Author's response to reviews

Title: Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant Vibrio cholerae O1

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Author's response to reviews: see over
To
Editor,
BMC Infectious Disease

Subject: Manuscript for review and publication

Dear Sir/M’m,
Please find the attached revised manuscript (MS) entitled “Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant *Vibrio cholerae* O1” you’re your review and publication. We have revised the MS in light of the reviewers’ comments. Please see below, our responses to reviewers are intercalated. May I now request you kindly to consider our MS for publication in your esteemed journal?

Sincerely yours,

Munirul Alam

**Reviewer 1**
Title: Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant *Vibrio cholerae* O1
Version: 2
Date: 22 October 2013
Reviewer: Iza Ciglenecki
Reviewer’s report:
My comments in the attachment
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: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests

Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant *Vibrio cholerae* O1
The paper describes the phenotypic and genetic characteristics of *V. cholera* isolated during 2012 cholera outbreak in Nepal. It adds important information regarding the cholera dynamics in Nepal, and it highlights the importance of regular monitoring of molecular characteristics both from clinical point of view in terms of monitoring the resistance to antibiotics and from epidemiological, as the changes in diversity of isolates open a lot of questions regarding cholera transmission. Although the title summarizes the main finding, in the article the research question seems blurred among other discussions less relevant to main topic. The paper would be much clearer with focus on
molecular biology only (concrete propositions are below). A shorter, more focused paper would be much easier to read. It would be also helpful if the language is simplified and more up to the point. I am not able to review the methods and results part for molecular biology point of view – the paper should be reviewed by an expert in molecular biology and/or epidemiology.

Comments
General comments - Major compulsory comments
My suggestion to the authors would be to focus the paper on molecular biology of 28 isolated samples as suggested in the title and omit parts not related to the main topic of the paper.

Abstract:
1. Line 53 it is not clear whether the cholera has been confirmed by culture prior to use of isolates for further analysis? Than aetiology would have been known?

**Our Answer:** We have rephrased the sentence to address this issue

2. Provide clear purpose of the study in the background part of abstract

**Our Answer:** We have modified the background to clarify the purpose, as suggested by the reviewer

3. The methods, results and discussion should focus on the same subject (the methods focus only on molecular biology, while the result section gives details of field investigation) – see other comments, I suggest to focus only on molecular biology

**Our Answer:** We have made this change as suggested

4. I would suggest to present the brief insight regarding the cholera outbreak in the background. While the details of the investigation itself are not important for understanding the paper (I would skip those for the purpose of clarity), some additional information might be helpful:

**Our Answer:** We have made the change as suggested

5. Information on how the surveillance data is reported – only number of acute watery diarrhea cases or suspected cholera cases? Could you provide case definition used? It seems as that watery and bloody diarrhea are reported together?

**Our Answer:** We have deleted this part to avoid confusion

6. Provide total number of suspected cases and deaths if available

**Our Answer:** Unfortunately, we do not have access to this info; we have omitted the epidemiology part to avoid confusion
7. Is there information on how many samples were collected and how many led to isolation of V. cholerae?

**Our Answer:** Unfortunately, We do not have any info on this

Methods
8. I suggest removing all parts unrelated to molecular biology from the methods (and provide brief description of the outbreak within background section). Methods related to field investigation are partially description of methods and partially providing results that are not sound enough - in particular the part on field observation (line 197) does not provide sufficient information on methodology used, and the results are too general (for example “majority of residents stated they never use soap” without providing total number of people interviewed and proportion of those stating it) – I suggest to remove this part, as it is not the focus of the paper

**Our Answer:** We have removed this part as suggested by the reviewer

9. Please provide information how many stool samples were collected and tested and how many out those were positive for V. cholerae.

**Our Answer:** We do not have this info; so, we have omitted the epidemiology part to address the issue raised

10. Line 257 – how was the randomisation of samples done?

**Our Answer:** It has been mentioned

Results
11. Remove “outbreak investigation” and “weekly trends” sections from the results and provide brief summary in the background (see above)

**Our Answer:** We have made this change as suggested

12. Figure 2 should provide data at least from the beginning of the year – otherwise it is not possible to judge what is “usual” number of cases and how the “peak” was defined

Discussion

**Our Answer:** We have deleted this part

13. Please start the discussion by summarizing the main findings, and focus discussion around this main finding.

**Our Answer:** We have made this change as suggested
14. Poor hygiene and sanitation do not appear in results section, this part can be easily omitted form the discussion

**Our Answer:** this part has been deleted

15. I don’t understand the discussion around fluoroquinolone resistance – I understand from the results section that the investigated samples were quinolone sensitive?

**Our Answer:** We have rephrased text to make it clear

Minor compulsory comments
1. Abbreviations: there are many abbreviations in the paper, it would help the clarity if the number is reduced. Please make sure that each abbreviation is explained when first appearing.

**Our Answer:** We have made this change as suggested

2. Line 133: annual mortality rate of 30’000? What does it mean? Rate should be expressed per Population

**Our Answer:** We have deleted this part

3. Line 136 – 3 days course of effective antibiotics? Could you specify what is the national protocol in Nepal (often a single dose of effective antibiotic is recommended)

**Our Answer:** We have rephrased the sentence

4. Line 162 – no abbreviation in the title

**Our Answer:** We have made this change

5. 165 – typo – consent (instead of content)

**Our Answer:** Corrected

6. Line 169 – increased number of diarrheal cases (instead of increased diarrheal cases)

**Our Answer:** This part has been deleted

7. Line 240 – spell out the abbreviation when first used

**Our Answer:** We have made this change

Discretionary comments
1. Is there any data or isolates available from the period between 2010 and 2012? This could potentially help understand the shift genetically divergent isolates in 2007-2010 period to clonal in 2012.
Our Answer: Unfortunately, we do not have data or access to isolates for that period

Reviewer 2

Title: Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant Vibrio cholerae O1
Version: 2 Date: 24 October 2013
Reviewer: Nur Hasan
Reviewer’s report:
In this manuscript presented by Dixit et al., phenotypic and genotypic analysis of diverse set of environmental and clinical V. cholerae isolates were carried out, and provided evidence of clonal transmission of multidrug resistance V. cholerae in 2012 cholera outbreak in Nepal. The study also identified mutational events in tcpA, gyrA and parC genes and reported the genotype distribution of major virulence associate genes or islands. This study as a whole offers valuable information to the filed particularly on cholera outbreak in Nepal and carries importance for future epidemiological, outbreak, and trace back study. I have few comments:
1. The overall presentation needs to be little more cohesive. There are too many paragraphs at methods and results sections, many of them could be condensed together under major categories like phenotypic, genotypic characteristics, virulence etc.

Our Answer: We tried our best to modify and improve the texts in method and result section as suggested by the reviewer

2. Abstract needs to be condensed for better clarity of major findings author wants to highlight

Our Answer: We have rephrased the abstract as suggested

Other minor comments:
P3. L70, “Vibrio” should italicized

Our Answer: Done, as suggested

P13, L344: distinct? ; Needs revision

Our Answer: We have revised this sentence

P14, L367: parenthesis needs to be corrected.

Our Answer: Corrected

P16, L411-413: the whole sentence needs revision for proper meaning

Our Answer: Revised as suggested
P17, L414: what other supporting data has been referred here? Once PCR provided amplification of wbe it should be O1? Too much redundancy all throughout the manuscript.

Our Answer: We have rephrased the sentence

P16, L434-437: this sentence needs revision for its actual meaning.

Our Answer: We have rephrased the sentence

Level of interest: An article of importance in its field
Quality of written English: Acceptable
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; However, I have collaboration and publication with some of the authors in this manuscript, and icddr,b.

Reviewer 3

Title: Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant Vibrio cholerae O1
Version: 2 Date: 31 October 2013
Reviewer: Alexander Rosewell
Reviewer’s report: Major compulsory revisions
1. The aim of the study is not clear.
Our Answer: We have tried to clarify the aim per suggestion

2. A major strength of the study is the laboratory component. This paper would benefit from focussing on the laboratory issues (resistance, clonal transmission etc.), rather than trying to address multiple audiences by providing information about outbreak investigations. The outbreak investigations and field observations do not add to the value of what could be an important laboratory focussed paper.

Our Answer: We have omitted the field observation part as per reviewer’s suggestion

3. If the authors choose to further explore the spread of clonal strains, more information needs to be provided about how isolates from other settings were accessed and/or compared (eg Bangladesh, Haiti etc) - ie this should be clearly outlined in the methods section. The discussion section should highlight some of the limitations in drawing conclusions about the source of the outbreak and the spread of cholera based on clonal data, when cholera isolates from many settings will never be analysed with comparable methods. It may be relevant to
refer to a recent Colwell paper that discusses the evidence relating to the source of cholera in Haiti.

**Our Answer:** We have mentioned it in the result part. The reference is sited

4. It is not clear if these 3 outbreaks were the only 3 outbreaks in Nepal in this year, or whether there were many other cholera isolates that reached laboratories in Nepal that the researchers did not have access to analyse to identify different clones. Ie are 28 isolates collected from 3 districts representative of all cholera in Nepal. This should be discussed further.

**Our Answer:** The outbreak results have been removed per reviewers’ suggestion

5. Some methods and results are mixed together.

**Our Answer:** we have revised the MS and hope that such concern is addressed

6. The significance of identifying clonal transmission of cholera in 3 village level outbreaks with 28 specimens collected in this setting is not clear. As previously stated, it would be useful to clearly state the objective of carrying out this work. The conclusions to the paper may flow more easily once the aim is clearly articulated.

**Our Answer:** Objective has been pinpointed per reviewers’ suggestion

Minor essential revisions
6. It would be useful to clarify whether antimicrobial sensitivity profiles for the 2012 cholera isolates were being compared with an identical cholera clone when comparing with a previous study with different sensitivity profiles?

**Our Answer:** unfortunately, we do not have access to 2010 Nepalese V. cholerae strains to compare.

**Level of interest:** An article of limited interest  
**Quality of written English:** Acceptable  
**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