Author’s response to reviews

Title: High prevalence of multidrug-resistant gram-negative organisms in HIV positive men

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Author’s response to reviews:

Reviewer 1

1. Study objectives should be included in the abstract

We changed the background section of the abstract into “Background: Routes of transmission of multidrug-resistant gram-negative organisms (MDRGN) are not completely understood. Since sexual transmission of MDRGN might represent a potential mode which has not been noticed so far, this study was started to evaluate if this route might additionally contribute to the spread of MDRGN” – done as suggested.

2. Include more details of the study design, criteria for selection for both cases and controls

3. Current study design is too scanty. More details of study design, inclusion/exclusion criteria for both cases and controls necessary.

We inserted a paragraph in the methods section to describe the design and criteria of inclusion and matching more detailed. Please find: “Identification of cases (HP) and controls (HN) was performed as follows. We first identified individuals serologically tested positive for HIV admitted to the department for Internal Medicine II/Infectious Diseases at UHF being screened for MDRGN between the observation period. We then identified controls tested serologically
negative for HIV admitted to any department at UHF and being screened for MDRGN between the observation period as well.” – done as suggested.

4. Why did you use only "age" as selection criteria? What about other parameters such as co-morbidities, antibiotics taken prior to hospital visit etc?

Due to possible restrictions concerning statistical findings given by introduction of different selection criteria, we decided not to use more than “age” as a selection criterion. Concerning the aspect of co-morbidities, please find our new table 1 in the manuscript. Please also find our statement concerning this aspect in L192-201: “Clearly, our study has some limitations. As data regarding the history of previous hospital stays nor antibiotic pre–treatment for male HP and male HN were not available, it cannot be ruled out, that male HP in the UHF–setting might have had a stronger history of antibiotic pre–treatment than male HN. Since all patients enrolled in this investigation fulfilled the conditions to be screened for MDRGN and this is usually limited to patients admitted to ICU or IMC at UHF, this might indicate that both male HP and male HN had a recent history of antibiotic treatment. However, HIV patients in particular might have had history of antibiotic pressure due to their HIV infection (e.g. administration of cotrimoxazole to treat Pneumocystis jirovecii Pneumonia) and might therefore have a higher risk for admittance to hospitals and health care facilities, where nosocomial transmissions can occur [23]. These aspects might therefore have introduced a source of biases in the selection of patients.” – done as suggested.

Since we absolutely agree with the reviewer’s comment we feel that a future investigation should also address aspects like co-morbidities or evaluation of the patients’ history of antibiotic treatment.

5. Why did you choose to use only data from Jan 2015-March 2016?

Thank you for this question. To keep the manuscript topically, we decided to present most current data and therefore restricted to the period from Jan 2015 – March 2016.

6. How did you determine sample size?

In our study, the basic population is identical with the statistical population we determined for statistical calculation. Therefore, all patients tested positive for HIV and screened for MDRGN are represented by the 109 individuals tested positive for HIV and screened for MDRGN. The number of patients tested negative for HIV and screened for MDRGN is higher, but we think inverse matching was not suitable for our investigation due to one formal regularity. The hygiene
plan valid for University Hospital Frankfurt is continual adjustment, particularly concerning for the patient groups who need to be screened for MDRGN (recently adjusted for refugee patients, for example). We therefore can only refer to patient groups who were admitted to UHF for identical conditions regulated by hygiene plan. This also contributed to the limitation on Jan 2015 to March 2016 (please see above, comment on 5.)

Sample size therefore resulted from two aspects, in particular: the basic population who was tested for HIV and screened for MDRGN, the hygiene plan valid for UHF. Furthermore, in Germany it is forbidden by law to investigate HIV status of any individual without given indication. We therefore were not able to expand the number of individuals for this study by testing them newly for HIV.

7. Give more details of the parameters that were analysed in each study group and the statistical test used

We improved the section Statistical analysis: “Chi squared test was performed for statistical analysis for association between two categorical variables (in this study: association between HIV status and MDRGN status). Exact confidence intervals (CI) for frequencies were calculated based on binomial distribution. P values (2-tailed) \( \leq 0.05 \) were considered as statistically significant.” – done as suggested.

8. A separate table on the clinical characteristics of the study population is necessary. Should be separated from the table on the prevalence of MDRGN.

Thank you for this suggestion. We added a table illustrating the departments HIV positive and HIV negative study individuals were admitted to. Please find the new table (new table 1) in the results section – done as suggested.

9. Titles of tables and figures need to be self explicit. Avoid the use of abbreviations on titles, include a legend at the bottom (not top) of each table or figure.

Please find our changes red-marked in the table 2 and figure 1. – done as suggested.

10. It's important to show the results (table) of antibody susceptibility testing and the distribution of bla genes coding for carbapenemases in both HP and HN.
This is a highly important aspect and we thank the referee for this suggestion. However, we did not observe any carbapenem resistant strains and carbapenemases, respectively, neither in HP nor in HN. We inserted a new table row in table 2 (red-marked) to clarify this item – done as suggested.

11. Check that the format of your tables is in line with the guidelines of the journal.
Corrected.

12. Since you excluded females from the study (lines 206-220), your discussion should focus more on males and the implications for HIV management/control. A recommendation could be made for similar studies in females.

We inserted a paragraph addressing a future setting focusing on females in the conclusions: “For a future setting, we feel that the evaluation should not only be expanded on patients’ co-morbidities and prior history of antibiotic administration but also should address a female cohort.” – done as suggested.

13. Why not discuss antibody susceptibility testing and bla genes?

We inserted in the conclusion: “For a future setting, we feel that the evaluation should not only be expanded on patients’ co-morbidities and prior history of antibiotic administration but also should address the distribution of bla genes found in HIV positive and HIV negative cohorts. We suggest that this will widen the knowledge of pathogenicity factors of MDRGN strains which might foster sexual transmission. Furthermore, we think that it is indispensable to additionally focus on a female cohort to clarify if results are comparable to the male cohort.” – done as suggested.

14. I think it’s important to acknowledge the patients whose samples/data you used for this study

We inserted a paragraph in the acknowledgement. – done as suggested.

15. Delete spaces between lines 158 &159. Position of table is already shown in line 157 (Table 1). Same comment applies to lines 152-153.

Corrected. Thanks.
Reviewer 2

1. The title needs modification concerning the abstract.

We truly apologize but we do not understand this comment.

2. Context and purpose of the study were not explained.

We apologize again but we strongly disagree. Context and purpose of the study is explained clearly in the abstract with “Background: Routes of transmission of multidrug-resistant gram-negative organisms (MDRGN) are not completely understood. Since sexual transmission of MDRGN might represent a mode which has not been noticed so far, this study was started to evaluate if this route might additionally contribute to the spread of MDRGN.” and was also mentioned in the Background section: “It was previously reported in several studies that the risk to acquire sexually transmitted diseases (STD) such as gonorrhea or syphilis, or infection with Human Immunodeficiency Virus (HIV) is higher in cohorts of men who have sex with men (MSM) than for the general population [11] and this has been demonstrated for Germany [12, 13]. Besides those “classical” STD, reports about the sexual transmission of Human Herpes Virus Type 8 (normally transmitted via saliva) [14], E. coli O117:H7 (normally feco-oral transmission route) [15] and Shigella spp. [16] demonstrate that agents can be sexually transmitted although their primary way of transmission may be different. In light of the persistent global spread of MDRGN, the role of sexual transmission has to be clarified. We present the first study which addresses this issue.”

3. The second sentence (line 41 of page 3) says "Sexual transmission of MDRGN might represent a mode which has not been noticed so far." It is not clear what the author want to say. If we take as it is, this study is not basic research that exhibiting us a unique route of transmission.

Again, we simply do not understand this comment. However, we tried to anticipate the meaning of this comment and changed this sentence into “Sexual transmission of MDRGN might represent a potential mode which has not been noticed so far”.

4. Multidrug-resistant microorganisms in addition to multidrug-resistant gram-negative organisms (MDRGN) have the same route of transmission with drug sensitive organisms within the same species. Nothing is unique for this organism except some virulent factors that help the for drug resistance.

Furthermore, concerning this aspect we discussed “As our findings indicate a pre-dominance of E. coli ESBL/FQ (Table 1), it might be interesting to investigate the E. coli-isolates isolated from male HP for expression of pathogenicity factors, e.g. type 1 fimbriae (fimH), pili associated with pyelonephritis (pap), S and F1C fimbriae (sfa and foc), afimbrial adhesins (afa), cytotoxic necrotizing factor (cnf), hemolysin (hly) or aerobactin (aer) using molecular techniques [29] and subsequently compare these findings with isolates isolated from HN. We therefore feel it is warranted to further characterize MDRGN isolates isolated from HP and HN by molecularbiological methods, preferentially by whole genome sequencing (WGS).”

5. Statistical test used was not stated.

We wonder about this comment as the test has clearly been given. However, to improve the section statistical analysis, we changed the sentence into: “Chi squared test was performed for statistical analysis for association between two categorical variables (in this study: association between HIV status and MDRGN status). Exact confidence intervals (CI) for frequencies were calculated based on binomial distribution. P values (2-tailed) ≤0.05 were considered as statistically significant.”

6. It didn't give us enough information how the study was conducted except study units.

We earnestly apologize but again we simply do not understand this comment.

7. The result didn't show us about sexual rout of transmission which is not consistent with the other part of the abstract. Rectal swab solely do not show us whether the organisms were from genitalia or gastro intestinal route (GIT).

Again, we do not understand this comment. However we try to anticipate: since stated in the methods section, the hygiene plan valid for University Hospital Frankfurt does not legalize the sampling of genital swabs for screening purpose (which is only legalized in case for pregnant
women under certain conditions). Due to the retrospective design we therefore can only assess rectal swabs.

8. The conclusion was not supported by the result and study design of the study. The results indicate the presence of high number of multidrug resistant Escherichia coli. But nothing is stated about sexual transmission. Furthermore E. coli is not known sexually transmitted pathogen (It is know faco-oral route transmission). The most probable source of these organisms is gastro intestinal source because they took rectal swab.

Again, we strongly disagree with this comment which is definitely not reflected by scientific literature. E. coli is not known as a sexually transmitted pathogen and would kindly like to remind on spread of the verocytotoxin-producing EHEC O117:H7 among men who have sex [see literature no. 15]. The sexual spread has been well documented and we would like to indicate the sexual transmission might have also occurred by both oral-genital or genital-rectal. Therefore, also E. coli is able to be transmitted by sexual intercourse.

Please find this discussed in our paragraph which says: “Several epidemiological events give an impression that sexual spread of organisms is possible, although their preferred way of transmission is reported to be non-sexual: Shigella sonnei, which is either none of the „classical“ STD-causing organisms, has been shown to spread among men who have sex with men (MSM) in Montréal, Canada, and Germany [24, 25] the verocytotoxin-producing EHEC O117:H7 has also been documented to spread among MSM in Great Britain [15] and, as well, no “classical” STD, meningococcal serogroup C diseases has newly been shown to spread among MSM [26]. This outlines that sexual transmission of Enterobacteriaceae is distinctly possible via translocation of intestinal bacteria. Sexual transmission of MDRGN should therefore not be dismissed in future comprehensive considerations on MDRGN transmission prevention.”

9. The context of the study was not stated

Not correct. Please see comment to 1.

10. Nothing is said about the gaps, the purpose and the aim of the study

We absolutely disagree and ask for editorial clarification. This comment is not reflected by our manuscript. Aims, background as well as limitations are clearly stated several times in our manuscript.

Concerning gaps, in our manuscript it is clearly said: “Clearly, our study has some limitations. As data regarding the history of previous hospital stays nor antibiotic pre–treatment for male HP
and male HN were not available, it cannot be ruled out, that male HP in the UHF–setting might have had a stronger history of antibiotic pre–treatment than male HN. Since all patients enrolled in this investigation fulfilled the conditions to be screened for MDRGN and this is usually limited to patients admitted to ICU or IMC at UHF, this might indicate that both male HP and male HN had a recent history of antibiotic treatment. However, HIV patients in particular might have had history of antibiotic pressure due to their HIV infection (e.g. administration of cotrimoxazole to treat Pneumocystis jirovecii Pneumonia) and might therefore have a higher risk for admittance to hospitals and health care facilities, where nosocomial transmissions can occur [23]. These aspects might therefore have introduced a source of biases in the selection of patients.”

Regarding purpose and aim, in our manuscript it is clearly said: “It was previously reported in several studies that the risk to acquire sexually transmitted diseases (STD) such as gonorrhea or syphilis, or infection with Human Immunodeficiency Virus (HIV) is higher in cohorts of men who have sex with men (MSM) than for the general population [11] and this has been demonstrated for Germany [12, 13]. Besides those “classical” STD, reports about the sexual transmission of Human Herpes Virus Type 8 (normally transmitted via saliva) [14], E. coli O117:H7 (normally feco-oral transmission route) [15] and Shigella spp. [16] demonstrate that agents can be sexually transmitted although their primary way of transmission may be different. In light of the persistent global spread of MDRGN, the role of sexual transmission has to be clarified. [Please find the following sentence changed into:] We present the first study which addresses this possible transmissibility of MDRGN by sexual intercourse.”

11. Nothing is said about design of the study and the setting.

Not correct. In our methods section we clearly describe the design and the setting of our study. Please find this paragraph in the methods section: “We retrospectively identified and included adult individuals having had both a rectal screening for MDRGN and were tested for HIV between January 2015 and March 2016. Patients having had a screening for MDRGN or HIV testing were excluded from this study. Patients tested positive for HIV and having had a rectal screening for MDRGN qualified for the case cohort, hereinafter referred to as HP. Vice versa, patients tested negative for HIV and having had a rectal screening for MDRGN qualified for the control cohort, hereinafter referred to as HN. Cases and controls were matched on +/- 2 years. By this approach, n=109 male HP and male HN each qualified for this study. In this assessment, only one female HP was found in the total group of HP (n=110, with n=109 males and n=1 female). Regarding to the over-representation of men, we decided to exclude women from this initial study, both from HP and HN. For note, this low proportion of HIV positive women within the study cohort reflects the demographic-epidemiological situation on new HIV infections in Germany [19].
12. The type of analysis used was not well stated.

See above, again not correct.

13. One subtitle under methods was "Definition of multidrug resistant gram-negative organisms (MDRGN)" the definition of MDRGN organism should be under background of the study.

Thank you for this comment, we moved this into the background section.

14. There was subtitle which say "Screening for MDRGN and testing for HIV" but nothing is explained about screening for MDRGN and testing for HIV methods

Not correct. Methods for screening were extensively stated in our manuscript from Lx to Ly:

15. The methodology part was generally not well explained. The study was retrospective as indicated but the methodology was not indicated in such away.

We strongly disagree. However, to cope with the referee’s comments we modified the paragraphs on MDRGN and HIV testing and now clearly stated that all results were retrospectively investigated.

16. The way authors present the result was not suitable for publication.

and

17. Table used including its title needs major revision

Again, we disagree. Both authors (first and senior author) published 133 original and peer reviewed articles. The comment, that we present our results not suitable for publication, is destructive. Nevertheless, we tried to improve table 2. Please see the changes red-marked in the table 2 and figure 1. Unfortunately, comment 17 is not helpful for us.

18. Figure one is not needed because the authors already indicated the magnitude of MDRGN by text. The authors should use illustration for a thing that is difficult to summarize by text.
We do not agree. Figure 1 is needed to illustrate one major finding of our study and is presented in the majority of epidemiological research worldwide.

19. I was not convinced that the literature quoted and compared to this study were a fair comparison

and

20. Please see comment given under conclusion section of the abstract above.

This comment itself is not fair. Since we present the first investigation in this field, there is a lack of literature. We however found literature, which was well suitable to approach our study’s subject – and this completely was a “fair” selection.