Author's response to reviews

Title: Effect of metallo-beta-lactamase production and multidrug-resistance on clinical outcomes in patients with Pseudomonas aeruginosa bloodstream infection: a retrospective cohort study

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Author's response to reviews: see over
Dear Mr. Nazareno,

The authors of the manuscript “Effect of metallo-ß-lactamase production and multidrug-resistance on clinical outcomes in patients with *Pseudomonas aeruginosa* bloodstream infection: a retrospective cohort study” would hereby like to thank the reviewers for the valuable recommendations and criticisms. We took every point into serious consideration in order to improve our manuscript and provided a point-by-point response. As part of this revision we removed table 6 and modified several other tables as requested by the reviewers. We hope that these modifications improve the understandability of the major results and conclusions presented in our study. Furthermore, we have provided the reader with the name of the ethics committee as requested by the editorial office. We would be grateful for your consideration of our revised manuscript for publication in BMC Infectious Diseases.

Yours sincerely,
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Response to reviewers’ comments

First Reviewer’s report

Reviewer: Barry Neish

Reviewer’s report:
This could be a very interesting piece of work. Unfortunately there are a number of major points outlined below that make interpretation of the data presented here difficult.

Major Compulsory Revisions

1. Study design and definitions paragraph 2-
The definition of multidrug-resistant Pseudomonas lacks an aminoglycoside. In the literature the most common definition consists of at least two beta lactams (usually a ureido-penicillin plus a cephalosporin and/or a carbapenem), a fluoroquinolone and an aminoglycoside. This definition is unusual because it lacks the aminoglycoside. Reference 18 used to define multidrug-resistance is in German, I am unable to comment on its appropriateness for this paper. See Falagas et al 2006, J.Med.Micro, 55(12):1619-1629 for a good review where the majority of definitions of multidrug resistance includes an aminoglycoside.

Response:
Our definition of multidrug-resistance is very close to the one suggested by the reviewer but does not include an aminoglycoside. We agree with the reviewers’ comment. While there are indeed several different definitions of multidrug resistance used in the literature, many but not all of them include an aminoglycoside. Although it is very desirable that only one universal definition would be used, there is a rationale behind our particular choice of definition. Reference 18 is a publication of the Robert-Koch-Institute and provides the definition of multidrug-resistance for Gram-negative rods as used in Germany. We apologize that it is only available in German. However, we are happy to provide a brief summary of this publication and its rationale. The definition was intended to represent a more clinically oriented view on multidrug resistance. For this purpose, only antimicrobial agents that have a bactericidal effect on *P. aeruginosa* and can be administered as effective monotherapy were included. Since aminoglycosides cannot be given as effective therapy on their own they are not a part of the definition. In Germany, all indications for infectious diseases control measures concerning multidrug-resistant Gram-negative rods are based on this definition. We added the following sentences in our patients and methods section to present the reader the appropriate explanation:

“For the definition of multidrug resistance we considered antimicrobial agents which have a bactericidal effect on *P. aeruginosa* and can be administered as effective monotherapy. Thus, aminoglycosides were not a part of the definition.” (page 6, paragraph 2)

Furthermore, to prevent confusion and to add a more familiar phrase we changed the term 34MRGN-PA to 3/4MDR-PA in the revised manuscript.
2. MBL detection and susceptibility profiles paragraph 2-

34 isolates were tested for MBL production, no statement is made regarding the reasoning for this number.

Response:
A total of 113 patients have been investigated. Thirty-four of the 113 isolates were tested due to the criteria mentioned in the patients and methods section (PCR assays and DNA sequencing) following the recommendations of our National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria (Bochum, Germany). We have modified the corresponding sentence because the importance of the EDTA combined disk test as a screening criterion was not included in our original manuscript. Also, we added the appropriate reference:

“P. aeruginosa isolates having a reduced meropenem zone size (defined as < 24 mm) and/or being positive in the EDTA combined disk test were further investigated for the presence of MBL genes [1].” (page 8, paragraph 1)

Of 113 isolates a number of 34 isolates met the criteria and were thus investigated for the presence of MBL genes.

For further clarification we have changed the following sentence in our result section:

“A number of 34 isolates met the criteria for a possible MBL production and were subsequently tested for the presence of MBL genes.” (page 10, paragraph 2)

3. Table 1-
Providing the zone sizes does not add anything to the interpretation, as a result this table is very confusing. Just providing number of antimicrobial resistant isolates with percentage in brackets would be enough.

According to EUCAST all Pseudomonas should at least intermediate to aztreonam, therefore using the authors definitions all Pseudomonas would automatically be reported as resistant to aztreonam prior to testing (intermediate reported as resistant).

Response:
We agree with the reviewer and have modified table 1 as asked and removed aztreonam since it would indeed not provide any additional information to the reader.

4. Table 2-
Please show number of patients with each parameter and the percentage (to 1 decimal place) in brackets, please be consistent with the presentation of data.

Response:
We have made the changes as requested by reviewer 1, rounding percentages up/down to the nearest whole number as asked by reviewer 2. These changes were applied to all tables.

5. The number of meropenem resistant isolates (28.32% =32 patients) does not match with the number of isolates tested for MBL=34 as stated in MBL detection and susceptibility profiles paragraph 2.
Response:

Thirty-four isolates were tested for MBL production while a number of 32 isolates were meropenem resistant. Two isolates were not meropenem resistant but positive in the EDTA combined disk test. Due to our criteria (see response to question 2) both isolates were further tested to exclude the possibility of a low level MBL production (such isolates might be \textit{in vitro} susceptible to meropenem). Both isolates turned out to be false positive in the EDTA screening test.

6. Study population characteristics paragraph 2 and Table 3- 
Is the 4MRGN-PA group necessary? It is a sub-group of 34MRGN-PA and does not show any extra significance or add additional discussion points. I recommend removal of this group and only present MBL-PA and 34MRGN-PA.

Response:

From a clinical point of view the 4MRGN are more important. Since we isolated two additional 4MRGN that did not produce a MBL we thought an additional analysis useful. However, we agree with the reviewer. Since no additional information can be gained with its inclusion we decided to remove the separate 4MRGN group from the tables to make the manuscript clearer and its major questions more discernible for the reader.

7. Table 4-
This is an overly large and confusing table. It would help immensely to have the HR consistently presented to 2 decimal places only.
Response:

These changes were made as asked by the reviewer. However, we kept 4 decimal places in all continuous variables when a very small increase in the HR was relevant (age, SAPS II).

I don’t think the HR for discharge including the deceased patients is necessary. The same impact would be generated using only the HR for deaths and the discharge time of survivors.

Response:

Although we get the point of the reviewer that it would be preferable to have a simpler presentation of the data, we would suggest that the results of HR for discharge in table 4 are kept. While it may be a possible to only present the discharge times, a more complex analysis that additionally accounts for possible confounders would not be possible (Table 5). Hence, misleading conclusion might be drawn from such a presentation of the results [2].

The laboratory parameters are not discussed at all in the text therefore they should be removed. Very few of the comorbid conditions show any significance, therefore they can be removed from the table and a line inserted in the results text saying that not significance observed with these conditions. Together this reduces the size and complexity of the table.

Response:
We have deleted the laboratory parameters and the non-significant comorbid conditions from the table, but kept those included in the final models. We hope that the reduction in size and complexity of the table will help the reader to focus on the major results without losing additional information of interest.

8. Table 5-

It would help immensely to have the HR consistently presented to 2 decimal places only.

Response:

These changes were made as requested by the reviewer.

I don’t think the HR for discharge including the deceased patients is necessary. The same impact would be generated using only the HR for deaths and the discharge time of survivors.

Response:

See response to question 7.

9. Discussion paragraph 1-

The authors state that MBL-PA BSI results in higher mortality, while this is true, no mention is made that there is also higher mortality with 34MRGN-PA, therefore this seems to bias the interpretation of the data towards increased significance of MBL and away from appropriate antimicrobial prescribing which
should be the more central theme.

Response:
We have written the following paragraph in the discussion section of our manuscript. It is slightly modified compared with the original manuscript (e.g. 34MRGN-PA changed to 3/4MDR-PA):

“In the case of 3/4MDR-PA, the observed difference in mortality compared to the Non-3/4MDR-PA (63% vs 30%, \( P = 0.002 \), Table 3) appeared to be due to the lower chance of patients with 3/4MDR-PA BSI to have received appropriate definitive treatment. This emphasizes the importance of reevaluating the initial therapeutic regimes in a hospital setting with a relevant incidence of multidrug-resistant \textit{P. aeruginosa}. It also stresses the importance of susceptibility testing results from the associated microbiology laboratories and the need to effectively and appropriately adjust empirical treatments where necessary.” (page 13, paragraph 2)

As we understand it, this would address the point the reviewer made and should reduce concerns that the presentation of the data would drive the interpretation towards an unduly increased significance of MBL-producers. We had the same concerns as the reviewer, which is why we chose to investigate various resistance phenotypes in the first place. In our opinion, the manuscript clearly states that – regardless of MBL production – the administration of appropriate treatment and the severity of underlying conditions are the major determinants of mortality in our study cohort.

Minor Essential Revisions
10. Table 1-
Add e to the end of ceftazidim

11. Table 4 and 5 titles-
It should read "Hazard ratios", not "rations".

Response:
The typing errors are corrected in the revised version of the manuscript.

12. Clinical outcomes paragraph 2-
No P-values mentioned for HR in multivariate models

Response:
Since the results from several multivariate models are presented in this section, mentioning every single p-value would probably overwhelm the reader with details. Thus, we presented the HR range of the significant mortality predictors and the exposures of interest.

Second Reviewer's report

Reviewer: Gavin Barlow

Reviewer's report:
Major compulsory revisions

1. For the non-expert reader, it would be useful to define MRGN and the relevance of this and also what the difference is between 34MRGN and 4MRGN and why this is important.

Response:
Our definition of multidrug-resistant *P. aeruginosa* is based on a clinical view of resistance, examining the four bactericidal antibiotics that can be administered as effective monotherapy for the treatment of a *P. aeruginosa* infection. The definition is mentioned in the patients and methods section (The term 34MRGN-PA was changed to 3/4MDR-PA in the revised manuscript):

“Multidrug-resistant *P. aeruginosa* (3/4MDR-PA) was defined as resistant to at least three of the following antimicrobial agents: piperacillin-tazobactam; ceftazidime; meropenem; and ciprofloxacin [3].” (page 6, paragraph 2)

From a clinical point of view it is especially important whether an isolate is still susceptible to at least one of these four antibiotics. This is also relevant when it comes to infection control measures. For this reason we found an additional analysis with 4MRGN as an exposure variable interesting. However, the 4MRGN (defined in the original manuscript as resistant to all of the antibiotics mentioned above) is a subgroup of the 3/4MDR. Since the analysis did not reveal further information when using 4MRGN as an exposure variable, we decided to follow
the recommendation of reviewer 1 and chose not to present the results of the 4MRGN in the revised manuscript to put a heavier emphasis on the major results of our study.

2. Only 34 isolates were tested for MBL - why - is this due to the retrospective nature of the study? How can we be sure the isolates not tested were not MBL - this is clearly a potential weakness of the study - I may be missing something (being stupid!), but I think, particularly for the non-expert reader, this needs to be explained more clearly/openly in the discussion.

Response:

The criteria for testing for MBL production are mentioned in the patients and methods section (PCR assays and DNA sequencing) following the recommendations of our National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria (Bochum, Germany). We have modified the corresponding sentence because the importance of the EDTA combined disk test as screening criterion was not included in our original manuscript. Also, we added the appropriate reference:

“*P. aeruginosa* isolates having a reduced meropenem zone size (defined as < 24 mm) and/or being positive in the EDTA combined disk test were further investigated for the presence of MBL genes [1].” (page 8, paragraph 1)

Only 34 isolates met these criteria for further testing. For clarification we have changed the following sentences in our result section and hope this helps the non-expert reader:
“A number of 34 isolates met the criteria for a possible MBL production and were subsequently tested for the presence of MBL genes.” (page 10, paragraph 2)

In summary, the selection of strains that were tested for MBL production had nothing to do with the study design but with accepted selection criteria [1, 4]. Furthermore, in cases where the PCR for VIM and IMP genes turned out negative in our laboratory, strains were sent to our National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria (Bochum, Germany) for further testing. Hence, we are very confident that the classification of MBL/Non-MBL *P. aeruginosa* is correct and no issue in our study.

3. In the tables please round percentages up/down to the nearest whole number (e.g. 47.79% becomes 48%). Likewise, HR do not need to be presented to 4 decimal points (e.g. 0.5469 becomes 0.54).

Response:

The changes were performed as requested by the reviewer.

4. Table 5 is quite confusing. It presents multiple multivariate analyses focusing on 7 resistance phenotypes with the other significant predictor variables in each model presented under the table, which is tricky to read. I would redraw this table focusing on 2 or 3 key resistance phenotypes and presenting all the significant predictor variables in each of those models and the corresponding statistics - any other relevant findings from additional models can be commented on in the results text.
Response:

We agree that the table is tricky to read. We followed the reviewers’ recommendation and removed all phenotypes except MBL and 3/4MDR in order to keep the readers’ attention on the major focus of the study. Since we reduced the number of presented multivariate models from 21 to 6 we are able to present the specific data for each model below the table and can thus provide the specific details the reviewer was asking for. Although we have not changed the overall design of the table, it is now clearer while still providing the important information.

5. I'm not sure table 6 adds much for most readers - I would take out.

Response:

We have removed table 6 as suggested.

6. The English is quite good, but the manuscript would benefit from being edited again by someone with English as their first language or who is highly fluent.

Response:

The manuscript was edited again for language issues. We hope that the current version of our manuscript is deemed appropriate for the journals readership.
References


3. Definition of multidrug-resistance to antimicrobial agents in Gram-negative rods with regards to preventative infection control measures
