Author's response to reviews

Title: Risk factors for multi-drug resistant Acinetobacter baumannii bacteremia in patients with colonization in the intensive care unit

Authors:

Ji Ye Jung (stopyes@yuhs.ac)
Moo Suk Park (pms70@yuhs.ac)
Song Ee Kim (dobie@yuhs.ac)
Byung Hoon Park (serandoc@yuhs.ac)
Ji Young Son (sonjyoung@yuhs.ac)
Eun Young Kim (narae97@yuhs.ac)
Joo Eun Lim (withjueun@yuhs.ac)
Sang Kook Lee (lskhi@yuhs.ac)
Sang Hoon Lee (tearpoem@yuhs.ac)
Kyung Jong Lee (drkyung@yuhs.ac)
Young Ae Kang (mdkang@yuhs.ac)
Se Kyu Kim (sekyukim@yuhs.ac)
Joon Chang (chang@yuhs.ac)
Young Sam Kim (ysamkim@yuhs.ac)

Version: 2 Date: 7 June 2010

Author's response to reviews: see over
June 7th, 2010

(Reviewer 1)

Dear Saad Nseir

RE: MS. No. 1044398363730395

Title: Risk factors for multi-drug resistant *Acinetobacter baumannii* bacteremia in patients with colonization in the intensive care unit

Thank you very much for reviewing our manuscript. We appreciate your advice about the revision and are very pleased to inform you that we are ready to resubmit our revised manuscript taking into account your comments. All significant changes have been written in red colored text. Thank you again for your kind review and we await your feedback and acceptance.

Sincerely yours,

Young Sam Kim M.D.

Assistant Professor
Division of Pulmonology, Department of Internal Medicine
Yonsei University College of Medicine
250 Seongsanno, Seodaemun-gu
Seoul 120-752, Republic of Korea
Telephone: 82-2-2228-1971, Fax: 82-2-393-6884
E-mail: ysamkim@yuhs.ac
Comments to the authors -
The authors performed a retrospective study to determine risk factors for bacteremia related to A. baumannii in ICU patients with A. baumannii colonization. 200 patients were included during a 2-yr period. Independent risk factors for A. baumannii bacteremia included infection and respiratory failure at ICU admission, mechanical ventilation, central venous catheter, bacteremia related to other microorganisms and prior antibiotic treatment.

1. Unfortunately, no new information is provided by the authors. All the risk factors identified by this study are well-known risk factors for A. baumannii, and other MDR bacteria. The conclusion provided by the authors is not applicable in clinical practice. They stated that ICU patients with colonization related to A. baumannii and these risk factors should be considered for early appropriate antibiotic treatment. However, all of these risk factors are present in all severely ill patients. The authors should better outline the need to remove mechanical ventilation and CVC as soon as possible since they are the only modifiable risk factors.

Reply : We agree with the reviewer that our conclusion seems to be off the point of the results. We have revised the discussion and conclusion more focusing on the results of our study. As reviewer suggested, we concluded that minimizing the invasive procedures and early removal of invasive devices are important efforts to prevent the development of bacteremia from colonization (page 16, line 4 & page 17, line 8).

2. It is unclear, at least to me, if colonization was taken into account at ICU admission or during ICU stay. In Methods (page 6 first para) we understand that only patients with colonization at ICU admission were taken into account. However, in table 2, the authors stated that time from ICU admission to colonization was 12 vs 6 days.

Reply : We are sorry to make you confused with the inclusion criteria. Only the patients with colonization “after” ICU admission were enrolled in this study, not those with colonization at the time of ICU admission. Therefore, the duration of 12 and 6 days are time from ICU admission to colonization in each group. We have corrected the sentence in the study subjects section of methods (page 6, line 19).

3. How colonization was diagnosed? Have the authors performed screening at ICU admission and during ICU stay?

Reply : Screening cultures were performed in blood, urine, and sputum/trans-tracheal aspirate for all the patients at the time of ICU admission. During the ICU stay, additional cultures were performed when infection signs such as systemic inflammatory response syndrome were newly developed, or sustained for more than 3 days after antibiotics change, or when clinical deterioration such as worsening of fever, respiratory condition, and/or radiographic status, requiring mechanical ventilation, requiring aggressive fluid resuscitation or vasopressors were observed. We have described how colonization was diagnosed in the method (page 6, line 10).

4. Time at risk was significantly shorter in patients with bacteremia compared with controls. Please explain this unusual finding.

Reply : As we mentioned on page 15, line 14, the severity of disease is a more important factor than the length of stay in the ICU for the development of MDR AB bacteremia. Patients with a high severity of disease require more acute care including invasive procedures and mechanical ventilation. The APACHE II score at the time of colonization, and the proportion of maintenance of mechanical ventilation and recent invasive procedure were higher in bacteremic group than in non-bacteremic group. We have described this explanation.
in little more detail (page 15, line 19).

5. The percentage of patients with A. baumannii colonization at ICU admission (46%) is impressive. Please comment.

Reply: We are sorry to make you confused with the inclusion criteria. As we have explained above, only the patients with colonization “after” ICU admission were enrolled in this study, not those with colonization at the time of ICU admission. We have corrected the sentence (page 6, line 19).

6. How mixed bacteremia were analyzed? Were patients with polymicrobial bacteremia excluded?

Reply: Bacteremia of at least one or more different kinds of microorganism was analyzed in this study regardless whether it was polymicrobial or not. The incidence of polymicrobial bacteremia was low without significant differences. We added the proportion of polymicrobial bacteremia in Table 4 (page 29 - 30).

7. Authors stated that continuous variables were analyzed using Student’s t-test. However, in non-normally distributed quantitative variables, they should have used a non-parametric test such as Mann Whitney U test. Was distribution of quantitative variables tested? In addition, the authors have included a very large number of variables in the logistic regression model. Were interactions between these variables tested?

Reply: We agree with the reviewer’s opinion and checked all the continuous variables again if they were normally distributed nor not. Several variables were non-normally distributed, so they were analyzed using Mann-Whitney U test. As a result, P-value of three variables below showed some minor numerical changes. We have revised the method of statistical analyses (page 9, line 10) and P-values in Table 2 (page 25).

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value (before)</th>
<th>P-value (after)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of hospitalization</td>
<td>0.137</td>
<td>0.333</td>
</tr>
<tr>
<td>Duration of exposure to risk</td>
<td>0.016</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of mechanical ventilation</td>
<td>0.018</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Moreover, as the reviewer pointed out, we performed the re-analysis to check if there was interaction between all the variables tested in the multivariate analysis. The interaction was observed only between the variables of maintenance of mechanical ventilation and maintenance of an endotracheal tube instead of switching to tracheostomy. The graph below shows the interaction of these two variables. However, combination of the two factors was a significant risk factor for MDR AB bacteremia in the multivariate analysis (odds ratio = 16.64; 95% CI 1.64-168.83; P = 0.017). These changes in data were fully reflected in this revised paper including adjustment of Table 6 (page 32) and texts in the results and discussion (page 12, line 18 & page 14, line 10). We have also revised the method of statistical analyses (page 9, line 11). Thank you for the comments.
8. Results are repeated in text and results. Result section should be substantially shortened.

Reply: We have deleted some redundant and repeated sentences as suggested. Thank you for the comments.

9. How many patients developed A. baumannii bacteremia without prior colonization, and how these patients were analyzed?

Reply: Of 257 patients without prior colonization, 23 patients developed A. baumannii bacteremia and they were excluded in this study. We have added information about these excluded patients in the method (page 6, line 22).

10. In Table 5, the authors provide results on prior antibiotic exposure. However, no information is provided on duration of this exposure. The duration of exposure to different antibiotics is an important risk factor for subsequent emergence of A. baumannii.

Reply: We have added duration of antibiotics exposure in Table 5 (page 31). Significantly shorter duration of exposure to quinolone, antipseudomonal penicillin, carbapenem, glycopeptide, and anti-anaerobe was observed in bacteremic patients than in non-bacteremic patients. However, this result was due to shorter duration of risk exposure in bacteremic patients than in non-bacteremic patients. Therefore, we analyzed the number of previous antimicrobials used at least 72 h within 2 weeks preceding the date of the positive culture for MDR AB for bacteremic patients, or the date of discharge from the ICU for non-bacteremic patients. We have added this explanation in discussion (page 14, line 6).

11. In background section, authors stated that multiple antimicrobial resistances in A. baumannii enhanced its virulence. I am not aware of studies suggesting such a mechanism. Please provide a reference for this statement.

Reply: We agree with the reviewer that multiple antimicrobial resistances in A. baumannii do not enhance its virulence. We have revised the sentence (page 4, line 4). Thank you for the comments.

12. Minor: page 7, last para: neutrophil count <1500?

Reply: We have corrected the direction of a sign of inequality (page 8, line 15).
June 7th, 2010

(Reviewer 2)

Dear Wen-Chien Ko

RE: MS. No. 1044398363730395

Title : Risk factors for multi-drug resistant Acinetobacter baumannii bacteremia in patients with colonization in the intensive care unit

Thank you very much for reviewing our manuscript. We appreciate your advice about the revision and are very pleased to inform you that we are ready to resubmit our revised manuscript taking into account your comments. All significant changes have been written in red colored text. Thank you again for your kind review and we await your feedback and acceptance.

Sincerely yours,

Young Sam Kim M.D.

Assistant Professor
Division of Pulmonology, Department of Internal Medicine
Yonsei University College of Medicine
250 Seongsanno, Seodaemun-gu
Seoul 120-752, Republic of Korea
Telephone : 82-2-2228-1971, Fax : 82-2-393-6884
E-mail: ysamkim@yuhs.ac
Comments to the authors -

The present study works on the retrospective analysis of risk factors for the development of MDR AB bacteremia among the ICU patients with MDR AB colonization. Much clinical information was collected and analyzed, and some risk factors were regarded to be independently associated with the occurrence of AB bacteremia among colonized patients. However, it is unusual that more than a half of colonized patients developed bacteremia in the study hospital.

Major Compulsory Revisions

1. For the multivariate analysis, though seven independent factors were related to the subsequent development of MDR AB bacteremia after colonization. However, these factors were not independent but interacting. ICU admission prior to infection and bacteremia due to other pathogens after MDR AB colonization will be possibly related to the number of prior antimicrobial agents. Also, ICU admission due to respiratory failure will be related to maintenance of mechanical ventilation or endotracheal tube. Particularly, all patients with respiratory failure received mechanical ventilation support, as mentioned in the Discussion. How could they be independently related to the primary outcome? Therefore, the interactions between these factors need to be examined, and another model of multivariate analysis should be considered to exclude their interactions between factors.

Reply: We agree with the reviewer’s opinion and have performed the re-analysis to check if there was interaction between all the variables tested in the multivariate analysis. The interaction observed only between the variables of maintenance of mechanical ventilation and maintenance of an endotracheal tube instead of switching to tracheostomy. The graph below shows the interaction of these two variables. However, combination of the two factors was a significant risk factor for MDR AB bacteremia in the multivariate analysis (odds ratio = 16.64; 95% CI 1.64-168.83; \( P = 0.017 \)). These changes in data were fully reflected in this revised paper including adjustment of Table 6 (page 32) and texts in the results and discussion (page 12, line 18 & page 14, line 10). We have also revised the method of statistical analyses (page 9, line 11). Thank you for the comments.
2. Clinical implication of the findings of the present study will be limited in clinical practice. The authors suggest in the end of Abstract that their findings will provide the identification of at-risk patients with MDR AB colonization for later development of bacteremia, and the former population can be benefited by early appropriate antimicrobial therapy. However, one important limitation of such a strategy will the timing of early appropriate therapy, since it remains impossible to clearly recommend when to give these antimicrobial agents, since there were chances of developing bacteremia, other than MDR AB bacteremia, esp. Enterococcus bacteremia, after AB colonization.

**Reply:** We agree with the reviewer that our conclusion seems to be off the point of the results. We have revised the discussion and conclusion more focusing on the results of our study for the prevention of the development of bacteremia from the colonization (page 16, line 4 & page 17, line 8). Thank you for the comments.

**Minor Essential Revisions**

3. Are these isolates genotyped for clonal relatedness to consider the possibility of an outbreak of MDR AB infections in the study ICUs?

**Reply:** The outbreak of MDR AB infection was developed in our ICUs from the early 2008. The fingerprint PCR for the isolated AB from the patients and from the environment was randomly performed and the concordance in the types of both samples was observed. We described about the situation of outbreak in the method in detail (page 6, line 7).

4. More than one hundred episodes of MDR AB bacteremia were present in the 2-year study period. What is the overall burden of AB bacteremia or infections in the study ICUs? Are there episodes of XDR or PDR AB infections or bacteremia?

**Reply:** As we mentioned about the outbreak above, because of the clonal spread of AB, most of the isolated AB was multi-drug resistant. During the study period of 2 years, 6 patients developed non-MDR AB bacteremia. Moreover, among 108 patients with MDR AB bacteremia, only 15 episodes were carbapenem sensitive AB. We have added this information in the method and result separately (page 6, line 22 & page 10, line 6).

5. The study population included those with MDR AB colonization at the time of admission. Do the authors indicate the ICU admission or hospital admission? Why did none become colonized with MDR AB during ICU admission? Are the former excluded from the study?

**Reply:** We are very sorry to make you confused with the inclusion criteria. In this study, only the patients with colonization “after” ICU admission were enrolled, not those with colonization at the time of ICU admission. We have corrected the sentence (page 6, line 17-20).

6. The isolation of coagulase-negative Staphylococcus (CoNS) from blood cultures before and after the MDA AB colonization was concerned by the authors. Please clarify the definition of significant CoNS bacteremia in the study. Was any episode of CoNS isolation from the blood culture included for analysis?

**Reply:** We defined significant CoNS bacteremia as isolation of the microorganism from 2 different blood cultures and showing clinical signs of infection at the same time. We added the definition in the methods (page 8, line 6).

7. The location and the number of colonization cites of MDR AB deserved a statistical analysis for their interactions with subsequent AB bacteremia, esp. for the mention of airway
AB colonization in the Discussion by the authors.

Reply: All of the colonization was diagnosed by trans-tracheal aspirate culture and 18 patients showed colonization in urine culture as well at the same time. We have added information about the location and the number of colonization cites in the method (page 6, line 25).

8. The authors confessed that there are several limitations in the study, but only one issue was discussed. Basically, the mentioned one is inherently methodological weakness of a retrospective study. Other issues should be concerned in the Discussion.

Reply: We have revised the limitation with more other issues as the reviewer suggested (page 16, line 10).