Author’s response to reviews

Title: Polymerase chain reaction ribotyping of Clostridium difficile isolates in Qatar: A hospital-based study

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Author’s response to reviews: see over
Bacterial and fungal diseases
BMC Infectious Disease

Dear Drs Garbino & Leung Ho,

Regarding the following manuscript: **Polymerase chain reaction ribotyping of Clostridium difficile isolates in Qatar: A hospital-based study**

On behalf of my co-authors, I would like to re-submit this manuscript with the second round of reviewers’ comments addressed (see specific answers to their queries below)

On behalf of my co-authors, I would like to thank you for reviewing the manuscript and look forward to hearing from you regarding publication

Yours sincerely,

Asma Althani
REVIEWER COMMENTS ROUND 2

Please include primer sequences used for PCR ribotyping.

These have been included under Materials & Methods section under “PCR Ribotyping”

REVIEWER COMMENTS ROUND 1

REVIEWER 1:
Major compulsory revisions

Abstract:
1) line 41-43: in the background, the authors specify that the aims of the study were to determine the prevalence of cdi and describe ribotypes. However, epidemiological information pertaining to the patient population was presented in the results section but not addressed in the objectives. objectives need to be re-written.
Done

Introduction:
2) line 61: “individuals with impaired antibiotic resistance tend to be....”. do you have a reference for this statement?
Sentence amended and referenced

3) line 68: “...despite over-prescription of antibiotics in this region”. do you have a reference for this statement?
A number of references added to support this statement

4) line 81: on line 74 you mention al-khor hospital but on line 81 you mention al-khor hospital laboratories. please clarify.
Amended to al-khor hospital

Materials and methods:

5) the authors should include information on each of the study hospitals [e.g., type of facility (academic, community), number of beds, services provided, population served (urban, rural), number of admissions on a yearly basis, etc.].
Information added to the Methods section

6) line 83: why did you include recent antibiotic exposure as part of the inclusion criteria for this study? how did the authors define “recent”? please specify.
Since recent exposure is linked to increased likelihood of CDI being present.
Recent was defined as antibiotics within the last 3 months

7) Could patients be included in the study more than once? this should be specified.
This was not the case in our study and this has been added in the Methods section

8) what about pediatric patients? the authors may want to provide exclusion criteria. Paediatric patients were included unless they were ≤12 months of age – see baseline demographics Table. Exclusion criteria added.

9) Line 94: include ‘by’ between “b” and “enzyme”. Done.

10) What about the binary toxin gene – was this investigated? The Binary Toxin gene was investigated. However the data is not available to add to the manuscript.

11) lines 95 – 97: additional information on culturing of c. difficile should be included in the manuscript. Done.

12) did the authors look at patient outcomes (e.g., 30 days after a patient was diagnosed with a cdi)? this should be addressed in the manuscript. These correlations were not made and are possibly a limitation of the study. This information has now been added to the manuscript.

13) did the authors also look at categorizing cases as hospital-associated, community-onset? why or why not? yes: In the RESULTS section under “Patients”: Of the 122 samples, 98 (80.3%) had hospital acquired CDI and 14 (11.8%) had community-acquired CDI. Data for the remaining 10 (8.2%) were not available to categorise them into hospital or community-acquired CDI.

14) statistical analysis section: the type of data that were analyzed should be specified (e.g., ribotypes, information pertaining to patient characteristics). the authors should provide information on the specific variables collected in this study (e.g., information presented in table 3), where this information was obtained (e.g., medical records), if information was gathered from all patients or only those identified with cdi, and what groups were being compared for the statistical analysis (e.g., cdi versus non-cdi). this information needs to be clear and match with the objectives of the study. This information was obtained for only samples identified as CDI positive. This has been added to the paper.

15) could the incidence rate of cdi for the study period, by hospital, be determined? this information should be presented. results: This has now been added to the manuscript Hamad General Hospital is 5.7 % AIlKhor Hospital 0.7%.

16) pcr ribotyping paragraph (lines 133 – 140): this paragraph can be shortened as information is repeated several times. for example: “hence pcr-ribotyping was performed on these 79 samples”.
information on the 79 samples was mentioned in the first part of the sentence. lines 138 – 139 = can be removed.

done

17) Correlating factors with cdi – this information is not presented prior to the results section. I believe the authors are using “correlation” and “association”, interchangeably, which can cause confusion to the reader (especially as the authors have not provided a specific explanation pertaining to their data analysis). For example: a. the investigation has not been designed to look at the association of specific risk factors (e.g., ppi) for cdi in a patient population. For the authors to determine if there was a statistical association between a variable and cdi, information would need to be collected on the non-cdi population. b. the authors discuss (lines 154 – 155) specific ribotypes being correlated with certain variables. therefore, information is only required on the cdi population.
Amended in the text to “correlations” where appropriate. Investigation of correlating factors also added to the introduction section

18) Line 156: information obtained from the patient’s medical history needs to move to the materials and methods section.
Amended

19) did the authors categorize the results by individual hospital? if so, why were they not presented and discussed in the manuscript?
The results were pooled due to the small sample number analysed
However, the incidence rate by hospital has now been added

20) As all patients included in this study had recent antibiotic exposure, analysis regarding antibiotic exposure and its association with cdi cannot be conducted (presented in the abstract under the results section and in the discussion lines 181 - 185).
We would like to leave this in as a correlation:
- Current or recent antibiotic use can increase a person’s risk of becoming infected with C. difficile
- All patients recruited had recent exposure (similar timeline). We understand that we cannot make an association as non-CDI patients were or patients with no recent exposure were not included. However, the purpose here is just to show amongst the recently used antibiotic which one correlated most with CDI
- The analysis is a correlation not statistical association

21) Results of ha-cdi and ca-cdi should be presented. discussion:
Done

22) The authors mention the difference in the prevalence of cdis between middle-eastern countries and europe (lines 162 – 164). why – study design? population investigated? sample size? different laboratory methods? the authors should elaborate.
Added

23) Limitations of this study need to be presented (e.g., testing regimen used at the hospitals for c. difficile, sample size, missing data).
These have been added

Minor essential revisions
Abstract:
24) line 34: remove “at”.
Done
25) lines 35 and 36: cdi was not identified. The bacterium was (clostridium difficile).
Amended
26) line 36: cdi samples were not cultured using pcr ribotyping. clostridium difficile was analysed, at the molecular level, using ribotyping.
The line reads “The C. difficile positive samples were then cultured for PCR-ribotyping.” As such, this sentence is correct.
27) lines 38 and 39: percentages should also be presented.
Added
28) line 45: change “commonest” to ‘the most common...’.
Done

Introduction:
29) line 57: remove “(c. difficile) strains”.
Done
30) line 59: need to re-word “...have resulted from c. difficile infection”.
Done
31) line 60: can a patient have an infection if they are asymptomatically colonized? need to re-word line 60.
Done
32) line 62: italicize c of c. difficile.
Amended
33) in line 62 you discuss c. difficile but in line 63 you discuss cdi. these are two distinct and different terms. please ensure you are using the correct terminology in the manuscript.
Checked and amended

Materials and methods:
34) line 80: should the “at” be replaced with “admitted”?
Amended
35) line 81: “a total of 1,532 patients with suspected cdi were recruited”. this information is part of the results and should not be presented under the materials and methods.
Amended
36) line 84: please add ‘that’ between “those” and “were”.
Amended
37) line 86: remove the “watery or bloody diarrhoea” as it was already defined earlier in the manuscript
38) line 92: specify the type of sample (e.g., stool). specify the laboratory – hospital? university?
Done

39) line 93: remove the sentence with 1,532 – this is a result.
Done

40) line 95: cdi should be changed to c. difficile.
Amended

41) lines 99 – 101: this can be removed or incorporated in the pcr ribotyping paragraph.
Done

42) line 105: is the “(2)” a reference?
No – removed

43) lines 106 – 112: this information can be removed and a reference provided.
The protocol was provided personally to us by Leeds Laboratory – we have added the protocol reference
though kept most of the methodology as we feel it will be useful to include it.

Results:
44) lines 124-125: reorganize sentence. start with the number of patients identified with diarrhea then
move onto those with cdi.
Done

45) line 126: 122 samples? 122 patients? 122 episodes of cdi? this should be specified and standardized
throughout the text.
Amended

46) lines 145 – 146: the breakdown in age is presented in table 1 so it can be removed from the main
text.
Done

47) lines 147 – 150. as the information is presented in the text, I would suggest that figure 1 be removed
from the manuscript (as you also have age information presented in table 1). would reword this section
so it flows better.
Done and figure removed

48) line 162: “c. difficile” infections should be removed and replaced with cdi.
Done

Acknowledgements

49) line 214: change assistant to assistance.
Done

References:
50) please ensure the references are in the correct format.
   a. #2: first names should not be spelled out.
   b. #16: title of article should be bolded.
   c. clostridium difficile should be italicized.
   All amends made

51) table 2: c. diff should not be shortened in the column heading.
   Amended

Discretionary revisions

Abstract:
52) line 45: change “commonest” to ‘the most common...’.
   Amended

Materials and methods:
53) further specifying the dates of the study (e.g., october 1, 2011 to august 31, 2012). however, i leave
   this decision to the authors.
   Have left the dates as they are

Discussion:
54) lines 172 – 179: remove the years of the studies in parentheses.
   Done

REVIEWER 2

Major Compulsory Revisions:

Materials and Methods

1. Second paragraph: the authors should use the standardized case definitions for CDI, hospital acquired
   (associated) diarrhea and community acquired (associated) diarrhea. And then some data need to be
   recalculated.

   The definition currently in the paper is what is used as a standard definition in Qatar and hence we
   based our results on this in order to make the data relevant for the Qatari healthcare professionals:

   “Hospital acquired diarrhoea was defined as diarrhoea which developed ≥48 hours after admission.
   Community-acquired diarrhoea was defined as diarrhoea, which developed before 48 hours of
   admission.”

2. Fourth paragraph: samples were tested by GDH assay, toxins A and B EIA, and PCR. If the results are
   not completely consistent, how to interpret it? Please provide the information.

   GDH assay, toxins A and B EIA were first-line screening tests. All samples were then tested using
   GeneXpert PCR. If the PCR did not show consistent results with the initial screening tests during the first
attempt, it was repeated using new reagents and a new cartridge. If the results were negative upon re-testing, the specimen was not considered *C. difficile* positive. Isolates that were proven not to be *C. difficile* during the initial screen were not characterised further.

Tests carried out to positively identify *C. difficile* from cultured cells were the following; morphology, odour (Horse manure odour), fluorescence and latex (when necessary).

3. Epidemiology terminology (such as prevalence and incidence) is used loosely since there is no clear denominator. For example: results, correlating factors with CDI, age, last sentence, “both the CDI infection rate and...in the elderly group”. In the manuscript we do not know how many elderly patients had diarrhea and how many were met the inclusion criteria and tested for CDI, therefore we do not know the infection rate.

The terms are now only used with respect to the overall study population. In terms of specific factors, “prevalence” or “incidence” has been replaced with “correlates”

4. The correlations made are weak since the sample size is small. Please consult the statisticians whether these data are suitable for the Chi-Square tests.

We have removed the statistical data and stated that due to the small sample sizes, statistical tests couldn’t be accurately performed on this data

5. The limitations of the work are not clearly stated.

These have now been added to the discussion section

Minor Essential Revisions

1. “*Clostridium difficile*” and “*C. difficile*” should be italic type.

*Amended*

Materials and Methods:

2. Please provide company location information. Please provide the company information of the software used in this study as well.

*Added*

3. First page, line 5: “Department of Laboratory medicine...” should be changed to “Department of Laboratory Medicine...”

*Done*

4. Introduction, first paragraph: Please provide the reference of “Individuals with impaired antibiotic resistance tend to be at higher risk of *C. difficile* infection.”
Sentence removed. Sentence added instead “Risk factors for C. difficile infection (CDI) include antibiotic exposure, hospitalisation, and advanced age” Ref 1

5. Introduction, second paragraph: As far as I know, ribotype 017 is common in many Asian countries, e.g. China and Korea. But 027 and 078 are not common.

Paragraph amended to reflect this

6. Materials and Methods, second paragraph: I suggest to delete the preposition “to”.

Done

7. Materials and Methods, second paragraph: “Recruited patients...” should be earlier than “the inclusion criteria...”

Done

8. Materials and Methods, 11th paragraph, last sentence: Do you mean you sent the strains with banding patterns that did not match with the references to UK for ribotyping? If not, is there a web link or a page you can site?

Yes – and sentence amended to reflect this

9. Results, 5th paragraph, and last sentence: do you mean you had typed several different C. diff colonies for each patient?

No – we are clarifying that only one ribotype was isolated from each C. difficile colony. There were no multiple ribotypes in one colony and each colony represented a single patient (i.e. 79 colonies represented 79 patients)

Discretionary Revisions:

1. The significance will increase if the authors can provide more information on the incidence of CDI in Qatar. Please provide the incidence of CDI per 10,000 admissions (or patient days).

There is virtually no data on CDI in Qatar outside our study and hence providing incidence per 10,000 is not possible at present based on just one study. We are planning to look at wider epidemiology of CDI in Qatar and hence need to demonstrate this data as evidence base for a rationale for larger studies.

2. Are there any outbreaks during the study period?

None that we are aware of

3. Table 3: Because of the sample size is small, I suggest to remove the column of P value (significance of correlation with ribotype).
Done