Author’s response to reviews

Title: An epidemiological and molecular study regarding the spread of vancomycin-resistant Enterococcus faecium in a teaching hospital in Bogotá, Colombia 2016

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REPLY TO REVIEWERS’ COMMENTS:

REVIEWER #1:

This manuscript describes an outbreak of vancomycin-resistant Enterococcus faecium (VRE) that occurred at the Méderi Teaching Hospital in Bogotá. The authors performed molecular analyses on strains of E. faecium to explain the transmissions.

It's an interesting study, but I have some major comments.

1. First of all, the manuscript is too long and should be shortened.

Reply: Thank you for your comment. The revised manuscript has now been shortened in the Background section, however, when clarifications suggested by the reviewers were included, it ended up being almost as long.
2. Second, the manuscript is not very clear. It is unclear whether the authors described the spread of VRE strains in their hospital or whether they describe an outbreak of healthcare-associated infection due to E. faecium, as strains included in molecular typing were present in infection and colonization and not all isolates are taking into account in the outbreak.

Reply: We aimed at describing the vancomycin-resistant Enterococcus faecium pattern within our hospital, including a pre-outbreak period for characterising the baseline, the outbreak period itself and a post-outbreak period to describe how it was controlled. Outbreak confirmation was based on guidelines provided by the Bogotá District Health Secretariat, as well as the hospital’s Epidemiology Department. Both colonised and infected patients were included in the outbreak report, mainly because four isolates came from patients considered as infected by the treating physician and upon reanalysis by the infectious diseases’ department, ended up being classified as colonised (Table 2A). Not all the isolates from the outbreak were analysed by molecular techniques, since samples from the first three patients were missing. To clarify this issue, a detailed description has now been included under the “Clinical setting and data collection” subheading (pages 6-7, lines 123-133), as well as the “Transmission analysis” subheading (page 9, lines 178-188).

3. In addition, it is unclear when the outbreak began and for how long. On Figure 1, a very high increase in isolated VRE strains can be observed in February 2016, but the authors wrote that the VRE outbreak began in May 2016 with the increase in VRE-related HAIs.

Reply: Figure 1 shows a peak in February for just isolates, but not HAIs. The increase in HAIs was identified in May 2016 following the guidelines provided by the Bogotá District Health Secretariat; this issue has now been clarified in the “Clinical setting and data collection” section (pages 6-7, lines 123-133).

4. Introduction. Too long. For example, fields L18 to 23 P4 could be deleted.

Reply: The introduction has been shortened.

5. L45 P4: "antibiotic misuse": not all antibiotics, probably avoparcin.

Reply: The manuscript has been corrected accordingly: “Vancomycin-resistant E. faecium (VREfm) was first isolated in Europe and the USA by the end of the 1980s and seems to have appeared as a consequence of avoparcin (growth promoter) misuse in livestock and antibiotic overuse in hospital settings.” (Page 4, lines 75-77).

6. L52 P4:"Efcm represented 3.7% of HAIs": where?
Reply: The paragraph has now been modified to read, “Efm accounted for 3.7% of HAIs according to the summary of data reported to CDC’s national automated biosurveillance system regarding HAI-associated antimicrobial-resistant pathogens from 2011 to 2014 in the USA, a tendency which has also been described in Colombia.” (Page 4, lines 79-82).

7. L12-17 P5: prevalence instead of probability?

Reply: Regarding this specific case, it means the probability of acquisition based on the acquisition rate. The phrase has been changed to read, “VRE acquisition percentage ranged from 1.9% to 37% amongst hospitalised patients (depending on length of exposure and proximity) and from 0.4% to 11.8% in the community”. (Page 5, lines 88-90).

8. Methods. It is difficult to understand why strain clonality was not used as the first criterion to confirm transmission. It will be clearer if authors could use CDC criteria to describe the outbreak as "confirmed", "probable", "possible" transmission (possible has fewer criteria than probable to suspect transmission), in that order and taking into account both patient location and strain clonality.

Reply: Thank you for addressing this point. Whereas the CDC uses these criteria to describe case definitions and source of infection, we used the TPS algorithm in our study mainly to identify transmission routes rather than sources of infection. This transmission analysis was performed by adapting an algorithm previously described for a long-term outbreak of Pseudomonas aeruginosa in Germany, leading to successful identification of relevant in-hospital transmission routes (direct patient contact, healthcare personnel transmission, environmental contamination). This paragraph has now been rewritten in the “Transmission analysis” section to clarify this issue (page 9, lines 178-188).

9. Result. L26-36 P10 "Hypothesis....program" is more a discussion than a result.

Reply: Thank you for pointing this out. The comment has been deleted.

10. Figure 1: Did the authors report several or only one isolated strain per patient?

Reply: We reported several strains per patient in our previous version. We have adapted Figure 1 to include just one isolate per patient.

11. Why is the figure 1 stopped in June, since the manuscript concerns the period from May to September?

Reply: All data up to September has now been included in updated Figure 1.
12. Figure 4: Same remark as for the methods part. Please use "confirmed, probable and possible" CDC criteria.

Reply: This issue has already been clarified in query 8. Figure 4 is now Figure 5, and a thorough description has now been included on pages 13-14, lines 272-300.

13. Table 2 Only one MIC dilution difference may be a technical artefact. I think Table 2 and the results concerning the prediction of the van gene with MIC of Vancomycin and teicoplanin should be deleted. Simply indicate that vanA genes are associated with MIC of Vancomycin from 64 to >512 and to teicoplanin from 8 to < 256 and describe the MIC of negative VanA genes.

Reply: Thank you for your comment. Table 2 has been deleted and information regarding this issue clarified in the “VanA” detection section. Table 3 is now Table 2 in the revised manuscript (Page 12, lines 250-256).

14. The MIC of teicoplanin could be added to Table 3A. Table 3 the name of the month would be clearer for the reader than the colours.

Reply: Thanks for your comment. The table has now been updated accordingly (now Table 2).

15. Discussion: The main limitation of this study is that we did not know whether systematic rectal screening for VRE was performed. If not, it is difficult to describe the outbreak because digestive colonization by VRE is much more frequent than infection. The unknown carriers are the hidden reservoir for the transmission and dissemination of VRE. These limits should be included in the discussion section.

Reply: Systematic rectal screening is not carried out in the hospital due to limited budget. Thanks for pointing this out, we have now referred to such limitation in the updated discussion (page 15, lines 330-332).

REVIEWER #2:

In this manuscript the authors describe the investigation of a nosocomial outbreak caused by VRE in a teaching hospital. The methods were well performed, and the data clearly presented. I have some points that need clarification:

1. Title: correct to 'in a teaching hospital'.
Reply: Thank you for your comment. The title is now, “An epidemiological and molecular study regarding the spread of vancomycin-resistant Enterococcus faecium in a teaching hospital in Bogotá, Colombia 2016”

2. Page 3, line 12: correct 'use of combined'.

Reply: This has now been corrected as follows, “This study supports the use of combined molecular and epidemiological strategies in an approach to controlling infectious diseases.” (Pages 2-3, lines 58-59).

3. Page 4, line 18: correct to 'beta-lactamase-encoding'.

Reply: This paragraph has been deleted, according to reviewer #1’s suggestion.

4. Page 6, line 48: delete 'has'.

Reply: This has now been corrected as follows, “however, it had a low pathogenic rate until 2016.” (Page 6, lines 122-123).

5. Page 8, lines 4-12: did the authors searched for the presence of vanB gene.

Reply: We did not assess vanB gene presence. We only aimed at identifying vanA which is the gene most frequently related to a high level of resistance to vancomycin.

6. Figure 1: In figure 1, the authors must show also the post-outbreak period. Since during the post-outbreak period were isolated 17 VREfm, the outbreak doesn't seem to have ended.

Reply: Thank you for your comment. Figure 1 has been modified and now contains the post-outbreak period.

7. Page 12, lines 25-30: What was the resistance mechanism for the three isolates that were negative for vanA gene?

Reply: Other van genes (vanB, vanC, vanD, vanE, vanG, vanL, vanM and vanN) were not evaluated since we did not have control strains carrying the different van genes to standardise the PCR. We therefore could not really know/identify the genetic resistance mechanisms for these isolates.
8. Page 13, lines 33-37: The index case must be the patient that was identified first.

Reply: The paragraph has now been modified to read as follows, “P1 was the first patient identified during the outbreak (index case), but P2 was the estimated case who infected the greatest amount of patients according to patient flow tracking.” (Page 13, lines 280-282).

9. Page 13, lines 12-58: Since there is lack of samples from the first three patients, and there is disagreement between the epidemiological data and the experimental findings, the transmission analysis doesn't seem accurate. Thus, it would better transmission analysis to be deleted from the revised manuscript.

Reply: We understand that the lack of samples from/for the first three patients could be a limitation. However, analysing their TPS epidemiological criteria enabled us to confirm that they had been in contact with the following cases; in fact, P2 seems to have been the one having the highest amount of contact with the remaining patients, considering the length of her hospital stay (Fig 5a). Regarding TPS analysis, we were able to trace both the transmission route and the clonal pattern, thereby enabling us to establish the proper control measures which finally led to controlling the outbreak; we thus respectfully disagree with your suggestion of removing it. Nevertheless, this segment of the manuscript has been rewritten for clarity and Figure 5 (previously Figure 4) has been modified accordingly (Pages 13-14, lines 272-300).

10. Page 14, lines 41-44: No data were rep-PCR analysis. The authors must delete this sentence, or they must present the findings of the rep-PCR analysis.

Reply: Thank you; this is also an important point. The sentence has now been deleted.

11. In Figure 3, it would be helpful, the authors to include the data for March and April.

Reply: Although we agree with the reviewer, unfortunately, it was only possible to recover VREfm strains from May onwards, so we were not able to include previous genetic relatedness data in this figure (this figure is now Figure 4).