

# Actinobaculum shaalii: a new uropathogen?

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## Summary

**Background and Aims.** *Actinobaculum shaalii* is a facultative anaerobic, Gram-positive rod-shaped species phylogenetically related to *Actinomyces*. *A. shaalii* is an emerging pathogen causing urinary tract infections (UTI) in both children and adults; although, as part of the human genitourinary tract flora, it is frequently overlooked or considered as a contaminant. While the phenotypic identification of *A. shaalii* is difficult, the recent Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight-mass spectrometry (MALDI TOF) technology could represent a promising tool for its identification.

**Materials and Methods.** This is a retrospective study including all known cases (n=7) of *A. shaalii* infections occurred (between July 2013 and November 2013) at the Microbiology Laboratory of the A. Cardarelli Hospital, in Campobasso (Italy).

**Results.** All the 7 *A. shaalii* collected strains, resulted *in vitro* susceptible to most of the drugs commonly used for urinary tract infections, but resistant to ciprofloxacin, a first-line antibiotic in the treatment of prostatitis. All isolates were susceptible to amoxicillin, amoxicillin-clavulanic, ampicillin-sulbactam, cefuroxime, gentamicin, piperacillin-tazobactam, vancomycin, tetracycline (no EUCAST breakpoints). All except two isolates were susceptible to cefotaxime; 3/7 and 5/7 strains were clindamicin and levofloxacin resistant, respectively.

**Conclusions.** As most antibiotics empirically prescribed for UTI (mainly fluoroquinolones or trimethoprim/sulfamethoxazole) are not

effective against *A. shaalii*, the appropriate onset of treatment was delayed by an average of 2.8 days. The implementation of the newer MALDI TOF technology in routine diagnostic procedures may allow a more reliable and rapid identification of *A. shaalii* in future.

## Introduction

*Actinobaculum shaalii* was first described in 1997 and named after Klaus P. Schaali, a German microbiologist specialized in Actinomycete microbiology. The genus *Actinobaculum* includes *A. shaalii* (1997), *A. suis* (1997), *A. massiliense* (2002) and *A. urinale* (2003) and is closely related to the genera *Actinomyces* and *Arcanobacterium* (12).

*A. shaalii* is a small Gram-positive, non-motile, asporigenous, facultative anaerobic, coccoid rod that requires CO<sub>2</sub> for optimal growth (Figure 1).

Hippurate is hydrolyzed whereas esculin (except a few exceptions) and gelatin are not (Table 1). Relatively few sugars are fermented (*i.e.* glucose, maltose, ribose, and D-xylose). The isolates produce  $\alpha$ -glucosidase, alanine-phenylalanine-proline arylamidase, and pyroglutamic acid arylamidase whereas alkaline phosphatase, arginine dihydrolyase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase, and  $\beta$ -mannosidase activities are not detected. They do not reduce nitrates to nitrites, and acetoin is not produced (7,12,15,16,20).

It grows slowly after 48h in anaerobic atmosphere or microaerophilic condition at 37°C as tiny grey colonies, less than 1 mm in diameter, and shows weak  $\beta$ -hemolysis on agar plates containing 5% horse or sheep blood after 3-5 days of growth (13,20) (Figure 2). It is catalase, oxidase and urease negative and resistant to trimethoprim and ciprofloxacin (15). It is easily overgrown by other bacteria, which are often found concomitantly. Because of its slow anaerobic growth and resemblance to the normal bacterial flora on skin and mucosa, *A. shaalii* is often overlooked or considered a contaminant. These difficulties regarding detection and identification prevent the evaluation of the clinical impact of this pathogen and of its potential to cause invasive infection.

Most laboratories do not look specifically for *Actinobaculum* species. Even in the case of *A. shaalii* growth, this microorganism may be overlooked either because of overgrowth of other common uro-pathogens or due to the bacteria's similarity to normal skin flora (3,15,19).

*A. shaalii* has been reported to be responsible for UTIs, mainly in elderly patients with underlying urological risk factors (2,7,13,15). Interestingly, a recent study showed that 22% of 252 urine samples from patients >60 years were positive for *A. shaalii* using real-time PCR. This microorganism may also cause septic complications such as urosepsis, abscess, osteomyelitis and endocarditis (2,9,10,13, 15,14,18). It has also been recovered from other human clinical specimens than urine such as blood, but its pathogenic potential remains unknown. Although *A. shaalii* is an emerging uro-pathogen, *in vitro* antimicrobial agents susceptibilities have been poorly evaluated, particularly in terms of MICs.

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Key words: *Actinobaculum shaalii*; urinary tract infection; antimicrobial susceptibility.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 28 October 2014.  
Accepted for publication: 18 December 2014.

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*Microbiologia Medica* 2015; 30:4801  
doi:10.4081/mm.2015.4801

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Difficulties identifying *A. schaalii* by using traditional phenotypic tests have obscured its pathologic role for many years. Our laboratory recently introduced the Bruker MALDI-TOF identification system which uses the Biotyper database. Given the ease and rapidity in species identification, this technology is now used to screen all blood culture samples (to evaluate the pure/predominant bacterial growth both from sterile and non sterile sites). Indeed, mass spectra (m/z range between 2000 and 13000) obtained for *A. schaalii* strains appear to be unique and reproducible. Notably, all the databases now available do not include the spectrum profile for this species and must be updated (11,17).

The aim of this study was i) to define the demographic and clinical characteristics of all the patients affected by an *A. schaalii* infection, ii) to identify the particular elements enabling clinicians to suspect an *A. schaalii* infection and consequently to request its specific detection.

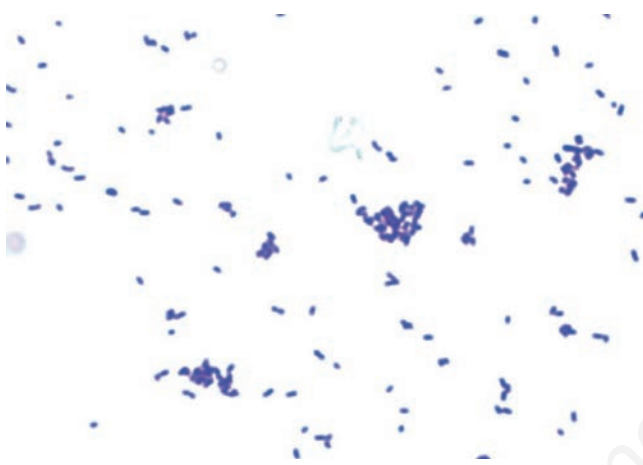


Figure 1. Gram stain of *Actinobaculum shaalii*.



Figure 2. Colonies morphology of *Actinobaculum shaalii* grown on Columbia agar medium with 5% sheep blood after 48h of anaerobic incubation at 37°C.

Table 1. Main phenotypic characteristics of *Actinobaculum shaalii* and related human pathogens.

Characteristic	<i>Actinobaculum shaalii</i>	<i>Actinobaculum massiliense</i>	<i>Actinobaculum urinale</i>	<i>Arcanobacterium haemolyticum</i>	<i>Trueperella pyogenes</i> *	<i>Trueperella bernardiae</i> *	<i>Actinomyces meyeri</i>	<i>Gardnerella vaginalis</i>
Catalase	-	-	-	-	-	-	-	-
$\beta$ -hemolysis (sheep blood)	-(w) <sup>a</sup>	-	w	+	+	v	-	- <sup>b</sup>
Nitrate reduction	-	-	-	-	-	v	-	-
Urease activity	-	-	v	-	-	-	-	-
Hippurate hydrolysis	+	+	+	-	+	-	-	+
Esculin hydrolysis	-(+) <sup>c</sup>	-	-	-	-	-	-	-
Gelatin hydrolysis	-	-	-	-	+	-	-	-
$\beta$ -galactosidase	-	-	-	+	+	-	+	v
$\alpha$ -glucosidase	+	+	-	+	+	+	+	v

+, positive; -, negative; v, variable; w, weak. \*Formerly: *Arcanobacterium pyogenes* and *Arcanobacterium bernardiae*.  
<sup>a</sup>Weak -hemolysis may be observed after a prolonged incubation; <sup>b</sup> $\beta$ -hemolysis on human blood agar; <sup>c</sup>Esculin hydrolysis has been reported for a few isolates.

## Materials and Methods

### Patients and data collection

This is a retrospective study including all known cases of *A. schaalii* infections (from July to November 2013) at the Microbiology Laboratory of Cardarelli Hospital in Campobasso. All the data on the patients, specimens and consecutive non-replicate isolates of *A. schaalii*, were obtained from the database of the Clinical Microbiology Laboratory.

Only the cases from patients with genitourinary site infections caused by *A. schaalii* were analyzed. An infectious diseases specialist reviewed the medical records of all the patients: patient's age and sex, underlying diseases or health condition, clinical signs at the time of detection of *A. schaalii*, administered antibiotic therapy, and clinical outcome. The antibiotic regimen chosen after the identification of the pathogen was considered as those related to the outcome. The cases were categorized as colonization or infection.

### Culture and species identification of *Actinobaculum schaalii*

We examined seven clinical isolates of *A. schaalii* recovered from urine, urethral swab and seminal fluid.

Grey, tiny colonies with weak  $\beta$ -hemolysis or without hemolysis on 5% Columbia sheep blood agar after 48h of anaerobic (or 5% CO<sub>2</sub>) incubation were further analyzed.

All isolates showing Gram-positive coccoid rods and a negative catalase reaction were identified by MALDI TOF system (Bruker-Daltonics) with a score of about 2.3 (*i.e.*, a reliable score to the species level) using the direct colony transfer method (5,6,11) (Figure 3). All bacteria growing as monocultures in normally sterile specimens and collected from unsterile body sites were considered to be clinically relevant.

### Antimicrobial susceptibility testing

Even though no recommendations have been established yet to perform antimicrobial susceptibility testing (AST) for *Actinobaculum schaalii*, the E-test method seems to be reliable (1,2,4,8,9,15).

MICs of the following 12 antibiotics were determined for all strains using E-test (Liofilchem): Amoxicillin, amoxicillin-clavulanic, ampicillin-sulbactam, piperacillin-tazobactam, cefotaxime, cefuroxime,

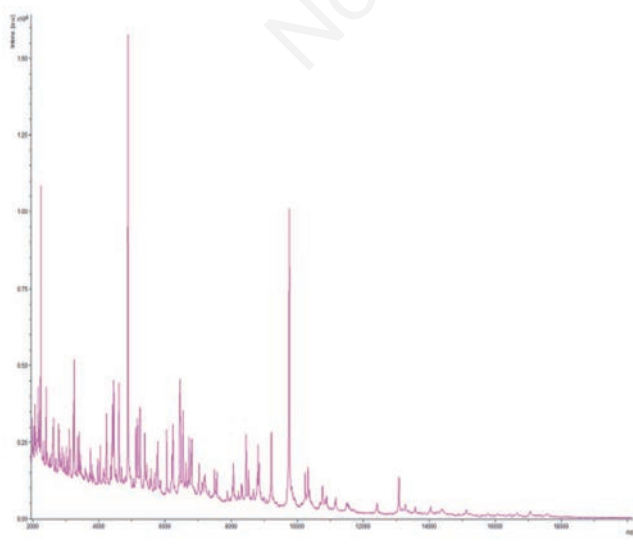


Figure 3. Mass spectra of *Actinobaculum schaalii*.

Table 2. *In vitro* activity of 12 antimicrobial agents against clinical isolates of *Actinobaculum schaalii*.

Antimicrobial agent	1 <sup>st</sup> case	2 <sup>nd</sup> case	3 <sup>rd</sup> case	4 <sup>th</sup> case	5 <sup>th</sup> case	6 <sup>th</sup> case	7 <sup>th</sup> case	Susceptibility breakpoint, µg/mL
Amoxicillin-clavulanic	0.094 (S)	0.094 (S)	0.19 (S)	0.19 (S)	0.19 (S)	0.38 (S)	0.38 (S)	≤4
Ampicillin-sulbactam	0.032 (S)	0.032 (S)	0.50 (S)	0.50 (S)	0.032 (S)	0.094 (S)	0.19 (S)	≤4
Amoxicillin	0.38 (S)	0.38 (S)	0.38 (S)	0.38 (S)	0.25 (S)	0.19 (S)	0.25 (S)	≤4
Piperacillin-tazobactam	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.016 (S)	≤8
Cefotaxime	32 (R)	32 (R)	0.125 (S)	0.125 (S)	0.38 (S)	0.094 (S)	0.38 (S)	≤1
Cefuroxime	0.016 (S)	0.016 (S)	0.064 (S)	0.064 (S)	0.032 (S)	0.016 (S)	0.25 (S)	≤4
Gentamicin	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	≤2
Vancomycin	0.094 (S)	0.094 (S)	0.125 (S)	0.125 (S)	0.064 (S)	0.064 (S)	0.125 (S)	≤2
Tetracycline	1 (S)	1 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	≤1
Clindamycin	64 (R)	64 (R)	128 (R)	128 (R)	32 (R)	0.032 (S)	256 (R)	≤4
Ciprofloxacin	32 (R)	32 (R)	4 (R)	4 (R)	16 (R)	4 (R)	4 (R)	≤0.5
Levofloxacin	32 (R)	32 (R)	2 (R)	2 (R)	0.75 (S)	0.75 (S)	6 (R)	≤1

ciprofloxacin, levofloxacin, clindamycin, gentamicin, vancomycin, tetracycline.

*In vitro* susceptibilities were performed on Mueller-Hinton blood agar in 5% CO<sub>2</sub> using an inoculum of 0.5 McFarland. The plates were incubated 48h in anaerobic conditions. *Bacteroides fragilis* ATCC 25285 was used as a quality control. No clinical breakpoints have been recommended yet for *A. schaalii*, but interpretation of MIC results may be done using non-species-related interpretative standards or those established for the other uro-pathogens (Table 2).

## Results

Between July 2013 and November 2013 we identified 7 *A. schaalii* positive samples in 7 male patients. Specimens positive for *A. schaalii* were found in urine, urethral swab and seminal fluid. All the patients were symptomatic for an UTI, showing urinary symptoms such as alguria and dysuria. All specimens resulted mono-microbial. The patient's ages ranged from 24 to 39 years (mean 30). All patients were not hospitalized.

The isolates were identified as *Actinobaculum schaalii* by MALDI TOF mass spectrometry, with a score from 2.15 to 2.36 (mean 2.25) using the direct colony transfer method.

Being informed on the prevalence of the causative UTIs pathogens (and their antimicrobial susceptibility) in different patient's types is of great importance to make a correct clinical and microbiological diagnosis. The *A. schaalii* isolates studied, *in vitro* susceptible to most of the drugs commonly used for UTIs, resulted resistant to ciprofloxacin, a first-line antibiotic in prostatitis treatment. All the isolates were susceptible to amoxicillin, amoxicillin-clavulanic, ampicillin-sulbactam, cefuroxime, gentamicin, piperacillin-tazobactam, vancomycin, tetracycline (EUCAST breakpoints). Five out of seven isolates were susceptible to cefotaxime, 3/7 and 5/7 were resistant to clindamycin and levofloxacin, respectively. As most antibiotics empirically prescribed for urinary infection (mainly fluoroquinolones or trimethoprim/sulfamethoxazole) are not effective against *A. schaalii*, the appropriate onset of treatment was delayed by an average of 2.8 days (range 1-13).

## Discussion

UTIs are predominantly caused by members of the *Enterobacteriaceae* family. However, Gram-positive bacteria and polymicrobial infections are not uncommon in patients with underlying urological conditions. *A. schaalii* is an underestimated opportunistic uro-pathogen and its search is relevant in cases of unexplained chronic pyuria, especially when there is discrepancy between smear microscopy findings and growth results under aerobic conditions (13,15).

Although *A. schaalii* is an emerging uro-pathogen, very little information are available about its *in vitro* antibiotics susceptibility, especially to those commonly used in UTIs treatment (e.g. ciprofloxacin). In addition slow growing bacteria such as *A. schaalii* can remain undetected for the overgrown of faster-growing Enterobacteria. Implementation of the newer MALDI TOF technology in routine diagnostic procedures may allow a more rapid identification of *A. schaalii*, which might increase the frequency of its detection.

*A. schaalii* has traditionally been thought to be part of the commensal flora of the human urogenital tract (12). Colonization seems to be a risk factor for an *A. schaalii* UTI both in children and adults, as most UTI case reports have been described in the same age groups in which colonization has been most frequently detected.

The vast majority of *A. schaalii* isolates were resistant to

ciprofloxacin, frequently used as empiric treatment in UTIs in children and adults. Current recommendations for the antibiotic treatment for *A. schaalii* infections are represented by amoxicillin or cephalosporins. Data suggesting that gentamicin, vancomycin, linezolid, nitrofurantoin and meticillinam are potential alternative treatment options are limited (1,4). The optimal length period of antibiotic therapy in the case of an *A. schaalii* infection is currently unknown. Most case reports suggest a treatment duration of 7 to 14 days.

Therefore we repeated the urine culture after eight days of antibiotic treatment, and stopped treatment after 14 days as soon as negative culture results were available.

## Conclusions

In conclusion, *A. schaalii* is an emerging pathogen in both children and adults. Colonization and subsequent infection seem to be influenced by a number of factors, in particular age of the patient. The spectrum of infections extends from benign cystitis to pyelonephritis with bacteremia, indicating that the pathogen can be invasive. *A. schaalii* is likely to have been under-diagnosed in the past, but with introduction of the MALDI TOF system, a rapid and inexpensive technology, *A. schaalii* should specifically be included in the microbiological search of pathogens instead of dismissing its presence as clinically irrelevant colonization by *Corynebacteria* or *Lactobacillus* spp.

Microbiology laboratory management include selective blood agar plates and identification by MALDI-TOF System.

A prospective study on *A. schaalii* infections should be performed to confirm these results and determine the appropriate treatment and its duration.

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