Author's response to reviews

Title: Tracing the emergence of multidrug-resistant Acinetobacter baumannii in a Taiwanese hospital by evaluating the presence of integron gene intI1

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Author's response to reviews: see over
Dear Dr. Haibo Qiu,

The authors thank your constructive comments. It is our pleasure to submit a revised manuscript that has been amended in light of the reviewer’ feedback. We appreciated your comments, and our answers are as followings:

Major Compulsory Revisions

#1
Question:
The authors mentioned that “the numbers of clinical AB isolates have risen since 2002, and the first MDRAB was reported in Taiwan that same year”, so it seems that 2002 is the beginning of the increment of AB infection. Please explain why the authors chose 2001-2002 as the study period and 2003-2004 as the control period, and what does the classification of study and control period mean because no comparison between these two groups was found in the results.

Answer:
Authors analyzed 401 A. baumannii isolates obtained at Changhua Christian Hospital between 2001 and 2002 [Chen CH, Young TG, Huang CC. Predictive biomarkers for drug-resistant Acinetobacter baumannii isolates with blaTEM-1, AmpC-type bla and integrase 1 genotypes. J Microbiol Immunol Infect. 2006 ;39:372-9.] , and we disclosed MDR A. baumannii , which were resistant to many broad-spectrum antibiotics (including ciprofloxacin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem-cilastatin, amikacin) since then (2002). So, we choose the study year is 2001-2002 with the hypothesis of study year is the beginning of the increment of resistant A. baumannii infection. If the hypothesis is correct, the presence of the intI1 integrase gene in class 1 integrons will increase between the study year and the observation year. Because it’s difficult to perform case-control study, we selected the cross-sectional method to perform this study. Concerning the observation year, we selected the 2003-2004 for comparison. Please see Page 5 Line 13- Page 6 Line 3.
In the line 2-3, page 7, “The ampicillin-sulbactam resistance phenotype, IntI1 integrase positive genotype, and strains isolated within season of high infection rate”. When is the season of high infection rate, please clearly definition.

Answer:

The reasons to choose ampicillin/sulbactam as the selection markers are according to following reasons. Ampicillin/sulbactam has been proven to be more efficacious than polymyxins in treating carbapenem-resistant A. baumannii infection [Betrosian AP, et al. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant Acinetobacter baumannii ventilator-associated pneumonia. J Infect, 2008;56: 432–436.]. Although resistance to ampicillin/sulbactam in A. baumannii has been reported in many countries [Perez F, et al .Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother, 2007;51:3471–3484 ] and authors disclosed one unique phenomenon is that some isolates of MDR A. baumannii, which were resistant to many broad-spectrum antibiotics (including ciprofloxacin, piperacillin-tazobactam, ceftazidime, cefepime, imipenem-cilastatin, amikacin) but susceptible to ampicillin/sulbactam between 2001 and 2002 [Chen CH, Young TG, Huang CC. Predictive biomarkers for drug-resistant Acinetobacter baumannii isolates with blaTEM-1, AmpC-type bla and integrase 1 genotypes. J Microbiol Immunol Infect. 2006 ;39:372-9.]. No resistance mechanism was described in those studies.

The reasons to choose the intI1 integrase gene in class 1 integrons as the selection markers are that is the target gene in this study. Because of a lot of clinical A. baumannii isolates, we needed to select the important isolates to study. According to the Additional files 2 (which shows the infection rate of the hospital and the ICU during 2001-2004), the strains isolated within season of high infection rate are from summer season during the four study period.

We add that explanation at Page 13-14.

Question:
In the additional files 2, the authors presented the infection rate of the hospital and the ICU of each year during 2001-2004 respectively. But the variation trend for the four years, which seems more significant than the monthly data in each year, should also
be explored.

Answer:

The cumulative dataset for nosocomial infection is big data, and the presentation of the time frame of nosocomial infection is controversy. [Hiroshi Nishiura. Time variations in the generation time of an infectious disease: implications for sampling to appropriately quantify transmission potential. Mathematical Biosciences and Engineering 2010;7,: 851-869.]

The purpose of this study is to assess the presence of the intI1 integrase gene in class 1 integrons as an MDRAB-associated biomarker.

According to the Additional files 2 (which shows the infection rate of the hospital and the ICU during 2001-2004), the ICU infection rate fluctuated and the hospital infection rate are stable. Several periods occurred during which the infection rate in the ICU increased, including June 2001, November 2001, February 2002, July 2002, September 2002, July 2003, November 2003, and March 2004. However, the reason for the gradual increase in AB infection is still unknown, despite investigation.

In brief, the ICU infection rate of summer seasons was higher during the four study period.

#4

Question:
Because it was a retrospective study, please illustrate how the samples were collected and preserved, and if there was any miss or lost sample. It is advised to provide a study flow to clarify the specific inclusion, exclusion and missing in data collection.

Answer:

Authors actively collected all AB isolates were collected during January 1st, 2001 to December 31st, 2004. All AB isolates were routinely examined at Medical Laboratory Department of CCH, and those isolates were stored in the Bacterial Bank of CCH.

Please see the Appendix 1 for study flow. And, the criteria for inclusion and exclusion are as follows:

(A)Inclusion criteria
1)Phenotypic methods were used to identify A. baumannii isolates using a Vitek-2 System (BioMerieux, Marcy l'Etoile, France).
2) Additionally, antimicrobial susceptibility was determined using an automated Vitek 2 (bioMe'rieux, Marcy l'Étoile, France) according to the recommendations of Clinical and Laboratory Standards Institute

(B)Exclusion criteria
1) If phenotypic methods (Vitek-2 System, BioMerieux, Marcy l'Etoile, France) for identifying A. baumannii isolates showed insistence with A. baumannii.

2) The AB isolates failed to survive from the stock of the Bacterial Bank of CCH

#5

Question:
In the line 15-16, page 10, “the relationships between the predominant clones and the presence of the IntI1 did not have a significant association”, please provide the statistics data.

Answer:

The statistic method is descriptive method, please see Table 2 and Additional file 3. The interpretation method for PFGE results is according to Tenover's criteria [Tenover FC, et al. Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing. Journal of clinical microbiology, 1995; 33: 2233–2239.]. Though PFGE results identified two predominant clones types during the study year (Table 2 and Additional file 3), the relationships between the predominant clones and the presence of the IntI1 did not have a significant association. We add the explanation at Page 10.

#6

Question:
It was mentioned that only eight samples of the clinical MDRAB isolates (two isolates each year) were chosen for PFGE study, please illustrate why only eight samples been drawn and how the eight samples been drawn for PFGE study

Answer:

We selected 12 multi-resistant A. baumannii (MDRAB) isolates with resistance to ampicillin-sulbactam to perform PFGE.

In order to evaluate the epidemiological features of the MDRAB isolates, selection criteria such as ampicillin-sulbactam resistance phenotype, IntI1 integrase positive genotype, and strains isolated within season of high infection rate were used for choosing candidates MDRAB strains (Table 2).

There were two isolates, which both carried IntI1 positive genotype and ampicillin-sulbactam resistance phenotypes, in each July during the four study years, so the total number of the clinical MDRAB isolates is EIGHT.

The left FOUR are those multi-resistant A. baumannii (MDRAB) isolates,
which are resistance to ampicillin-sulbactam, but do not carry \textit{IntI1} gene.

So the total multi-resistant \textit{A. baumannii} (MDRAB) isolates are TWELVE.

Minor Essential Revisions

#1
Question:
In table one, what is the ICU infection rate and hospital infection rate. Does it mean the rate of MDRAB infection rate or AB infection rate or the total infection rate? Please make it clearly

Answer:
The Infection Control Office of CCH began to investigate the infection rate in the hospital and the ICU (Additional files 2).
The ICU infection rate is the nosocomial infection rate of overall pathogens.
The hospital infection rate is the overall nosocomial infection rate.
Because denominator is big number with stable patter and numerator is small number with fluctuated characteristic, the overall hospital infection rate at study hospital was 5-6% in recent 15 years, but the infection rate in the ICU fluctuated.

#2
Question:
In the figure one, what did those lines and bands represent, please indicate Clearly

Answer:
We did not provide Figure one in manuscript. If you are mentioned Additional files 2 (the infection rate of the hospital and the ICU during 2001-2004). The specifications of graphics in tables are the band for the \textit{A. baumannii} isolates, dotted line with triangle for ICU infection rate, dotted line with square for hospital infection rate, solid line with round for integrin carrying rate.

These changes having been made, we hope that Journal of Negative Results in BioMedicine will accept and publish our manuscript.

Thank you for your consideration.
Sincerely yours,

Chang-Hua Chen, M.D., M.Sc., Ph.D.
On behalf of Chieh-Chen Huang, Ph.D.
Appendix 1
The study flow of *A. baumannii* isolates

All GNB isolates were routinely examined at Medical Laboratory Department of CCH during January 1st, 2001 to December 31st, 2004

Is phenotypic type of isolates using a Vitek-2 System (BioMerieux, Marcy l'Etoile, France) compatible with AB or not.

Exclude in this study

No

Yes

All enrolled AB isolates were stored in the Bacterial Bank of CCH

Is inoculated AB isolates from the Bacterial Bank survival?

Exclude in this study

No

Yes

AB isolates perform the molecular study