicrobial course for UTIs is not clearly established, it should include a β-lactam antibiotic, such as penicillin, amoxicillin or a cephalosporin (especially cefuroxime) for uncomplicated or complicated UTIs for at least two weeks since treatment failure has been observed with short course therapy [10].

Conclusion

We conclude that susceptibility testing should be routinely performed when *A. schaalii* is isolated in relevant clinical specimens.

The purpose of this study was to design a flowchart that would allow routine microbiology laboratories, unable to perform molecular identification, to identify this emerging pathogen in a simple and inexpensive way. If the case cannot be solved using the proposed flowchart, the strains should be sent to a reference laboratory for definitive diagnosis using 16S r RNA sequencing.

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References

1. Bank S, Jensen A, HansenTM, Soby K, Prag J (2010). *Actinobaculum schaalii*, a common uropathogen in elderly patients. Emerg Infect Dis 16: 76-80.
2. Beguelin C,Genne D,Varca D, Tritten ML, Siegrist H, Jaton K Lienhard R (2010) *Actinobaculum schaalii*: clinical observation of 20 cases. Clin Microbiol Infect 17: 1027-1031.
3. Zimmerman P, Berlinger L, Liniger B, Grunt S, Agyeman P, Ritz N (2012) *Actinobaculum schaalii* an emerging pediatric pathogen?. BMC Infect Dis 12, 201.
4. Greub G, Raoult D (2002) *Actinobaculum massiliae,* a new species causing chronic urinary tract infection. J Clin Microbiol 40: 3938-3941.
5. Hall V, Collins M, Hutson R, Falsen E, Inganas E, Duerden B (2003) *Actinobaculum urinale* sp nov, from human urine. Int J Syst Evol Microbiol 53: 679-682.
6. Waghorn D (2004) *Actinobaculum massiliae*: a new cause of superficial skin infecion. J Infect 48: 276-277.
7. Gomez E, Gustafson D, Rosenblatt J, Patel R (2011) *Actinobaculum* bacteremia. J Clin Microbiol 49: 4311-4313.
8. Haller P, Bruderer T, Schaeren S, Laifer G, Frei R, Battegay M, Fluckiger U, Bassetti S (2007) Vertebral osteomyelitis caused by *Actinobaculum schaalii:* a difficult-to-diagnose and potentially invasive uropathogen. Eur J Clin Microbiol Infect Dis 26: 667–670.
9. Vanden Bempt I, Van Trappen S, Clenwerck I, DeVos P, Camps K, Celens A, Van De Vyvere M (2011) *Actinobaculum schaalii* causing Fournier's gangrene. J Clin. Microbiol 49: 2369–2371.

10. Reinhard M, Prag J, Kemp M, Andresen K, Klemmensen B, Hojlyng N, Hildebrand Sorensen S, Jorgen Christensen J. (2005) Ten cases of *Actinobaculum schaalii* infection: clinical relevance, bacterial identification and antibiotic susceptibility. JClin Microbiol 43: 5305-5308.
11. Riegel P, de Briel D, Pre´vost G, Jehl F, Monteil H (1994) Genomic diversity among *Corynebacterium jeikeium* strains and comparison with biochemical characteristics. J Clin Microbiol 32: 1860-1865.
12. Weisburg WG, Barns SM, Pelletier DA, Lane LD (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173: 697-703.
13. Rosenblatt J, Gustafson E (1995) Evaluation of the E-test for susceptibility testing of anaerobic bacteria. Diagn Microbiol Infect Dis 22: 279-284.
14. Tschudinn-Sutter S, Frei R, Weisser M, Goldenberger D, Widmer A (2011) *Actinobaculum schaalii-invasive pathogen or inn*ocent bystander? A retrospective observational study. BMC Infect Dis 11: 289
15. Hesstvedt L, Hasseltvedt V, Aandahl E, Caugant D, Hoiby EA (2006) Septicaemia due to *Actinobaculum schalii*.Scand J Infect Dis 38: 735-737.
16. Martinaud C, Gaillard T, Maslin J, Riviere P, Fournier R, Trueba F (2008) *Actinobaculum schaalii* bacteremia in an aged male patient. Med Mal Infect 38: 617-619.
17. Hoenigl M, Leitner E, Valentin T, Zarfel G, Salzer HJ, Krause R (2010). Endocarditis caused by *Actinobaculum schaalii*, Austria. Emerg Infect Dis 16: 1171-1173.
18. Reimer LG, Reller LB (1985) Use of a sodium polyanetholesulfonate disk for the identification of *Gardnerella vaginalis*. J Clin Microbiol 21: 146-149.
19. Cattoir V, Varce A, Greub G, Prod´hom G, Legrand P, Lienhard R (2010) In vitro susceptibility of *Actinobaculum schaalii* to 12 antimicrobial agents and molecular analysis of fluorquinolone resistance. J Antimicrob Chemother 65: 2514-517.

Corresponding author

Claudia Barberis
Avenida Córdoba 2351. Departamento de Bioquímica Clínica.
Ciudad Autónoma de Buenos Aires. Zip Code: 1120, Argentina
Phone: (5411) 5 950 8665.
Fax: (5411) 5 950 8694.
E-mail: claudiabar07@gmail.com

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