Author's response to reviews

Title: Dissemination of Imipenem-Resistant Acinetobacter Baumannii with New Plasmid-Borne BlaOXA-72 in Taiwan

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Author's response to reviews: see over
Dear Editor,
Thanks for your kind consideration of our manuscript and your comments. All the comments are highly appreciated and they help to greatly improve our manuscript. We have our best to respond to the comments point by point in the below. We believe the manuscript is now suitable for publication in your esteemed Journal.

Q1: a. Throughout, please use “pulsed-field gel electrophoresis”
   b. Consider replacing “spreading” with “spread” and “spreading mechanisms” with “mechanism of spread”.
   c. Line 61 Consider replacing “the one similar to that shown in” with “one described in previous literature.”
   d. Tables 1 and 2 Please replace “MICs of Acinetobacter baumannii” with “MICs of antibiotics for Acinetobacter baumannii” or similar.
A1: Thanks for your comments. We had replaced all of them according to your suggestions. We also found the number of IRAB was wrong and had corrected it (142 instead of 115 isolates).

Q2: References 22 and 23 are the same.
A2: We are sorry for the mistake. We had corrected it and revised the references.

Q3: PFGE dendrogram: images would be better than scored bands.
A3: We had used images rather than scored bands.

Q4: Please remove some of the repetition from the Discussion.
A4: We had removed the repetition according to the reviewer’s suggestions.

Q5: Line 59 Abstract and lines 179-180: they don’t look closely related to me!
A5: Thank you. We had deleted the description of “closely related”.
Reviewer 1


A1: Thank you. The definition of MDR in the study was non-susceptibility to $\geq 1$ agent in $\geq 3$ antimicrobial categories. These isolates were at least resistant to two agents in antipseudomonal carbapenems (imipenem and meropenem), one agent in extended-spectrum cephalosporins (ceftazidime) and one agent in antipseudomonal fluoroquinolones (ciprofloxacin). Therefore, they were multidrug resistant. We had added the reference.

Q2: Lines 179-182. (Line 58 in abstract): Why do you say that defined clones are closely related? The clones were defined with a percentage of genetic similarity above 85%. Suggestion: refer only two distinct and major clones (A and B). In this section, the authors should mention that isolates carrying OXA-72 determinant did not belong exclusively to these two clones, and indicate the percentage of homology of the remaining isolates relatively to these two major clones. Isolate 1765 should be highlighted, since it carries a plasmid not identical to the other isolates (and it will introduce the next section).

A2: Thanks for your reminding. We had deleted the description of “closely related”. We added “the other six isolates carrying blaOXA-72 (including isolate 1765) did not belong to the two major clones and had only 65-75% homology related to the major clones”.

Q3: Line 210. Indicate something about strain Ab290 (clinical, previous study…).

A3: We had added “Ab290 was a clinical isolate from Taipei Veterans General Hospital and has been used for transformation in the previous study [Antimicrob Agents Chemother. 2008 July; 52(7): 2573–2580]”.

The discussion begins with a summary of the results of the study, the main conclusions.
Q4: The discussion would be improved if re-organized. For example, the first sentences of the second paragraph (line 228) could be the beginning of the discussion and introducing the topic. Not all the readers will analyze carefully the results, and some points should be clearer.
A4: We had moved the first sentences of the second paragraph to the beginning of the discussion.

Q5: The authors concluded that both clonal spread and plasmid dissemination played a role in the spreading of $\textit{bla}(\text{OXA-72})$. This conclusion is mentioned three times in the discussion (line 225, line 246, line 262). This should be corrected (re-organization).
A5: We had deleted and corrected the statement in line 225 and 246, respectively. However, we thought statement in line 262 would be necessary for the conclusion. We asked for your permission to keep them.

Q6: Line 220. The first sentence refers that 291 $A. \text{baumannii}$ were collected and two major clones of IRAB were disseminated. The sentence should be precise. Not all the 291 isolates were imipenem-resistant, and of those, just a small percentage carried OXA-72 determinant. This must be clear.
A6: We have rephrased them to “in this study we collected 142 IRAB from different geographical regions of Taiwan. Among them, 27 IRAB carrying $\textit{bla}_{\text{OXA-72}}$ gene had successfully spread throughout Southern Taiwan and extended into Central Taiwan”

Q7: Line 224. Here, the authors mention the presence of OXA-72 determinant in other clones. See comments in Results.
A7: We had replaced “two closely related clones” with “major clones”.

Q8: Line 229. It is stated that class D beta-lactamases are the main mechanism of resistance to carbapenems in $A. \text{baumannii}$. However, of the 115 IRAB only 27 carried the $\textit{bla}(\text{OXA-72})$, and other OXA beta-lactamases and metallo-enzymes were not found. This should be discussed. OXA-72 may be emergent, but it is not prevalent relatively to carbapenem resistance.
A8: The resistance mechanism varied among areas. In Southern Taiwan, IRAB carrying
\textit{bla} \textsubscript{OXA-72} accounted for around 60\% of all IRAB. In Central Taiwan, more than 50\% of
IRAB carried \textit{bla} \textsubscript{OXA-23}. In Taiwan as a whole, the \textit{ISAb1} - \textit{bla} \textsubscript{OXA-51} was the main
mechanism. We had mentioned these in the third paragraph and addressed the \textit{bla} \textsubscript{OXA-72}
was mainly in Southern Taiwan.

Q9: What is the relevance of knowing the genetic environment of the \textit{bla}(OXA-72)?
A9: Our previous study showed the genetic environment affected the expression of
carbapenemase. For example, the plasmid-borne \textit{bla} \textsubscript{OXA-58} was inserted upstream by
\Delta \textit{ISAb3} and \textit{IS}1008, which provided two promoters [Antimicrob Agents Chemother.
2010 Aug;54(8):3107-12]. The expression of \textit{bla} \textsubscript{OXA-58} driven by two different promoters
contributes to different levels of antimicrobial MIC. We found the \textit{bla} \textsubscript{OXA-72} with
different genetic environment still results in high degree of carbapenem resistance.

Minor essential revision
Abstract

Q1: Line 52: “Clonality” and “pulse field gel electrophoresis”
A1: Thank you. We had corrected them.

Q2: Line 55: “imipenem resistance”
A2: “The contribution of \textit{bla} \textsubscript{OXA-72} to imipenem was determined by transforming plasmids
carrying \textit{bla} \textsubscript{OXA-72} into imipenem-susceptible \textit{A. baumannii}.” We meant Ab290. If we
transformed plasmid into imipenem-resistant strains, it would not reveal the contribution
of \textit{bla} \textsubscript{OXA-72}.

Q3: Figure 1. Pulse field gel electrophoresis, Figure 2 should not be figure 3?
A3: Thank you. We had corrected them.

Q4: Figure 2. The legend should indicate the isolates tested, and the type of Marker.
A4: We had indicated the isolates and the type of marker.
References

Q5: Check italics and incomplete references, namely in references: 5, 7, 16, 24
A5: Thanks for your review. We had checked these references.

Q6: References 22 and 23 are the same. Correct the list of references, including in the manuscript text (in Material and Methods, lines 148-155)
A6: Thanks for your careful review. We had corrected it.

Reviewer 2
1) In the third paragraph of results, 50% should be revised to 50.0%;
2) In the first sentence of discussion, for should be revised to from;
3) In table 1, n should be revised to \( n \) and Antimicrobial should be revised to Antimicrobial agent;
4) In table 2, OXA72 should be revised to OXA-72 and there should be no space between Ab and 290;
5) in figure 4 legend, \( A. \ baumannii \) should be revised to \( Acinetobacter \ baumannii \);
6) In figure 1 legend, reference 14 should be deleted;
7) P value should be \( P \).

Please double check the other paragraphs to find the problem like those

Thanks for your careful review. As for point (1) to (7), we had corrected them accordingly. We are sorry for the mistakes.