Author's response to reviews

Title: Clonal spread of Acinetobacter baumannii CC92 in a teaching hospital in Guangzhou and amlodipine reverses its imipenem-resistance in vitro

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Version: 4 Date: 11 July 2013

Author's response to reviews: see over
Dear Mr Nathaniel Nazarenor:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Antimicrobial activity of amlodipine against extensively drug-resistant Acinetobacter baumannii isolates in vitro” (ID: 1989897354876175). According with your advice, we amended the relevant part in manuscript. Revised portion are marked in red in the paper. Some of your questions were answered below.

**Reviewer 1: Professor Howard H Xu**

**Major Compulsory Revisions**

1. **Comment**: Using multilocus sequence typing (MLST) to determine molecular epidemiology of these isolates.
   
   **Response**: Considering the Reviewer’s suggestion, we have performed MLST to determine molecular epidemiology of 42 isolates. According to the MLST, 42 A. baumannii isolates can be grouped into 10 STs. ST195 and ST208 were the most common STs accounting for 68.05% (29/42) of the isolates. Clonal relation analysis showed that both ST195 and ST208 belong to the CC92.

2. **Comment**: The conclusion reached by the authors "AML alone or combined with imipenem showed antibacterial activities against clinically resistant Acinetobacter baumannii isolates in vitro" cannot be supported by results presented.
   
   **Response**: Considering the Reviewer’s suggestion, conclusion had been amended as “CC92 were the major clone that spreads in our hospital, and AML can improve the activity of imipenem against A. baumannii isolates in vitro which doesnot work by inhibiting MBL”.

**Minor Essential Revisions**

1. **Comment**: the title is inappropriate.
   
   **Response**: Considering the Reviewer’s suggestion, title has been amended as “Clonal spread of Acinetobacter baumannii CC92 in a Teaching Hospital in Guangzhou and Amlodipine reverses its Imipenem-resistance in vitro”.

2. **Comment**: in the Methods section, sources of other antibiotic drugs should have been described.

   **Response**: In the Methods section, we have described that sources of antibiotics disc (OXOID) were obtained from Melone Pharmaceutical Co. Ltd. (China).

3. **Comment**: in the Results section, Table 1 was never cited; it was initiated mentioned in Methods section

   **Response**: We are very sorry for our negligence. In results section, Table 1 has been amended as “Table 3” and described in paragraph 3.

**Reviewer 2: Professor Michael McConnell**

**Major Compulsory Revisions**

1. **Comment**: The strains used in the study are 42 consecutive clinical isolates from a single hospital. In many hospitals there is a dominant clone of A. baumannii that produces the majority of infections. Do the authors have any data with regard to the clonal relationship between the strains included in the study (i.e. PFGE or REP-PCR data).

   **Response**: Considering the Reviewer’s suggestion, we have performed MLST to determine molecular epidemiology of 42 isolates. According to the MLST, 42 A. baumannii isolates can be grouped into 10 STs. ST195 and ST208 were the most common STs accounting for 68.05% (29/42) of the isolates. Clonal relation analysis showed that both ST195 and ST208 belong to the CC92.

   PFGE, REP-PCR and MLST are all typing systems suitable for clonal relationship analysis. MLST has its advantage in global epidemiological investigation of A. baumannii. Typing data of MLST are translated into a numerical code that can be obtained in an identical manner at different laboratories by using the same protocol. It provides a portable method that may be suitable for global epidemiologic study and allow the recognition of epidemic, multiresistant, and virulent A. baumannii clones and the monitoring of their national and international spread.
Minor Essential Revisions

1. **Comment:** Figure 1 could easily be incorporated into Table 1 by placing an additional line of “% susceptible” at the bottom of the table

   **Response:** Considering the Reviewer’s suggestion, the original “Figure 1” has been deleted and its data is incorporated into Table 3 (original Table 1 had been amended as “Table 3”) by placing an additional line of “% susceptible” at the bottom of the table.

2. **Comment:** Figure 2 shows routine results of Etest experiments. Since the authors explain the results in the test, this figure may not be necessary.

   **Response:** Considering the Reviewer’s suggestion, the Figure 2 has been deleted.

Discretionary Revisions:

1. **Comment:** Data addressing how AML affects the growth of A. baumannii would be interesting to include (i.e. growth curves of A. baumannii in the presence and absence of AML).

   **Response:** Special thanks to you for your good comments. In our study, we mainly evaluate the synergism between imipenem and the cardiovascular agent amlodipine by checkerboard method. We would like to carry out a separate but more extensive experiment on this topic. Accordingly, we have modified the sentence to address the reviewer’s point. (Discussion section Paragraph 3) However, if the reviewer feels that it is essential to add this result in this manuscript, we would be willing to carry out the additional experiments.

2. **Comment:** In the abstract and the introduction the authors mention that they provide information on the possible mechanisms of action of AML. The data presented does not seem to shed much light on this issue so the authors may wish to soften these statements.

   **Response:** Considering the Reviewer’s suggestion, We have re-written this part according to the Reviewer’s suggestion. And “and its potential mechanism of action was explored” has been deleted

3. **Comment:** It would be interesting if the authors could comment on the relationship between the AML MIC values and the doses of AML permitted in
Response: Considering the Reviewer’s suggestion, We have comment on the relationship between the AML MIC values and the doses of AML permitted in humans in paragraph 4 of discussion section.

Reviewer 3: Professor Shan-Chwen Chang

Major Comments

1. **Comment**: The title of this manuscript is not good.
   
   **Response**: Considering the Reviewer’s suggestion, title has been amended as “Clonal spread of Acinetobacter baumannii CC92 in a Teaching Hospital in Guangzhou and Amlodipine reverses its Imipenem-resistance in vitro”.

2. **Comment**: According to the study results, AML need to be used up to 40 ug/ml to improve the activity of imipenem against these resistant A. baumannii isolates. The authors should discuss the possibility to achieve this kind of AML concentration in human and its possibility of clinical application.
   
   **Response**: Considering the Reviewer’s suggestion, We have comment on the relationship between the AML MIC values and the doses of AML permitted in humans in paragraph 4 of discussion section.

3. **Comment**: The numbers of MDR and XDR strains were not correct. Besides, the definition of MDR and XDR used in this study should be put in the “Methods” section, not in the “Background” section.
   
   **Response**: Considering the Reviewer’s suggestion, we calculate and check the S, I, R results of individual isolates again. Phenotype results of isolate 23, 25 and 36 were not correct. The wrong data had been corrected in new Table 3. The correct numbers of MDR and XDR strains were 10 and 32 respectively.

   The definition of MDR and XDR used in this study has been put in the “Methods” section,

4. **Comment**: It is better to do the time-killing study to demonstrate the time-killing curve, which may prove or disprove the killing synergistic effect against the specific isolates.
Response: Special thanks to you for your good comments. In our study, we mainly evaluate the synergism between imipenem and amlodipine by checkerboard method. We would like to carry out a separate but more extensive experiment on this topic. Accordingly, we have modified the sentence to address the reviewer’s point (Discussion section Paragraph 3). However, if the reviewer feels that it is essential to add this result in this manuscript, we would be willing to carry out the additional experiments.

5. Comment: It’s better to do the molecular typing of these 42 isolates, such as PFGE, to know whether there was clonal spreading in this hospital or not, how many isolates were belonged to the same clone.

Response: Considering the Reviewer’s suggestion, we have performed MLST to determine molecular epidemiology of 42 isolates. According to the MLST, 42 A. baumannii isolates can be grouped into 10 STs. ST195 and ST208 were the most common STs accounting for 68.05% (29/42) of the isolates. Clonal relation analysis showed that both ST195 and ST208 belong to the CC92.

PFGE and MLST are all typing systems suitable for clonal relationship analysis. MLST has its advantage in global epidemiological investigation of A. baumannii. Typing data of MLST are translated into a numerical code that can be obtained in an identical manner at different laboratories by using the same protocol. It provides a portable method that may be suitable for global epidemiologic study and allow the recognition of epidemic, multiresistant, and virulent A. baumannii clones and the monitoring of their national and international spread.

6. Comment: Only checked MBL production by E-test MBL and without checking other carbapenem resistance mechanism or the antimicrobial mechanism of AML. It is not enough to explore the potential mechanism of action for either AML alone or AML in combination with imipenem. In addition, the MBL production was checked by E-test MBL method only. It is not enough. They should check what kind of MBL was produced in this isolate by other methods, such as PCR with appropriate primers.
Response: Special thanks to you for your good comments. In our study, data associated with possible mechanisms of AML is limited. We have re-written this part to soften these statements. However, if the reviewer feels that it is essential to add this result in this manuscript, we would be willing to carry out the additional experiments.

7. Comment: Fig. 2 can be deleted. The results of Table 3 can be demonstrated in figure showing the distribution of imipenem MICs.

Response: Considering the Reviewer’s suggestion, Original Fig. 2 has been deleted. The change of imipenem MIC with adding different concentrations of AML has been demonstrated in a new “figure 2” and original table 3 has been amended as “Table 4”.

Minor Comments

1. Comment: In “Methods” section Paragraph1, “The First People’s Hospital” in what city or what province should be clarified.

Response: Considering the Reviewer’s suggestion, “The First People’s Hospital” had been amended as “The Guangzhou First People’s Hospital”.

2. Comment: In “Discussion” section Paragraph 1, the authors should not extend the description to Acinetobacter infection. They should only say A. baumannii infection because this study only tested the isolates of A. baumannii.

Response: Considering the Reviewer’s suggestion, In Discussion”section Paragraph 1., All “Acinetobacter” have been replaced by A. baumannii.

3. Comment: In “Discussion” section Paragraph 1, the sentence “According to our data, A. baumannii is widely involved in hospital infections, resulting in respiratory, blood, wound, urine and cerebrospinal infections, ……” is not correct because the authors mentioned that they collected consecutive isolates from clinical specimens of different patients, but did not mention whether these patients had true A. baumannii infection or not, especially when the isolates were from respiratory specimens.

Response: Considering the Reviewer’s suggestion, the sentence has been re-written as “According to our data, A. baumannii is widely distributed in our
hospital, isolated from respiratory, blood, wound, urine and cerebrospinal fluid, especially from intensive care unit (ICU) patients, who were older and had a longer inpatient stay, which is similar to many other studies.”

4. **Comment**: In “Discussion” section Paragraph 2 : “staphylococcus aureus NCTC6571” should be “Staphylococcus aureus NCTC6571”. “Escherichia coli” and “Klebsiella pneumoniae” should be “E. coli” and “K. pneumoniae” when they appeared second time in the text.

**Response**: They have been corrected according to the Reviewer’s suggestion.

5. **Comment**: In “Discussion” section Paragraph 2 : After the sentence “A further study demonstrated that AML in combination with streptomycin has a synergistic effect.” references should be added.

**Response**: The reference had been added according to the Reviewer’s suggestion.

6. **Comment**: Typo error in the sentence “Our research demonstrates the antimicrobial activities of AML against clinical A. baumannii strains, and the MICs ranged from 40 to 320 ug/ml in combination with imipenem. Half of the isolates (N=21, 50.0%) demonstrated synergy or partial synergy.” in “Discussion” section, Paragraph 2.

**Response**: Considering the Reviewer’s suggestion, the sentence has been re-written as “Our research demonstrates the antimicrobial activities of AML against clinical A. baumannii strains, and the MICs ranged from 40 to 320 ug/ml. In combination with imipenem, half of the isolates (N=21, 50.0%) demonstrated synergy or partial synergy.”

7. **Comment**: In “Conclusion” section, the sentence “Our research indicates that AML alone or in combined with imipenem showed antibacterial activities against clinically resistant Acinetobacter baumannii isolates in vitro.” is not correctly described, because AML alone did not have good antibacterial activity. “Acinetobacter baumannii” in this sentence should use abbreviation as “A. baumannii”.

**Response**: Considering the Reviewer’s suggestion, conclusion has been amended as “CC92 were the major clone that spreads in our hospital, and AML can improve
the activity of imipenem against A. baumannii isolates in vitro which does not work by inhibiting MBL”.

8. **Comment:** The detailed antibiogram of individual isolates in Table 1 can be put in the “Appendix”. Table 1 can be modified to show only the statistical results of “S, I, R” percentage of total 42 isolates for individual antibiotics tested. In this way, data in Fig. 1 can be showed in this new Table. Therefore Fig. 1 can be deleted.

**Response:** Considering the Reviewer’s suggestion, the “Figure 1” has been deleted and its data is incorporated into Table 3 (original Table 1 had been amended as “Table 3”) by placing an additional line of “% susceptible” at the bottom of the table. However, we would be willing to carry out the additional modification if the reviewer feels that it is essential.

In all, I found the reviewer’s comments are quite helpful, and I revised my paper point-by-point. Thank you and the review again for your help!

Yours sincerely,
LI Yu-jun

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