Author's response to reviews

Title: A retrospective study of the incidence, clinical characteristics, phenotypic identification, and antimicrobial susceptibility of bacteremic isolates of Acinetobacter ursingii

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Author's response to reviews: see over
To the Editor,

We wish to re-submit the revised manuscript (A retrospective study of the incidence, clinical characteristics, identification, and antimicrobial susceptibility of bacteremic isolates of Acinetobacter ursingii). The invaluable comments and suggestions are highly appreciated. We have addressed the reviewers’ suggestions as shown below. The changes we made in the manuscript are highlighted. All the authors have read and agreed to the re-submitted version of the manuscript.

Response to Referee 1:

Major compulsory revisions:
1. Line 62: please properly cite website in references
   # Response: Revised as suggested (line 68 and line 274-275).

2. Line 142: please describe the hospital's population. Veterans hospitals often have predominantly male patient populations. Could this have impacted the demographics of the study?
   # Response: Taipei Veterans General Hospital is a 2980-bed medical center which serves about 120 thousand person-times per year. It is one of the most well established hospitals in Taipei which serves not only veterans but also their families and all other people. We think that the demographics of patients was not affected by the patients population in our study (line 83-85), as the gender of the patients was similar in A. ursingii bacteremia, while most of the patients with A. baumannii bacteremia were male.

Minor essential revision:
1. Line 55: please clarify hospital only or all health care associated personnel from reference 3.
   # Response: The authors investigated the colonization with Acinetobacter spp. of the skin and mucous membranes of 40 patients hospitalized in a cardiology ward and 40 healthy controls (9 of whom were laboratory workers and others are not mentioned clearly). Thirty patients (75%) and 17 controls (42.5%) were found to be colonized with Acinetobacter spp. We do not know whether the 40 healthy controls were all hospital personnel. Thus, I’ve deleted the term “personnel “ at line 62.

2. Line 65: Causative of what? Bacteremia, or other disease? Please clarify, though may be easier to just state that A. ursingii is rarely reported as a pathogen.
3. Line 154: please add p-value to mortality  
   # Response: Revised as suggested (line 170).

4. Line 170: perhaps resistant or intermediate susceptibility could replace "unsusceptible"  
   # Response: the term is changed to "intermediate susceptible" according to second reviewer (line 50, 187,189,190)

5. Line 216: isolates were, not was  
   # Response: Revised as suggested (line 234).

Discretionary revisions:
1. Line 67: describe may be a better verb than delineate  
   # Response: Revised as suggested (line 74).

2. Figure 1 does not add much to the article. It is evident that the A. ursingii incidence is low. Especially given the low percentage, the figure is difficult to read with the current y-axis  
   # Response: The figure was deleted as suggested
Response to Referee 2:

Major comments/revisions:

1. Is 16S rRNA gene sequencing sufficient to discriminate *A. ursingii* from other *Acinetobacter species*? Do additional loci need to be sequenced. Please comment.

# Response: Although it has been demonstrated that 16S rRNA gene sequence data on an individual strain with a nearest neighbor exhibiting a similarity score of <97% represents a new species, the meaning of similarity scores of >97% is not as clear (Petti, C. A. 2007. Detection and identification of microorganisms by gene amplification and sequencing. Clin. Infect. Dis. 44:1108–1114.). Thus, we identified these 19 isolates again by using 16S-23S rRNA internal transcribed spacer sequence analysis. All these 19 isolates were identified as *A. ursingii* with a similarity scores of >98% compared to the ITS sequence of reference strains reported on NCBI website (line 124,148 ).

2. Where the isolates from a single medical institution? If yes, the authors must assure that the isolates are not clonal in nature; i.e., through the use of a molecular epidemiological technique: PFGE. If they are, it is strongly recommended that the study be widened to include additional medical centers and to attempt to obtain non-clonal isolates.

# Response: The isolates were all isolated from a single medical institution. We performed PFGE and found that except 2 out of 19 strains exhibiting smeared DNA, two of 19 strains exhibiting 93% similarity of PFGE patterns, all the PFGE patterns of remaining strains were smaller than 80%. Except the 2 strains with high PFGE pattern similarity(93%), all the remaining isolates should not be clonal in nature. (line 33-35, 125-126, 149-151 and Fig.1 ).
3. Line 43 and elsewhere: the authors repeatedly use unsusceptible. If there are INT and RES categorical interpretations for a given drug/microorganism combination and the isolate(s) tests as such, intermediate or resistant can be used. If only SUS categorical interpretations are available non-susceptible should be used for those isolates that do not test susceptible.

# Response: Revised as suggested (line 50, 187,189,190)

4. Line 55: do the authors mean hospital personnel? Please indicate.

# Response: The authors investigated the colonization with Acinetobacter spp. of the skin and mucous membranes of 40 patients hospitalized in a cardiology ward and 40 healthy controls (9 of whom were laboratory workers and others are not mentioned clearly). Thirty patients (75%) and 17 controls (42.5%) were found to be colonized with Acinetobacter spp. We do not know whether the 40 healthy controls were all hospital personnel. Thus, I’ve deleted the term “personnel “ at line 62(line 62).

5. Line 68: did the authors have access to mass spectrometric platforms? It is highly recommended that the isolates are assay on both the Bruker Biotyper and VITEK MS platforms to allow readers an understanding of the performance of the various mass spectrometry platforms for A. ursingii identification. This is important as mass spectrometry is becoming increasingly utilized globally and is much more accurate than biochemical-based methodologies.

# Response: Matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) had been performed for these 19 isolates. All
the isolates were identified as *Acinetobacter ursingii* by MALDI-TOF-MS (line 128, 177,182-183, 229-231, table 2 ). Belows are examples of the identification results.

### Table 2: Identification Results

<table>
<thead>
<tr>
<th>Number of identifications:</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>✔</td>
</tr>
<tr>
<td>Analysis Data</td>
<td>4/10/13 5:08 PM</td>
</tr>
<tr>
<td>Organism Name</td>
<td><em>Acinetobacter ursingii</em></td>
</tr>
<tr>
<td>Pathogenicity</td>
<td>N/A</td>
</tr>
<tr>
<td>Confidence Value</td>
<td>99.9</td>
</tr>
<tr>
<td>Confidence Level</td>
<td>✔</td>
</tr>
<tr>
<td>Acquisition/Computation message(s)</td>
<td>7</td>
</tr>
</tbody>
</table>

6. Throughout the text the authors indicate that certain populations had an association or not, but no statistical calculation (e.g., P value) is provided. Please include these data. For example, "Patients with A. ursingii bacteremia were also less likely to be admitted to the intensive care unit (18.8%) than patients with A. baumannii bacteremia (63.5%)."

# Response: Revised as suggested (line 42,44,45, 161,162,163,165, 166,167, 170).
7. Table 1: indicate where appropriate, if the mean or median value is tabulated.
   #   Response: Revised as suggested (table 1).
8. indicate the resultant identification biotype for all identifications on the Phoenix and VITEK platforms. Were there biochemicals that were highly discriminatory (sensitive and specific) for A. ursingii compared to other Acinetobacter species; especially A. baumannii.
   #   Response: Phoenix platform rarely correctly identify A. ursingi to the genus level. Although VITEK II can identify A. ursingi to the genus level, about 50% of the isolates were identified as A. lwoffii. According to the previous study of Laurent Doretet in 2006 (cited ref), A. baumannii has biochemical features with assimilation of glucose, arabinose, gluconate while A. ursingii has not. In our study, all these 19 isolates revealed not only the ability of enzymatic hydrolysis of L-leucine 7-amino-4-methylcoumarin, but also using acetate, malonate, adonitol, D-mannitol, citrate, and α-ketoglutaric acid as a carbon source. Fourteen isolates had the ability of using tiglic acid as a carbon source. Fourteen isolates hydrolyzed L-proline-Na. However, it is difficult to figure out which biochemicals can precisely identified A. ursingii from other Acinetobacter species due to limited data. Thus, protein fingerprinting using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) represents a promising molecular method for rapid identification of Acinetobacter ursingi.

Best regards
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