Author’s response to reviews

Title: An outbreak of multidrug-resistant Acinetobacter baumannii associated with bronchofiberscopy in an intensive care unit in Beijing, China

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Author’s response to reviews: see over
Dear Dr. Philippa Harris,

Thank you for giving us the opportunity to revise our manuscript entitled "An outbreak of multidrug-resistant Acinetobacter baumannii associated with bronchofiberscopy in an intensive care unit in Beijing, China", by Xia et al. According to the correspondence of the 23rd of August 2012, our manuscript should be revised for improvement according to the suggestions of the reviewers.

We have gone in details what the reviewers have suggested and revised the article to solve the major concerns of the reviewers. We performed additional statistical analysis which resulted in a new organization of the tables and description/discussion of the results. Detailed accounts of the changes adopted in the revised manuscript are given in the point-by-point account below.

We thank you and the reviewers for the careful handling of our manuscript and constructive comments that we believe has improved the scientific quality of this report.

**Reviewer 1: Anke Kohlenberg**

**C1 [Comment 1]:**

1. Methods / results / discussion section. It does not become clear, how the authors reached the main conclusion of the manuscript that the outbreak was related to bronchoscopy. In my opinion the main reason for this conclusion was not the case-control study, but the temporal association between bronchoscopy and culture positivity for MDR-Ab, the detection of MDR-
Ab on the bronchoscope and the evidence of faulty cleaning. Only few risk factors are presented as results of the case-control study in Table II and no multivariable analysis could be performed, therefore it is not possible to draw any firm conclusions from this study. In addition, evidence is missing in the manuscript such as a detailed list of results of the environmental cultures and the number of patients who received bronchoscopy during the outbreak period.

A1 [Answer1]:

In this manuscript, our main conclusion was an association of bronchofiberscopy with the outbreak. It was supported mainly by the case-control study (Table 2) and the temporal association between use of bronchofiberscopy and culture-positivity of MDR-Ab (Figure 2). Multivariable analysis was not applied due to the small sample size. Due to the methodological weakness of case-control study, we admitted that we could not draw any firm conclusions regarding to causality. However, case-control study is a classic epidemiological method for generating hypotheses or describing a new phenomena and has been used in many outbreak investigations of MDROs, such as an outbreak of MDR-Ab associated with pulsatile lavage wound treatment, et al (Maragakis LL, et al. JAMA. 2004, 292(24)).

Additionally, we have isolated four outbreak strains from the bronchofiberscope and disclosed several potential administrative and technical problems in bronchofiberscope reprocessing. As suggested, a detailed list of results of the environmental cultures and the number of patients who received bronchofiberscopy during the outbreak period have been included in the new revised manuscript.

C2: To enable the reader to understand the main conclusion of the manuscript, Please - Include the duration of the observation period of risk factors for the case-control study. Were data on risk factors in cases and controls collected for the complete ICU stay or for a limited interval like for example one week? Were all risk factors determined for the period before detection of MDR-Ab or also afterwards (for example was septic shock only a risk factor or could it also be a consequence of MDR-Ab?) How was ICU and hospital stay defined, stay until detection of MDR-Ab or stay until discharge?

A2: The duration of the observation period for different risk factors was different. The presence of primary diseases or medication history including septic shock, multiple organ failure, pulmonary diseases, renal diseases and surgical operation was determined at the time of ICU admission. For the invasive procedures and other bedside procedures, such as blood transfusion, mechanical ventilation, bedside diagnostic ultrasound, bedside chest X-ray, bronchofiberscopy, electrocardiogram, venipuncture, gastric lavage, urinary catheterization, hemodialysis, presence of a central line and antibiotic use, the duration of the observation period was was from ICU admission to outbreak strain detection for the cases, whereas it was from ICU admission to patient discharge for the controls. For the cases, ICU stay and hospital
stay were defined as the stay until outbreak strain detection, whereas for the controls they were defined as the stay until patient discharge. In brief, the duration of the observation period for the case control study was the total period in risk during the MDR-Ab epidemic period, except for the presence of primary diseases and medication history at admission. Therefore, the septic shock couldn’t be a consequence of MDR-Ab. The duration of the observation period of risk factors for the case-control study was included in the Methods Section of revised manuscript; the explanation of risk factors of the case-control study was added in the Discussion section.

C3: Only the results of about half of the risk factors on which data was collected according to the list in the methods section (page 7) are shown in Table II. Please include all risk factors in this table.

A3: Yes, all risk factors were included in Table 2.

C4: Include a list of the environmental cultures with their exact sites of isolation in the ward. According to Figure 3 the outbreak strain was also isolated from common ward areas such as the medication room and nurses station, why do the authors believe it was the bronchoscope and not transmission via the hands of health care workers? Please also include the microbiological methods used for environmental sampling in the methods section.

A4: Twenty-two outbreak strains have been isolated from the environmental samples, their exact sites of isolation were as follows: bronchofiberscopy (n=4), bed sheets (n=4), bedrails (n=4), invigilator or blood filtering machine’ keyboard (n=4), nurse’ notebook, desk and calculator (n=3), dispensing table, scrub sink and the medical treatment room (n=3). A list of the environmental cultures with their exact sites of isolation in the ward has been added in the figure legends and notes of the revised manuscript. Due to the low level of hand hygiene compliance of healthcare workers before our intervention, transmission via the hands of health care workers may be one of the reasons for this outbreak, however, no culture of samples from the hands and nasal cavity of healthcare workers was positive for MDR-Ab, so it was not supposed to be the main reason. In addition, microbiological methods used for environmental sampling has been included in the methods section.

C5: - Include the number of patients who received bronchoscopy during the outbreak period and did not show cultures positive for MDR-Ab. If bronchoscope cleaning was faulty also other bacteria (such as P. aeruginosa) could have been transmitted, did the authors find any evidence for transmission of other bacteria?

A5: There were four patients who received bronchofiberscope during the outbreak period but did not show culture positive for MDR-Ab, which has been shown in the Results section and Table 2. Definitely the reviewer is right, it could be possible to find out other bacteria to be
transmitted; however, we did not, at least in this study, find any evidence for transmission of other bacteria, such as *P. aeruginosa*.

**C6:** *Include more information about the index case. The first case (patient D) with the outbreak strain in Figure 2 also became positive for MDR-Ab after bronchoscopy. Was patient D the index case who “imported” the outbreak strain into the ICU (no association to bronchoscopy) or was there an undetected index case from whom MDR-Ab was transmitted via bronchoscopy to patient D? How did the outbreak strain enter the ICU and were there any cases in other wards of the hospital?*

**A6:**
Yes, patient D was the first case with the outbreak strain during the outbreak period, but patient D could not be considered as the index case who imported the outbreak strain into the ICU because the acquirement of outbreak strain of patient D was associated with bronchoscopy. According to the clinical information and environmental sampling, it was very hard to confirm that there was an undetected index case from whom MDR-Ab was transmitted via bronchoscope to patient D. Since two MDR-Abs had been indentified as other PFGE types before the emergence of outbreak strain, it could be only deduced that the emergence of the outbreak strains might be associated with the natural microevolution or mutation of bacteria. In addition, no any similar case was reported in other wards of the hospital, so the possibility of transmission from other wards was very less. This kind of information has been added in the discussions section.

**C7:** *State clearly in the beginning of the discussion section, how the main conclusion was reached and all evidence in support or against it. Please mention that different risk factors were significant in the univariate analysis and not only bronchoscopy and that the outbreak strain was not only isolated from the bronchoscope.*

**A7:** Thanks for the suggestions. We have reorganized the beginning of the discussion about how the main conclusion was reached. The evidences above have been mentioned in the discussion section.

**C8:** *Rephrase the abstract because it puts too much emphasis on the case-control study which did not contribute much to solve the outbreak.*

**A8:**
Yes, the abstract has been rephrased now, evidences other than case-control study, such as the temporal association between use of bronchoscopy and culture positivity of MDR-Ab and environmental culture results, have been added.

**C9:** *2. All sections. Please use the checklist of the ORION statement (for example available at*
http://jac.oxfordjournals.org/content/59/5/833.full) to make sure that the outbreak is reported according to current guidelines for transparent reporting. For example, this guideline asks for description of the objective of the investigation in the introduction (item 5), the number of patients admitted during the study period (item 7) and details of environmental sampling (item 10).

A9: Thanks for the suggestions. According to the checklist of the ORION statement, the following information has been included now, such as study design, the objective of the investigation, the start and finish dates of the study, the number of patients admitted during the study period and details of environmental sampling.

C10: Minor comments
3. Methods section, page 8. Please include the information when the interventions were implemented so that their effect on outbreak control could be judged.

A10: The interventions were implemented on 21st October 2009. This information had been described in the Results section of the old manuscript and now has been removed to the Methods section.

C11: 4. Results section, Figure 1. Table 1, Figure 2 and the text state that there were seven cases with the outbreak strain of MDR-Ab, but the epidemic curve shows only six cases.

A11: We are terrible sorry for this mistake. There were seven cases with the outbreak strain of MDR-Ab, the number of cases in October was five, but not four. The figure 1 has been corrected in the revised manuscript.

C12: Level of interest: An article whose findings are important to those with closely related research interests

A12: Thanks for the comment.

C13: Quality of written English: Needs some language corrections before being published

A13: It has been done.

C14: Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

A14: The statistical analysis in the revised manuscript has been reviewed by a qualified statistics professional, and we hope it would reach the criteria of BMC Infectious Diseases.
Reviewer: 2 (Giovanni Battista Orsi)

C15: Major Compulsory
Introduction
Page 4, lines 5-6: the authors first state that “five patients have healthcare-associated bloodstream infection caused by MDR-Ab” and thereafter refer to “six of seven clustered cases had received Bronchofiberscopy” please clarify the exact number of the cluster cases;

A15:
Sorry for these confused expression in the introduction section. It was just a report we received from the hospital that five patients have healthcare-associated bloodstream infection caused by MDR-Ab. Then we performed the investigation and found out that six of seven clustered cases had received bronchofiberscopy. To make it clear, we deleted the later statement and rewrote this sentence.

C16: Page 4, lines 7-8: please include reference “Young LS et al. Infect control Hosp Epidemiol 2007; 28 (11): 1247-1254” explaining the difference. You might include this comment in the discussion;

A16: The reference reported a large outbreak of infection due to clonal MDR-Ab transmission and found that widespread environmental contamination was perhaps promoted by aerosolization of organisms during pulsatile lavage debridement of infected wounds and during the management of respiratory secretions from colonized and infected patients, whereas our study demonstrated an association of bronchofiberscopy with the MDR-Ab outbreak. Both studies highlights the importance of appropriate infection control measures when invasive medical procedures are performed. The comments have been included in the discussion.

C17: Methods
Setting
Setting paragraph needs to be shortened;

A17: It has been shortened in the revised manuscript.

C18: Epidemiological investigation
Page 6, lines 3-5: the epidemic period and pre-epidemic periods are not clearly defined, in particular how long was the pre-epidemic period;

Page 6, lines 5-7 MDR A. baumannii definition: this is a very important point. The authors need to refer correctly to preceeding papers (Kallen AJ, Hidron AI, Patel J, Srinivasan A. Multidrug resistance among gram-negative pathogens that caused healthcare-associated infections reported to the National Healthcare Safety Network, 2006-2008. Infect Control
As resistance to #3 of the following: penicillins, cephalosporins, aminoglycosides, fluoroquinolones, carbapenems and sulbactam;

**A18:**

The epidemic period and pre-epidemic periods were clearly defined in the methods and results section now.

We did not list all of the antibiotic susceptibilities in the old manuscript. The multi-drug resistance of *A. baumannii* in our study was defined as the resistance to at least three kinds of following antibiotics, *penicillins, cephalosporins, aminoglycosides, fluoroquinolones, carbapenems*. It was quite similar with the preceding papers the reviewer suggested. We have checked the original data of susceptibility of all strains and charged the MDR strains according to suggestion of the reviewer.

**C19:** Case-Control Study

*This is a critical point of the study. The definition of infection or colonization as adopted by the authors is not correct. First of all urine is not a sterile site. Secondary not all catheters can be considered sterile sites.*


Patients with *A. baumannii* positive cultures, but no infection, would be considered colonized. *It must be considered that among ICU patients it may be very difficult to discriminate between infected and colonized. Therefore patients infected or colonized by *A. baumannii* could be defined as carriers, whereas all the other as non-carriers. This definition would be methodologically correct and would allow case-control analysis between carriers (infected and colonized) and non-carriers. See also paper by “Orsi G, Franchi C, Giordano A, Rocco M, Ferraro F, Mancini C, Venditti M. Multidrug-resistance Acinetobacter baumannii outbreak in an intensive care unit. J Chemother 2008; 20 (2): 219-224”.*

**A19:** Thank for the comments and suggestions. We totally agree with the reviewer. In the revised manuscript patients infected or colonized by *A. baumannii* were defined as carriers, whereas all the other as non-carriers.

**C20:** Results

*Page 10, lines 1-3: the authors need to define exactly the pre-pidemic period. In Figure 1 they show only year 2009 (pre-epidemic period January-September 2009 – seven months and three “cases”), whereas in the text they refer to two preceding years with eight “cases”. The reported data needs to be more consistent. Although already clear, the authors should add*
that the increase was statistically significant ($p<0.05$);

A20: Thank for the comment. The period between 1st January 2009 and 4th August 2009 (seven months) was treated as pre-epidemic period. In the text, two proceeding years with eight “cases” were just historical data. The statistically significant increase of MDR-Ab incidence during the epidemic period has been described in the results.

C21: Page 10, lines 3-7: the authors refer to 12 patients infected or colonized (Table I), but in Figure 1 only 11 patients are reported (months August to November 2009), please correct;
Page 10, lines 3-7: data on gender, age, infection/colonization status, chronology should be included in Table I and deleted from the text;
Page 10, line 7: Figure 2 see comments on Figures;
Page 10, lines 13-16: all data on patients underlying conditions should be included in Table I and deleted from the text;
Page 10, lines 16-17: authors refer to seven patients harbouring outbreak strain A, therefore this comment should be postponed after REP-PCR analysis description (page 11);
Page 10, line 23 and page 11 lines 1-2: all data on culture sites is included in Table I and should be deleted from the text;
Page 11 lines 2-3: Data on strains antimicrobial pattern is very important. Did all strains show the same pattern or were there differences?

A21: Page 10, lines 3-7: The 12 patients carrying the MDR-Ab in Table 1 was right and one case was left in October in old Figure 1. We are so sorry for the mistake. We have corrected the Figure 1 in the revised manuscript.
Page 10, lines 3-7 and lines 13-16: Initially we presented the data on gender, age, infection/colonization status, chronology in Table 1 for the preparation of manuscript; however, we were requested by the journal to revise Table 1 to ensure that individual patients can not be identified and more detailed information about underlying disease and infected/colonized, gender, age, etc, could only be generally, but not individually, described in the corresponding text.
Page 10, line 23 and page 11 lines 1-2: All data on culture sites was deleted from the text.
Page 11 lines 2-3: In the present study all of outbreak MDR-Ab strains showed the same antibiotic susceptibility pattern, at least, to the listed drugs in the study, no significant differences were found.

C22: Discussion
Page 14, lines 12-14: “85% MDR-Ab-positive isolates from the environment were outbreak strain”, this is a very important point that needs to be underlined, because it includes several implications. Authors should include here the following reference "Orsi G, Franchi C, Giordano A, Rocco M, Ferraro F, Mancini C, Venditti M. Multidrug-resistance Acinetobacter

**A22:** Thank for the comments. The implications and reference were included.

**C23:** Discussion: as MDR-Ab-positive strains were isolated from two patients (2\textsuperscript{nd} November and 30th November) after intervention was implemented (21\textsuperscript{st} October) some comment on this should be added to the discussion;

**A23:**

First, we had to admit that although several interventions were implemented on 21\textsuperscript{st} October, the transmission of MDR-Ab could not be exclusively stopped in the ICU. On the other hand, the MDR-Ab-positive strains isolated after implementation of intervention were not outbreak strains, so the intervention was definitely effective on reducing the transmission of outbreak MDR-Ab strain. This comment has been added to the discussion.

**C24:** Table I

Data on gender, age, infection/colonization status, patients underlying conditions and isolation chronology should be included;

**A24:** Thank for the comments and please see A21 (Page 10, lines 3-7 and lines 13-16).

**C25:** Figure 1 Authors refer to 12 patients infect or colonized (Table I), but in Figure 1 only 11 patients are reported (months august to november 2009), please correct. The figure shows only year 2009 (pre-epidemic period january-september 2009 – seven months and three “cases”), whereas in the text authors refer to two preceeding years with eight “cases”.

**A25:**

The mistake of figure 1 has been corrected (see also A21: Page 10, lines 3-7). In the text, two preceding years with eight “cases” was the historical data, not the pre-epidemic period. To avoid the confusion, we have deleted the description of two preceding years with eight “cases”.

**C26:** Figure 2

This Figure should be deleted, as information is already included in Table I;

**A26:**

Figure 2 demonstrated clearly the temporal association between use of bronchoscopy and culture positivity of MDR-Ab, which was not included in Table 1. So we hope to get the understanding of the reviewers and keep it in the manuscript if possibly.

**C27:** Minor Essential Revisions
Abstract

Pag 2, line 2: “has been reported” please correct;

Methods

The authors state that the study “was approved by the Institutional Ethic Committees of the Academy of Military Medical Sciences and 309 hospital”, as the study was carried out in one hospital, why it was approved by other 308?

A27: Page 2, line 2: it has been corrected. Our institute belongs to the Academy of Military Medical Sciences, which is also called by the Center for Disease Control & Prevention of Chinese PLA. This investigation was implemented by the Academy of Military Medical Sciences and 309 hospital together, so it had to be approved by these two institutions.

C28: Epidemiological investigation
Page 6, line 1: please modify “Medical records including paper and electronic charts were reviewed.”
Page 6, lines 7-8: Samples from Healthcare personnel are not environmental;

A28: Yes, the text has been corrected according to the review’s advices.

C29: Results
Page 10, lines 17-18: authors refer to interval between ICU admission and MDR-Ab identification (6.3±3.8 days) and after declare (data not shown). Why as data are shown?
Page 10, lines 18-21: Rewrite after considering modifications to Table I;
Page 10, lines 23: Rewrite after considering modifications to Table I;
Page 11 lines 6-12: Please consider if this period may be more adequate for the discussion;
Page 11 lines 13-15: Please modify as follows “Twenty-six out of 78 (33.3%) environmental strains were MDR-Ab-positive and 22 of them (84.6%) were identical to the outbreak strain A. Four of these 22 strains were recovered from the biopsy forceps and the bronchofiberscope tip that was used to treat one case patient, and from the bronchofiberscope surface after reprocessing in the ICU”;
Page 12 line 4: Please correct “… patients who received bronchofiberscopy were …”;

A29:
Page 10, lines 17-18: “(data not shown)” were deleted.
Page 10, lines 18-21 and lines 23: No modification to Table 1 has been done.
Page 11 lines 6-12: This period has been removed to the discussion section.
Page 11 lines 13-15 and Page 12 line 4: These sentences have been modified or corrected according to the reviewer’s advice.

C30: Case-Control study
Page 12 lines 1-3: Cases and controls selection was described in the methods, therefore
please delete the period “Seven cases ... outbreak strain”;
Page 12 lines 7-8: Please delete because redundant (Table II) “Six ... bronchofiberscopy”;

A30:
The sentences of Page 12 lines 1-3 and lines 7-8 have been deleted as suggested.

C31: Discussion
Page 15, line 3: bronchofiberscopy, please correct;
Page 15, line 10 to page 16 last line: this part of the discussion may be a bit shortened;
Table II
P values should be written in a column after Odds Ratios;
Figure 3
No change is needed;
Figure 4
No change is needed;

A31:
Page 15, line 3: It has been corrected.
Page 15, line 10 to page 16 last line: The part of the discussion has been shortened now.
Table 2: P values were written in a column after Odds Ratios.

C32:
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.

A32: Thanks for the positive comments and the revised manuscript has been further polished by English-writing service company.

We hope that our revised manuscript is now acceptable for publication in the *BMC Infectious Diseases*, and eagerly await your response.

Sincerely yours,

Li Han