

Actinobaculum schaalii: identification with MALDI-TOF

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Abstract

Actinobaculum schaalii is an emerging uropathogen. So far, its identification has been performed with 16S rRNA gene sequencing or PCR. The diagnosis has often been delayed due to fastidious growth and identification problems. Eleven clinical isolates of *A. schaalii* from bloodstream infections that were initially identified with 16S rRNA sequencing analysis were recovered and later identified with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). We present a review of bacteriological data of these patients, an algorithm for fast laboratory work-up and advocate the use of sensitized culture of urine to allow better recovery of *A. schaalii* in susceptible patients.

Keywords: *Actinobaculum schaalii* infection, bacteriological diagnostics, emerging infections, Gram-positive rod, urinary tract infection

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Introduction

Actinobaculum schaalii is not yet a well-known bacterium but has been increasingly reported as an emerging pathogen causing urinary tract infections (UTIs) in patients with underlying uropathologies [1–8] or infections of other organs [9–11]. This pathogen has also been reported to cause urinary infections in children with renal or neurological disorders [12, 13]. Due to its fastidious growth in microaerophilic

environments *A. schaalii* may be overlooked when traditional culture methods are applied. In addition, *A. schaalii* may be neglected and misinterpreted as a representative of normal microbiota of mucosa or skin [3, 6, 7], especially when present as a co-isolate with ‘classical’ pathogens of UTIs, such as *Escherichia coli*. In many reported cases diagnosis was delayed, and the final identification to the species level was achieved after 16S rRNA gene sequencing [1–13]. What makes this pathogen important is its peculiar antimicrobial resistance to ciprofloxacin and co-trimoxazole, the drugs often prescribed empirically for the treatment of UTIs.

Urine from urological or immunocompromised patients can be cultured using a sensitized procedure. This procedure includes, in addition to the conventional agar for Gram-negative bacteria, the cultivation on a chocolate agar with a 10 µL loop to increase sensitivity.

Below we present results that advocate the use of a sensitized culture in risk-group patients. We also demonstrate the key metabolic reactions, an algorithm to increase the rate of laboratory work-up, and the suitability of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) for identification of *A. schaalii*.

Materials and Methods

Eleven clinical isolates collected from 2008 through 2012 were from five district laboratories. All were isolated from the routine blood culture (bioMérieux, Marcy L’Étoile, France) and initially identified using 16S rRNA gene sequencing (Huslab, Helsinki, Finland) according to the previously published protocol [14]. Ethics approval was not requested because the data presented were collected only from laboratory records.

All isolates were stored at –70°C before being retrieved and cultured on anaerobe basal agar with 10% horse blood (ABA; Oxoid Ltd, Basingstoke, UK), chocolate, and sheep blood agar at +35°C in a CO₂ atmosphere. Retrospectively, phenotypical identification of all the strains was performed with Rapid ID32A; API Coryne and VITEK 2 (bioMérieux). The susceptibility testing was performed from a suspension of McFarland 6 with E-tests (bioMérieux) or with disc diffusion on ABA, 48 h.

Before MALDI-TOF analysis the isolates were sub-cultured on fastidious anaerobe agar (FAA) with 5% sheep blood and incubated for 48 h. Each isolate was initially inoculated directly from a colony onto a steel target plate on two consecutive spots. One microlitre of matrix solution (α -cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid; Bruker Daltonics, Bremen, Germany) was added. The identification was performed by MALDI-TOF MS using Microflex LT

instrument with Flex Control 3.0 software and Maldi Biotyper DB Update_V3.2.1.1 (Bruker Daltonics) at the Laboratory of Clinical Microbiology, Turku University Hospital. If both scores from the initial run gave a value of <2.0, the bacterial material was extracted as specified by the manufacturer and re-analysed.

To test the sensitized culture midstream urine from a young healthy male was collected and artificial urine samples were prepared from three isolates with serial dilutions of 10^3 – 10^6 CFU/mL. These samples and unspiked urine were cultured on ABA, chocolate agar and ChromID™CPS3 (bioMerieux) with a 10 µL loop. The results were read 24 and 48 h later.

Results and Discussion

The patient-related data and laboratory identification of recovered isolates are presented in Table 1. All 11 strains were successfully identified to the species level (correct identification with a score value ≥ 2.0) but 10/11 strains required an extraction step with 70% formic acid for reliable identification. Only one strain (S-21528) was reliably identified to species level directly from the colony.

The simulated urine samples produced visible growth on ABA already after 24 h but on the sheep blood and chocolate agars the cultivation required 48 h. No growth was detectable on the CPS3. The number of colonies was proportional to the strength of the spiked urine. The algorithm for the identification of *A. schaalii* is presented in Fig. 1.

The retrospective analysis of the patient data indicated that urinalysis was requested from all the patients in whom *A. schaalii* was isolated from blood; however, aerobic mixed culture and non-significant growth of *Corynebacterium* species or *Lactobacillus* was reported in 6/11 patients. In the remainder (5/11) the urinalysis resulted in identification of bacteria associated with UTIs but as expected, *A. schaalii* was not recovered. In 4/11 patients co-infection with other pathogens was detected in the bloodstream (Table 1). RAPID ID 32A provided the most sustained identification profile of 04XXX77717, where the X stands for any reaction (Table 1). This profile suggests *Capnocytophaga* spp. or *Micromonas micros*, but these species can easily be ruled out. This commercial test strip can also misinterpret *A. schaalii* for *Actinomyces meyeri*; however, the latter can be ruled out on the basis that (1) this pathogen is seldom recovered from blood culture, (2) the origin of infection is often related to oral cavity,

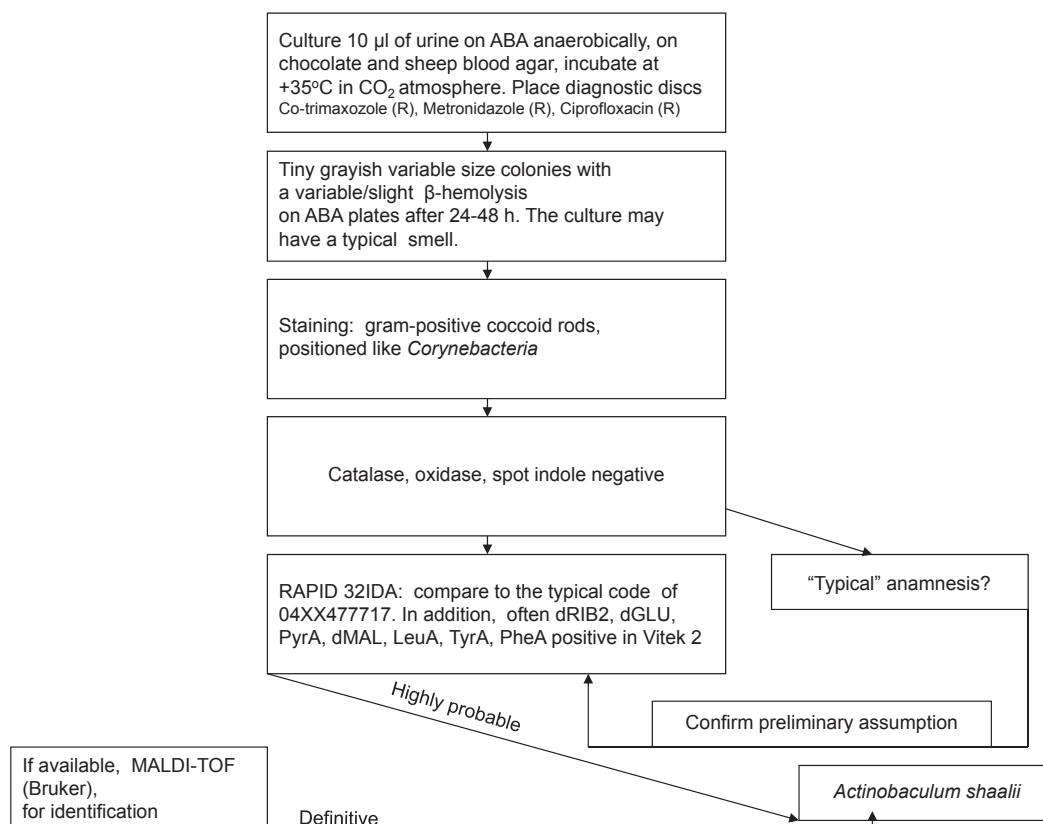


FIG. 1. A proposed algorithm for identification of *Actinobaculum schaalii* from clinical specimens. ABA, anaerobe basal agar.

TABLE 1. Patient-related data, biochemical and MALDI-TOF identification of 11 *Actinobaculum schaalii* isolates

Isolate	Reported results from urine within 2 week proximity	Growth from aerobic or anaerobic bottle	Concomitant isolates in blood	Maldi Biotyper <i>A. schaalii</i> confirmed, score	Vitek	APICoryne	Rapid ID 32A
S-21495	<i>Corynebacterium</i> spp.	1 anaerobic bottle	<i>Peptoniphilus asacchari</i> in 1 anaerobic bottle	2.219	6764100010641	2050721	0410477717
S-21528	<i>Oligella urethralis</i> and <i>Aerococcus</i> spp.	1 anaerobic bottle	<i>Aerococcus</i> species in 1 aerobic bottle	2.263	2601000000401	0010721	0426477717
M-21846	1. No growth 2. <i>Lactobacillus</i> spp.	1 aerobic and 1 anaerobic bottle each	No	2.124	6727102010661 6764102010621	2050721	0436477717
J-20772	Aerobic mixed culture	1 anaerobic bottle	No	2.070	6765100010441	2050721	0416477717
P-132	<i>Klebsiella pneumoniae</i>	2 aerobic bottles	No	2.117	6625100010401	2050721	0436077717
P-77	<i>Klebsiella oxytoca</i>	1 aerobic bottle	No	2.115	6625000010401	0050220	0436477717
K-26636	Aerobic mixed culture	2 anaerobic bottles	No	2.202	6724100010641	0050721	0414477717
K-27888	<i>Klebsiella pneumoniae</i>	2 aerobic and 1 anaerobic bottles	<i>Enterococcus faecalis</i> in 1 anaerobic bottle <i>Atopodium parvulum</i> in the same anaerobic bottle as <i>A. schaalii</i>	2.004	2721000000401	4050320	0400271717
K-27197	1. No growth 2. Mixed aerobic culture	1 aerobic and 1 anaerobic bottle	<i>Peptostreptococcus tetradicus</i> in 1 anaerobic bottle	2.151	6725102010661	4010621	0434477717
T-41163	1. Mixed aerobic culture 2. <i>Lactobacillus</i> spp.	1 anaerobic bottle	No	2.056	6764100010401 6764100010421	4150621	0434477717
T-42442	<i>Enterococcus faecalis</i>	1 anaerobic bottle	No	2.183	2721000000001	4010000	0416073714 variability in reactions

and (3) the bacteria are not aero-tolerant in comparison with *A. schaalii*.

All tested isolates were susceptible to penicillin (MIC below 0.008 g/L), cephalexin (disc diffusion, mean 32, range 26–38 mm) and cefuroxime (MIC below 0.016 µ/L) being in agreement with Cattoir *et al.* [15]. In one study this pathogen was reported susceptible [16] but in another study [5] 28% of strains were resistant or intermediately susceptible to pivmecillinam. Our strains had an inhibition zone to pivmecillinam in the range 28–38 mm but were unambiguously resistant to co-trimoxazole with no zone of growth inhibition whereas for ciprofloxacin a slight inhibition zone was detectable for some strains (data not shown).

In our opinion culture, phenotypical examination and MALDI-TOF identification are superior to PCR because the genomic material of *A. schaalii* can be detected with PCR in up to 13% of asymptomatic patients [17]. We do not perform microscopy of urine samples of all patients aged over 60 years, as recommended by Nielsen *et al.* [5]. We believe that screening the urine using flow cytometry sorting (FACS) [18] will help identify those samples that require special culture.

In conclusion, we advocate the sensitized culture to increase the yield of *A. schaalii* recovery from urine in risk-group patients and MALDI-TOF for fast identification.

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Conflict of Interest

None declared.

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