Author's response to reviews

Title: Actinobaculum schaalii - invasive pathogen or innocent bystander?: a retrospective observational study

Authors:

Sarah Tschudin-Sutter (TschudinSar@uhbs.ch)
Reno Frei (FreiR@uhbs.ch)
Maja Weisser (WeisserM@uhbs.ch)
Daniel Goldenberger (GoldenbergerD@uhbs.ch)
Andreas F Widmer (WidmerA@uhbs.ch)

Version: 2 Date: 30 June 2011

Author's response to reviews: see over
Dear Editor,

Please find enclosed our revised manuscript entitled “Actinobaculum schaalii – invasive pathogen or innocent bystander?”

We would like to reply to the reviewers’ comments as listed below:

**Reviewer 1 (Dasja Pajkrt)**

**Major compulsory revisions**

1. I believe table 4 provides no added value. I would suggest to either simplify the table (categorizing only the antibiotic treatments into main groups, with duration of treatment depicted as: for example duration with amoxicillin/clavulanic acid ranged from 5-61) or simply described the data in the text.

**Response:** We agree that it is sufficient to simply describe the most important data of table 4 in the text and have omitted table 4 accordingly.

**Minor essential revisions:**

2. The most striking issue I found is the low number of isolates with detection of A. schaalii in such as big tertiary center in Switzerland; only 40 isolates were detected. Could the authors indicate what percentage of positive isolates from patients whose cultures were detected from any sterile body site? In the event the authors cannot reproduce these numbers from all positive cultures from sterile body site, please elaborate in the discussion section more on the low numbers of isolates of A. schaalii.

**Response:** We agree that 40 isolates in 11 years is a very low number. We cannot reproduce the percentage of positive isolates from patients whose cultures were detected from any sterile body site. However, our laboratory processes around 60’000 isolates per year enhancing the fact the percentage is indeed low. In order to assess the incidence of *Actinobaculum schaalii* prospective studies with a longer cultivation period, as stated under point 3 by the reviewer would be needed.
Bank et al used PCR to detect *A. schaalii* and found in 22% of routine urine samples of patients aged >60 years in Denmark to be positive [1]. This finding supports the statement of the reviewer made in point 3 that the low incidence in our study is mainly due to the difficulties to detect this pathogen by culture rather than it really being rare. This finding has been highlighted in the “discussion” section: “As this pathogen is difficult to detect in the laboratory and is easily overgrown by other bacteria, awareness of its existence is crucial. Bank et al. analyzed 252 urine samples for presence of *A. schaalii* by a real-time quantitative PCR they developed and found 41 of 252 samples (16%) to be positive with bacterial concentrations >10^4 CFU/mL”.

3. Another reason for the low numbers of *A. schaalii* isolates could be that the urine were only cultures for 2 days (as is routine practice), but it is know that it may take more than 2 days for *A. schaalii* to grow even in 5% CO2. Why was the cultivation not prolonged to at least 3 days?

**Response:** We agree with the reviewer that in order to rule out the presence of *Actinobaculum schaalii* in any specimen, it would make sense to prolong cultivation time. Additionally, if the main aim of the study would be to determine the true incidence, PCR, as described by Bank et al. would be a valuable alternative [1]. However the aim of our study was not to determine incidence but clinical significance of *A. schaalii*. In addition, space and human resources restriction did not allow to routinely extend the time of culturing urine samples. Approximately 10’000 urines are sent every year to the laboratory, and this would add at least 10’000 incubation days, requiring additional space and equipment, currently not available in the microbiology laboratory.

**Minor consideration:**
1. I would suggest another English language check.
2. Textual revisions:
   a. *urin* should be *urine* in the tables.
   b. **Discussion:** “Cattoir et al described similar results when testing 48 clinical isolates” please change this sentence, this is not proper English

**Response:** We have corrected “urine” in the table accordingly and have rephrased the sentence to “Similar results were described by Cattoir et al. after testing 48 clinical isolates [Error! Reference source not found.].”

Reviewer 2 (Vincent Cattoir)

**Specific comments**
- What was the bacterial concentration of *A. schaalii* recovered from urinary samples? What was the cut-off value the authors used?

**Response:** *A. schaalii* was recovered in a total of 11 urinary samples at concentrations ranging from 10^3/CFU/ml to 10^6/CFU/ml. Colonization was differentiated from urinary tract infections using the CDC criteria as stated in the methods section [2]. In patients classified as having an urinary tract infection *A. schaalii* was recovered at a concentration of 10^5/CFU/ml to 10^6/CFU/ml.
All deep tissue samples were polymicrobial, but what was the relative proportion of *A. schaalii* in these specimens? Was *A. schaalii* predominant?

**Response:** Semi quantitative cultures (categorized as light, moderate or heavy) from deep tissue samples were performed, the relative proportion of *A. schaalii* varied from light to heavy growth. *A. schaalii* was recovered from deep tissue samples from 7 patients. Semi quantitative cultures revealed light growth and moderate growth in one patient, respectively (*A. schaalii* was not predominant in the former and found in the same quantities as the other pathogen — *Corynebacterium* spp. — in the latter). In the remaining 5 patients, growth of *A. schaalii* was quantified as heavy and the proportion of the other pathogens was equal (other pathogens detected were *Proteus mirabilis*, *Citrobacter kooseri* and Enterococci in one patient, anaerobes and *Actinomyces* spp. in two others, and only anaerobes or anaerobes and *Staphylococcus lugdunensis*).

Did any patients suffer from endocarditis?

**Response:** Endocarditis was not diagnosed in any of our patients. In the patient reported with spondylodiscitis, one blood culture in addition to the biopsy of the vertebra was positive for *A. schaalii* and spondylodiscitis was considered a complication of urinary tract infection as he suffered from dysuria some weeks before.

Tables 3 and 4 could be omitted.

**Response:** We agree that table 4 can be omitted as also stated in response to the first reviewer’s comments. We however believe, that table 3 helps illustrate the principal findings of our study.

Reviewer 3 (Val Hall)

1) In ‘Background’ (main text and Abstract) the initial sentence is erroneous. *A. schaalii* did not ‘formerly belong to the species Actinomyces suis’. The organism was newly described in the referenced manuscript. Also, the derivation of its etymology is irrelevant in this MS.

**Answer:** We agree that the initial sentence is wrong and have corrected it accordingly. We have rephrased the introductory sentence to “*Actinobaculum schaalii* is a Gram-positive, facultatively anaerobic, non motile coccoid rod, classified as a new genus in 1997” in the “Background” section and in the abstract of the manuscript. We agree with the reviewer that the description of its etymology is not relevant to the manuscript and have therefore omitted it accordingly. We thank the reviewer for this valuable correction.

2) Throughout the MS, the authors appear to confuse the term ‘isolate’ with ‘specimen or sample’. E.g. ‘40 isolates with detection of *A. schaalii*’ and ‘Table 3: Isolates with detection of …’ this table is probably superfluous anyway.
Answer: We agree that the use of the terms “isolate”, “specimen” and “samples” seems confusing in our manuscript. We have corrected this in our manuscript accordingly. We have also corrected the title of table 3 to “specimens with detection of…” accordingly.

3) In ‘Results’ the numbers of blood cultures sampled are confusing. For one patient results are listed as ‘F. magna from 2 of 2 and A.schaalii from 4 of 4 blood cultures’. Were 2 or 4 blood cultures drawn from this patient? The authors have not specified whether blood cultures were anaerobic or aerobic bottles and this may be highly relevant to the isolation rates (and may clarify the stated numbers).

Answer: Four blood cultures were drawn from this patient in total (2 pairs consisting of one anaerobic and one aerobic bottle each). Two of the four blood cultures drawn grew F. magna (both anaerobic cultures) and four of the four blood cultures grew A. schaalii, i.e. both pathogens grew simultaneously in two blood cultures. At our institution blood cultures always include one aerobic and one anaerobic culture. We have included this statement in the “Methods” section of our manuscript for clarification.

4) In the same paragraph (and the Abstract) ‘… were polybacterial (all deep tissue samples, …’ ‘All’ should be replaced with numbers e.g. ‘7 of 7 deep tissue samples’.

Answer: We have replaced “all” by numbers in the “Results” section and in the abstract for clarification.

Overall, the MS is too long and somewhat repetitive. I recommend that the results, discussion and conclusions are combined and that results are detailed either in tables or text but not in both.

Answer: We agree that the conclusions were somewhat repetitive in summarizing findings already highlighted in the “discussion” section. We have therefore omitted the conclusions at the end of the “discussion” section. We would however rather not combine the “results” and “discussion” sections to clearly distinguish our results from consecutive comparison and discussion of results obtained by others. We have modified the “discussion” section by not repeating findings from the “results” section.

We thank all the reviewers for their valuable contribution to our manuscript. Thank you for considering our manuscript for publication.

Sincerely

Prof. Dr. med. Andreas F. Widmer  
Head Hospital Epidemiology

Dr. med. Sarah Tschudin-Sutter  
Staff Physician Hospital Epidemiology
