Author's response to reviews

Title: Actinobaculum schaalii an emerging pediatric pathogen?

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Response to Reviewers’ Comments

MS: 1673470924697166 - *Actinobaculum schaalii* an emerging pediatric pathogen?

Reviewer 1:
Major comments:

C1) It is hard to differentiate cystitis from asymptomatic bacteruria in this case. The infant was afebrile and otherwise asymptomatic. In addition, the number of WBC in the urinalysis was not very impressive. Why did the author think the patient had cystitis and needed treatment?

R1) Reviewer 1 is correct that differentiation of cystitis from asymptomatic bacteriuria is challenging in paediatric patients. However, in the reported case, elevation of the leukocyte esterase activity in a dipstick test and leukocyturia of >20 leukocytes per high power field (hpf) from a catheter specimen were suggestive for a UTI. In addition the diagnosis of cystitis was subsequently supported by the growth of $10^4$ CFU/ml *A. schaalii* in monoculture. Finally the urinalysis normalized and urine culture was sterile at the end of antibiotic treatment. Previous seminal studies investigating the diagnosis of UTI in children including those by Hoberman et al (Pediatr Infect Dis, 1996) and by Shaw et al (Pediatrics, 1998) suggest that the combination of > 10 leukocytes/hpf and bacteriuria had a specificity for UTI of 84-93%. These findings were confirmed in two reviews suggesting each test individually (ie either leukocyte esterase activity or leukocyte count in microscopy) had a specificity for UTI of approximately 80% (Downs ME, Pediatrics 1999 and Finnell et al, Pediatrics 2011).

Therefore the following sentence has been added to the manuscript in the last paragraph of the case presentation: “The combination of a repeatedly abnormal urinalysis and growth of *A. schaalii* in monoculture was considered diagnostic for a UTI.”

C2) Were previously reported pediatric cases also asymptomatic? Please comment on that in the Discussion section.

R2) All previously reported pediatric cases were symptomatic. Our case is the first reported case in which *A. schaalii* UTI has been treated in an asymptomatic child. The following sentences have been completed/added to the discussion section: "Of these, 41 cases (87%) were reported in adults and only six cases in children under the age of 15 years. All of those six children were symptomatic and treated with antibiotics. Two of the three children with cystitis and the child with pyelonephritis were febrile. In the case of the 15-year old boy who presented with cystitis and the girl with the intradural abscess the temperature was not specified in the case report. Our case is to our knowledge the youngest child currently described with an *A. schaalii* UTI. It is also the first case where *A. schaalii* was treated in an asymptomatic child."

C3) Is urine Gram-stain useful to screen patients who are at risk of *A. schaalii* UTI?

R3) Gram-stain can indeed be useful in the detection of *A. schaalii* in particular when culture remains negative. The following sentence has been added to the manuscript: “The presence of Gram-positive rods in microscopy with a negative culture result should prompt the search for unusual pathogens including *A. schaalii*.”

C4) For the most part, microorganisms with > 100,000 CFU in urine culture are considered significant. Less colony counts may be considered contaminants. Since *A. schaalii* is hard to culture, how can physicians differentiate a significant colony count from a non-significant one?
The concept that > 100 000 CFU/ml indicates a UTI is based on morning collections of urine from adult women considered clinically to have pyelonephritis with specimens from women without symptoms; the transition range, in which the proportion of women with pyelonephritis exceeded the proportion of women without symptoms, was 10 000 to 100 000 CFU/ml (Kass E. Trans Assoc Am Phys. 1956). To establish the diagnosis of UTI the American Academy of Pediatrics suggests in their recently published guideline the growth of > 50 000 CFU/ml in combination with pyuria (defined as more than 5 leukocytes/hpf) (AAP Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management, Clinical practice guideline for the Diagnosis and Management of the Initial UTI in Febrile Infants and Children 2 to 24 Months. Pediatrics. 2011). In the case of results in the 10 000 to 100 000 CFU/ml these “need to be evaluated in context, such as whether the urinalysis findings support the diagnosis of UTI and whether the organism is a recognized uropathogen.”

We have therefore added a small paragraph in the discussion section to clarify how to differentiate a significant colony count from a non-significant one: “In our patient urine culture grew 10^4 CFU/ml A. schaalii and in contrast to previous reports in children with A. schaalii UTI there were no clinical symptoms [4,10,21]. Similar colony counts have been found in children considered to be colonized with A. schaalii, however none of these children had concomitant positive leukocyte esterase test or pyuria [21]. In our patient the combination of a repeatedly pathologic urinalysis together the with growth of 10^4 CFU/ml of a recognized uropathogen in monoculture was considered sufficient evidence for the diagnosis of a cystitis.”

Please comments on how in vitro antimicrobial susceptibility testing can be done in the lab.

In the publication by Cattoir et al. (Reference 5) the Etest method was applied to 48 clinical isolates of A. schaalii. In brief, a strip impregnated with an antimicrobial is set on top of a culture with known quantities of the bacterial isolate. The resulting zone of inhibition decreases along the gradient of antimicrobial concentration and allows the determination of the minimal inhibitory concentration (MIC). In the above mentioned publication the interpretation of the MIC was done according to EUCAST clinical breakpoints. A more in depth explanation of this method can be found in the publication of Cattoire et al. (Reference 5).

Minor comments:

First sentence of the last paragraph in the Discussion section: please add “respectively” at the end of the sentence. This is important since many pediatricians do not use ciprofloxacin in children.

Changed as suggested.
Reviewer 2:
Major corrections:

C1) In discussion you write: "There is limited data from in vitro susceptibility testing suggesting that gentamicin, vancomycin, linezolid, and nitrofurantoin are potential alternative treatment options [5]."
You should complete the list by adding mecillinam which has been examined in a supplementary study to reference 5 [22]. Pivampicillin is widely used in the scandinavian countries.

R1) Changed according to suggestion.

Minor corrections and comments:

C1) Abstract/Background: 16S rRNA sequencing is usually slow. Since new techniques like real-time PCR and Maldi Toft allow fast identification it could be interesting to know how many days went before you got the 16S rRNA result (if you have the data)?
"Sequencing of the 16S rRNA gene confirmed an A. schaalii infection XX day later."
R1) Sequencing took 9 days. This information has been added to the corresponding part in the abstract and the case presentation section.

C2) Key words: I just wonder why you use "Urinary Tract" instead of "Urinary Tract Infections".
R2) Changed according to suggestion.

C3) In discussion you write: "The optimal duration of antibiotic treatment for A. schaalii infection is currently unknown. Most case reports suggest a treatment duration of seven to 14 days."
Either write seven to fourteen days or 7 to 14 days.
R3) Changed according to suggestion.

C4) In discussion you write: "Implementation of the newer technology MALDI-TOF in routine diagnosticprocedures will allow a fast and easy identification of A. schaalii and might change its the frequency of detection."
This sentence has to be corrected.
R4) The sentence has been corrected.

C5) In discussion you write: "Incubation may need 48 to 72 hours as a result of its slow growth and 16S rRNA sequencing is required for identification."
I think you should emphasize that it requires incubation in CO2.
R5) The corresponding paragraph has been changed and we included the information that incubation with CO2 is needed.
C6) In discussion you write: "PCR for A. schaalii from urine was positive in 36% (5 of 14) of children below three years of age but negative in all 15 children tested between three and 15 years of age." Either write all numbers with arabic numerals or all with letters (do not change "36% (5 of 14)").

R6) Changed according to suggestion.

C7) In the abstract, Case presentation, you write: "Urinalysis showed leukocytosis....." I might be wrong, but how does Urinalysis relate to leukocytosis? Can you clarify the sentence?

Definition: Leukocytosis is a raised white blood cell count (the leukocyte count) above the normal range in the blood.

R7) The sentence in the abstract has been modified including the corrected term “pyuria”.

C8) Background, you write: "Like other Actinomyces-like organisms it is suspected to be part of the commensal flora of the human urogenital tract." You should add a reference to this sentence. You might have taken that information from your reference 1!!

R8) The correct reference has been added.

C9) In case representation you write: "(Becton Dickinson AG, Basel, Switzerland and a Columbia-Colistin-Nalidixic acid agar (CNA)."

I believe it should be (CNA) not (CAN). You also use CNA (correct) two lines later.

R9) The typing error has been corrected.