Author's response to reviews

Title: Enterobacter nimipressuralis as a cause of Pseudobacteremia

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

**Re: MS: 1786881123546087**

“Enterobacter nimipressuralis as a cause of Pseudobacteremia”

Dear Editor

We have revised our paper. Please see attached one main text file.

We believe that we have revised our manuscript as your suggestions, but we would be happy to provide further information or revision if necessary. Thank you for your consideration and please feel free to contact us if we can help you in any way.

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Reviewer: Armand Paauw

Reviewer's report:

Dear author,

The manuscript is very informative and shows that reliable identification of Enterobacter species is important. Also, it is clearly shown that phenotypic identification of Enterobacter species is unreliable, as well. As has been shown recently, a better identification could potentially lead to a better understanding of which Enterobacter species are more infectious, or pathogenic than other Enterobacter species (1,2,3)

1. I’m convinced that the isolated Enterobacter species are all identical and most likely they are also E. nimipressuralis. However, recent papers showed that identification with 16S partial sequencing is not sufficient to differentiate between all Enterobacter cloacae complex species. Therefore, I think it will improve the manuscript if you add sequencing a fragment of hsp60 or rpoB as recently be described (1,2,3).

Responds:

Dear reviewer, we would like to give our sincere thanks for your outstanding remarks in our manuscript. We tried our best to give answer of your each and every point more clearly in our reviewed manuscript. We hope, our new manuscript is better and helpful for the readers of BMC infectious diseases.

From our experimental data, we also believe that 16S rRNA is not fully sufficient to differentiate the closely related members of the Enterobacter members and performed sequence analysis of the protein encoding gene hsp60 and draw phylogenetic tree as per your suggestion.

The gene sequencing and phylogenetic analysis of amplification products of 16S rRNA revealed that Enterobacter amnigenus (clinical isolate) shared maximum of 99.308%, 99.239 %, 99.166 % and 99.093% sequence similarities with Buttiauxella izardii, B. noackiae, Enterobacter nimipressuralis and E. amnigenus respectively. However, according to the Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline of CLSI (Formerly NCCLS), only 99.0 % identity can be used for species identification (with greater than 0.8% separation between different species based on 16S rRNA sequences. Hence, our 16S rRNA sequencing data is not reliable for the species identification. Additionally, no rpoB gene was amplified from the PCR products and could not be applied at least in our present case and no described in the manuscript.

Furthermore, when we performed hsp60 gene sequencing and phylogenetic analysis of amplification products, our clinical isolates shared maximum of 96.7%, 94.5 %, 93.7% and 92.8 % sequence similarities with Enterobacter nimipressuralis ATCC 9912T (GenBank accession number AJ567900), E. hormaechei sub sp. hormaechei EN- 449T (GenBank accession number AJ866491), E. cloacae ATCC 46162T (GenBank accession number AJ417108), E. nickellidurans LMG 23000T (GenBank accession number AMO76892) and E. dissolvens ATCC 23373T (GenBank accession number AJ417143) respectively. Hence, this hsp60 gene sequencing might be more authentic for species identification and draw phylogenetic tree.
1. Possibly the case descriptions can be shorted because these are of little relevance. It is enough to mention the reason of hospitalization and that there were no clinical signs of infection.

**Responds:** *It has been done as per the reviewer’s suggestion.*

**Furthermore some small issues**

Page 2 line 5 and is in italic

**Responds:** *It has been done in manuscript.*

Page 3 line 5: etc. complete the list that is monitored

**Responds:** *Now, it has been fully written as per the suggestion*

Page 5 line: 2, page 6 line 12; specie to species

**Responds:** *It has been corrected.*

Page 8 line 10 Enterobacter in italic

**Responds:** *It has been corrected.*
Responds to the reviewers comments:

Reviewer: Zhiyong Zong

Reviewer's report:

This study described two cases of *Enterobacter nimipressuralis* pseudobacteremia due to the contamination of saline cotton swabs. This paper is easy to understand but a native English speaker is required to correct the written English. As *E. nimipressuralis* is rarely associated with clinical samples and the origin of the contamination had been identified, this paper is of interest and may be suitable for publication. However, several questions need to be answered and some revisions are required before being considered for acceptance.

Major Compulsory Revisions

1. According to the authors, the two patients had normal temperatures on admission, though they complained “fever sensation”, and had no signs of bacteremia, it seems difficult to justify the necessity of the blood cultures at the very beginning. Did the patients and their doctors really need blood cultures for the diagnosis?

   Responds:
   
   Dear reviewer, we would like to give our sincere thanks for your valuable suggestion in our manuscript. We modify our manuscript as per your guidance and remarks. We hope now it is more attractive and helpful for the readers of BMC infectious diseases.

   We agree with your opinion that it seems difficult to justify the necessity of the blood cultures at the very beginning. The two patients with febrile sensation at the beginning visited our outpatient clinic such as Division of Gastroenterology and Hepatology, and OBGY department respectively. We have tendency of doing blood culture for patients who had complained of febrile sensation in our hospital especially in outpatient clinic since we have not enough time to assess the patients. (In Korea, we have a lot of patients to deal with within limited time. We used to see about one hundred patients a day).

   As you know, the best time to do blood culture is at very before when some one has fever. As for professor, we have educated that kind of things to medical students. When above two patients visited outpatient clinic, the doctors who were in charge just ordered blood culture as well at routine labs at the situation when they did not know the lab results such as CBC, U/A, etc.

2. Both patients received ciprofloxacin though they did not have any relevant manifestations. This suggests that pseudobacteremia was not recognized at the early stage though an infectious diseases physician was monitoring the data everyday. It seems in contradiction with the conclusion that “The microbiologic cultures monitoring system will probably help us detect early pseudobacteremia.” The authors should provide explanations or need to revise the conclusions.

   Responds:
   
   As for infectious disease clinician, I recommended the doctors who took care of the two
patients in ward to adminster ciprofloxacin. We thought that there was little chance that Enterobacter spp. were pathogens. However, which clinicians dare bravely just observe without administration of antibiotics when Enterobacter spp. were isolated from blood culture?

What we want to say is that this pseudobacteremia outbreak wouldn’t have finished with just two cases, if we did not have the microbiologic cultures monitoring system.

Minor Essential Revisions

3. The authors should provide more details about the 16s rRNA sequences including the identities to those of other E. nimipressuralis strains deposited in GenBank to prove the species identification.

Responds:

We incorporated more information about the 16S rRNA. Additionally we draw phylogenetic tree based on the protein encoding gene hsp60 that help to establish a well-accepted classification for the members of the family enterobacteriaceae. We hope, this new manuscript will be more interesting for the reader of BMC infectious diseases.

We compared the sequence to those available in the GenBank and EMBL databases by using the Clustal N program with the BLAST package (http://www.ncbi.nlm.nih.gov/BLAST/BLAST.cgi). Based on the 16S rRNA gene sequences, Enterobacter clinical isolates shared maximum of 99.308%, 99.239 %, 99.166 % and 99.093% sequence similarities with Buttiauxella izardii, B. noackiae, Enterobacter nimipressuralis, and E. amnigenus respectively. However, according to the Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline of CLSI (Formerly NCCLS), only 99.0 % identity can be used for species identification (with greater than 0.8% separation between different species based on 16S rRNA sequences; and becomes less authentic at least in species identification aspects. Additionally, no rpoB gene was amplified from the PCR products and could not be applied at least in our case.

Similarly, the hsp60 gene sequencing and phylogenetic analysis of amplification products revealed that clinical isolates shared maximum of 96.7%, 94.5 %, 93.7% and 92.8 % sequence similarities with Enterobacter nimipressuralis ATCC 9912T (GenBank accession number AJ567900), E. hormaechei sub sp. hormaechei EN- 449T (GenBank accession number AJ866491), E. cloaceae ATCC 46162T (GenBank accession number AJ417108), E. nickellidurans LMG 23000T (GenBank accession number AMO76892) and E. dissolvens ATCC 23373T (GenBank accession number AJ417143) respectively.

4. Page 6, line 7, a reference should be cited for the IRS-PCR.

Responds: The manuscript has been updated as per the suggestion made by the reviewer.

5. As E. nimipressuralis has not been associated with human infections, it may not be appropriate to call it as pathogen at all.

Responds: It has been incorporated in the manuscript.
6. Table 1, for combinations such as piperacillin, the MIC values are not in the right formats.

Responds: Correction has been done in the manuscript.