Author's response to reviews

Title: Outbreak of Clostridium difficile PCR ribotype 027 - the recent experience of a regional hospital.

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Version: 3  
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Author's response to reviews: see over
We appreciated the careful review and greatly acknowledge the comments provided by the editor and reviewers. We made the recommended changes and responded in detail to the questions raised.

The reviewers suggested some changes in order to improve the manuscript. The changes made are detailed in the following items and highlighted in yellow in the manuscript.

R1:
Reviewer’s report

Title: Outbreak of Clostridium difficile PCR ribotype 027 - the recent experience of a regional hospital.
Version: 2 Date: 8 January 2014
Reviewer: Marya D Zilberberg

Reviewer's report:

Thank you for the opportunity to review this paper.
General impression: The paper is interesting, but unfocused. The authors describe the characteristics, interventions and outcomes of 53 patients with CDI during a period of time that included a multi-month outbreak in 2012 at a single center in Portugal. They further describe a subset of 22 patients who underwent further investigation, a portion of whom tested positive for ribotype 027. Since the study is a research article, it is unclear to me what the research question is. The bulk of the paper is spent on a descriptive analysis of all 53 cases detected. There are comparisons, but none is adjusted for confounders. Furthermore, there are several ways in which the authors subdivide the cohort, and this is confusing.

Is the primary interest death vs. no death? Or is it the 027 ribotype vs. other? The countefactuals seem to shift. The conclusions authors draw do not seem valid in the absence of adjustments for confounding. Yet their sample size if too small to permit meaningful adjustments. Care needs to be taken on what is reported and the strength of the conclusions drawn. The Discussion is also a bit thin, as there are plenty of data to compare this study to. Figures 2A ad 2B are confusing – don’t you want to report the severity itself, not just how many had
markers of severity collected or what they were? Seems that this would be more useful. The paper needs to be much more focused before it can be reviewed cogently. I suggest that you report it either as an outbreak investigation and how it was addressed or as a descriptive study of those infected with the 027 ribotype.

Reply: We appreciated the general comments of the reviewer. We would like to clarify some points:

- This study is a CDI outbreak report, which we have emphasized more in the abstract and in the background of the revised manuscript.
- This study is the first report of an outbreak due to the hypervirulent *C. difficile* 027 strain in Portugal, with high mortality rate. Indeed, to date there is no report of this strain circulating in Portugal. Therefore, we consider of utmost importance to report this outbreak, in the context of the *C. difficile* infection in Europe.
- Regarding the fact that only 22 patients were further investigated, it has to be taking into account that, to date, in Portugal, there is no guideline to send strains for the national reference centre to ribotype determination, therefore, the 22 samples sent for ribotyping were not randomly chosen, they were sent when the hospital administration gave its permission. The fact that only a subset of cases were studied for the strain type, has direct implications in the way we subdivide the cohort, either we consider the all 53 cases, or the 22 cases, depending on what we are analysing.
- Concerning the primary interest of the study, death vs no death, or 027 ribotype vs other, according to our view, both are the interest of the study, since, as previous mentioned, the fact that we have a subset of cases more fully studied implies different analyses with different aims. Of course we didn’t previously know what dominant strain type we would find. But what is interesting is that, in contrast to the decline of the 027 strain reported in other countries, the present outbreak occurring in 2012 in Portugal, was due to this epidemic strain, and not other, further suggesting that this strain is still a problem in our country. Moreover, the high 30-day all-cause mortality and a 027-CDI-related mortality rate reported in our study is higher than that reported in other 027-associated outbreaks, a pint that it was fully discussed, and associated not only to
the strain, but to problems related to patients, CDI diagnosis, clinical management of the cases and lack of awareness and knowledge of the institutional guidelines by the hospital staff.

- As the reviewer points, the sample size of our cohort was too small to allow for confounding adjustments’. Accordingly, we discussed the results on a basis of suggestions and avoiding drawing conclusions.

- Since many variables have contributed to the outbreak, we tried to discuss them all, making comparisons with other countries. A lot more could be discussed, however that could turn out to be deleterious for the paper, since too long discussions should be avoided. However, the comments of the reviewer conduct us to discuss further the implicated strain, which contrasts to the declining trend reported for the incidence of this strain across Europe. Nevertheless, according to other reviewers, we tried to shorten the discussion, avoiding redundant information.

- Regarding the comment on Figures 2A and 2B, we understand the concerning of the reviewer and we agree that in a normal situation, i.e., when the severity criteria were correctly evaluated and registered in the medical records, the data could be compiled in a single Figure, reporting the severity criteria only. However, in the case of the present outbreak, one of the factors contributing to worst outcome was likely the lack of an initial severity evaluation for a considerable number of patients, a fact that we want to underline, and is fully discussed. Therefore, and even that the figure may be more difficult to read at a first glance, we would like to keep it as it is, since we really want to highlight the reality of situation.

**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.
Declaration of competing interests:
I have received research and consulting funding from Optimer and Cubist
Minor Essential Revisions
1. The paper has several typographical and some grammatical errors here and there.
   Reply: According to this comment, the revised manuscript was reviewed concerning grammar and clarity.

2. American and British spellings are interspersed. Use the one required for the journal
   Reply: According to this comment, the revised manuscript was carefully reviewed by a copy editor with English language experience. As a result, the revised version of the manuscript is largely improved regarding the English, as it can be easily checked.

Major compulsory revision
3. In Abstract: Lines "Outbreak control measures.... antibiotic stewardship"
   Actually as these form part of the methods employed, it should be put under Method section. Here again period at which control measures were taken is not clear.
   Reply: We agree with this comment. Accordingly we transferred this part of the text to the “Methods” section of the abstract, and add the information that the control measures were taken immediately after the outbreak was declared, in April 2012.
4. Under the section C. difficile culture and strain characteristics, it is not clear whether the work was prospectively done or was retrospective data. However on page 13 it is mentioned that the strain typing was started from 1st July 2012. This needs to be mentioned under the Method section.

Reply: We agree that mentioning under the method section the period for which strain typing started will clarify the readers that the typing was done prospectively.

5. On Page 8 under Result section again, the Lines "Antibiotics were fluoroquinolones mainly ciprofloxacin and penicillins..." is better written as "Antibiotics were fluoroquinolones (mainly ciprofloxacin) and penicillins..."

Reply: This correction was performed according to the reviewer suggestion.

6. The outbreak control measures should be mentioned under the Method section. Extraneous explanation should be deleted and an algorithm for the same would be more appreciable.

Reply: We agree with this comment. Accordingly, we transferred all this subsection to the materials and methods, and gave it a more comprehensive title, replacing “Outbreak control measures” by “Outbreak Control Measures”. We have also made an effort to reduce the description of the measures taken, in order to be more objective and avoiding redundant information. We have kept the format of text instead of presenting the measures as an algorithm, which we consider more appropriate to give particular details, and according to other similar papers, such as Muto et al, Clin Infect Dis, 2007 and Aldeyab MA et al, Infect Control Hosp Epidemiol, 2011.

7. Under Discussion in the first paragraph references should be given for reports mentioned.

Reply: The reports mentioned in the first paragraph of the Discussion are papers published on local Portuguese journals, which are not indexed to PubMed. We therefore add this information in the text in order to clarify that those papers cannot be referenced in the References section.
8. The Discussion should be improved by removing irrelevant discussions and repetitions.
Reply: We agree with the reviewer that the discussion is too long. Accordingly, an effort was made to reduce it, and repetitions and less relevant discussions were removed.

9. Under Conclusions, no reference should be included
Reply: this correction was performed according to the reviewer suggestion.

10. In Table 1 include the word 'CDI' for attributable cause and contributing cause
Reply: this correction was performed according to the reviewer’s suggestion.

Discretionary Revisions
Definition of McCabe score should also be given under Definition Section.
Reply: according to the reviewer’s suggestion, the McCabe score definition was given under definition section.

If possible the modified McCabe score (the Sabadell score) may be used as it works effectively to stratify patients according to hospital outcome and is a newer approach.
Reply: Actually, the Portuguese General Health Direction recommends the use of the McCabe classification to evaluate the vital prognosis of the underlying clinical situation, a recommendation that is followed by all the Portuguese hospitals, enabling comparison between different hospitals.

On Page 8 under Result section, in para 3 it is mentioned that CDI was diagnosed by means of detection of pseudomembranous colitis and biopsy. Both are nonspecific diagnosis, though may aid in CDI diagnosis. The stools of these patients were negative for toxins (ELISA may sometimes miss out toxin positivity). However simultaneous stool culture for C. difficile could have been done to confirm CDI and type the strain involved.
Reply: Indeed for three patients the diagnosis of CDI was performed by means of detection of pseudomembranous colitis by fibrosigmoidoscopy and biopsy,
since two of these patients presented ileus, and therefore no stool samples could be collected. For the third patient, the stools were negative for toxins, but there was a high clinical suspicion of CDI, In addition, no other diagnostic test, like a molecular method, was available at the hospital laboratory at that time. Although both methods are nonspecific since they do not target the bacterium, they are recommended in severe cases of CDI, according to the recent update of ECCMID (Debast et al, Clin Infect Dis, 2014). This information was lacking in the manuscript, but it was added in the revised version.

**Level of interest:** An article of importance in its field

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

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

**Declaration of competing interests:**
I declare that I have no competing interests
Reviewer's report

Title: Outbreak of Clostridium difficile PCR ribotype 027 - the recent experience of a regional hospital.

Version: 2 Date: 2 February 2014
Reviewer: Jean O'Driscoll

Reviewer's report:

Minor essential revisions:
I recommend that

• More detailed information is given about the antibiotics received by the patients prior to onset of C. difficile infection (First part of results section).

  Reply: Concerning this comment we feel that giving more detailed information about the antibiotics received by the patients would be irrelevant since it is well documented in many casuistic that all antibiotics can be implicated, although some can more implicated than others, that’s why, and according to recent published papers, we tried to reduce mainly the consumption of fluoroquinolones and third generation cephalosporins as an outbreak control measure.

• There should be a separate epidemic curve for the ribotype 027 cases only.

  Reply: We agree with this comment, therefore, in Figure 3 a separate epidemic curve for the 027 ribotype cases was added.

• The use of English should be improved in places. I don’t have time to go into this in detail. For example, the use of trimester should be replaced by quarter.

  Reply: According to this comment, the revised manuscript was carefully reviewed by a copy editor with English language experience. As a result, the revised version of the manuscript is largely improved regarding the English, as it can be easily checked.

Level of interest: An article of importance in its field
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.

Declaration of competing interests:
I declare that I have no competing interests.