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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 (bioMerieux, Marcy L’Etoile, 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 (bioMerieux). The susceptibility testing was performed from a suspension of McFarland 6 with E-tests (bioMerieux) 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 $\geq 2.0$) 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 04XXXX77717, 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,

Culture 10 μl of urine on ABA anaerobically, on chocolate and sheep blood agar, incubate at +35°C in CO₂ atmosphere. Place diagnostic discs Co-trimoxazole (R), Metronidazole (R), Ciprofloxacin (R)

Tiny grayish variable size colonies with a variable/slight β-hemolysis on ABA plates after 24-48 h. The culture may have a typical smell.

Staining: gram-positive coccoid rods, positioned like Corynebacteria

Catalase, oxidase, spot indole negative

RAPID 32IDA: compare to the typical code of 04XX477717. In addition, often dRIB2, dGLU, PyrA, dMAL, LeuA, TyrA, PheA positive in Vitek 2

If available, MALDI-TOF (Bruker), for identification

Highly probable

Actinobaculum shaalii

Confirm preliminary assumption

Typical anamnesis?

“Typical” anamnesis?

Definitive

FIG. 1. A proposed algorithm for identification of Actinobaculum shaalii from clinical specimens. ABA, anaerobe basal agar.
TABLE 1. Patient-related data, biochemical and MALDI-TOF identification of 11 Actinobaculum schaalii isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Reported results from urine within 2 week proximity</th>
<th>Growth from aerobic or anaerobic bottle</th>
<th>Concomitant isolates in blood</th>
<th>Maldi Biotyper A. schaalii confirmed, score</th>
<th>Vitex</th>
<th>API Coryne</th>
<th>Rapid ID 32A</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-21495</td>
<td>Corynebacterium spp.</td>
<td>1 aerobic bottle</td>
<td>Peptostreptococcus tetradius</td>
<td>2.115</td>
<td>6761600010401</td>
<td>0410477717</td>
<td></td>
</tr>
<tr>
<td>S-21528</td>
<td>Oligella urethralis and Aerococcus spp.</td>
<td>1 aerobic bottle</td>
<td>Agenes strains</td>
<td>2.100</td>
<td>6761600010401</td>
<td>0410477717</td>
<td></td>
</tr>
<tr>
<td>M-21846</td>
<td>1. No growth</td>
<td>1 anaerobic bottle</td>
<td>No</td>
<td>2.200</td>
<td>260100000000401</td>
<td>0010721</td>
<td></td>
</tr>
<tr>
<td>j-20772</td>
<td>Aerobic mixed culture</td>
<td>1 aerobic bottle</td>
<td>No</td>
<td>2.070</td>
<td>6761600010401</td>
<td>2050721</td>
<td></td>
</tr>
<tr>
<td>P-77</td>
<td>Klebsiella pneumonia</td>
<td>2 aerobic bottles</td>
<td>No</td>
<td>2.115</td>
<td>6625000010401</td>
<td>0050200</td>
<td></td>
</tr>
<tr>
<td>K-26636</td>
<td>Aerobic mixed culture</td>
<td>2 anaerobic bottles</td>
<td>No</td>
<td>2.202</td>
<td>6761600010401</td>
<td>2050721</td>
<td></td>
</tr>
<tr>
<td>K-27888</td>
<td>Klebsiella pneumonia</td>
<td>2 aerobic and 1 anaerobic bottles</td>
<td>Enterococcus faecalis</td>
<td>2.004</td>
<td>272100000000401</td>
<td>4050320</td>
<td></td>
</tr>
<tr>
<td>K-27197</td>
<td>1. No growth</td>
<td>1 aerobic and 1 anaerobic bottle</td>
<td>Agenes strains</td>
<td>2.151</td>
<td>6761600010401</td>
<td>0410477717</td>
<td></td>
</tr>
<tr>
<td>T-41163</td>
<td>Mixed aerobic culture</td>
<td>1 anaerobic bottle</td>
<td>No</td>
<td>2.056</td>
<td>6761600010401</td>
<td>4150621</td>
<td></td>
</tr>
<tr>
<td>T-42442</td>
<td>Enterooccus faecalis</td>
<td>1 anaerobic bottle</td>
<td>Peptostreptococcus tetradius</td>
<td>2.183</td>
<td>272100000000401</td>
<td>4010000</td>
<td></td>
</tr>
</tbody>
</table>

Conflict of Interest

None declared.

References


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