Author's response to reviews

Title: Antibiotic susceptibility of Clostridium difficile is similar worldwide over two decades despite widespread use of broad-spectrum antibiotics. An analysis done at the University Hospital of Zurich.

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Version: 6 Date: 23 October 2014

Author's response to reviews: see over
Reviewer's report

Title: Antibiotic susceptibility of Clostridium difficile is unchanged despite widespread use of broad spectrum antibiotics.

Version: 3 Date: 5 August 2014

We highly appreciate that the referees accepted the replies to revision one of our manuscript. We made again a major effort to improve the quality of the manuscript and gave a detailed point-by-point reply. In addition, the manuscript was checked for grammar and style by a native American professional editor.

We hope that the manuscript now meets the criteria for acceptance in the BMC Infectious Diseases.

Reviewer: Nitish Khanna

Reviewer's report:

Overall

The authors have answered most of the queries relating to the paper, however, in doing so, numerous grammatical and spelling mistakes have been created. In addition, grammatical issues relating to colloquialisms not detected on the first draft have been found. Therefore, I would recommend a detailed grammatical and spelling check be performed ensuring that each sentence reads well and contains only the essential information. Pay close attention to antibiotic names as still numerous spelling mistakes, also in supplementary material as well. Advised to review all documents and amend as necessary. Examples found include nitromidazoles, lincosamid, cephalosporines, glycopeptides.
**Major Compulsory Revisions**

None

**Minor Essential Revisions**

Line 61 – “exemplary” does not make sense: Should be “For example”
- We corrected the usage accordingly (in the current version line 54).

Line 63/64 – Review English as does not make sense. Should be: “All strains were susceptible to metronidazole. One strain was resistant to vancomycin”
- We corrected the usage accordingly.

Line 89 – „Irrespective of ribotype 027, the mortality rate appears to have increased in other parts of the world“ – Can you reference this?
- We re-examined the literature, and we agree that this issue is somewhat controversial. Some papers report new outbreaks with other ribotypes [1, 2], but an overall increase in mortality is not convincingly demonstrated. Thus, we removed this sentence.

Line 96 – Review English – Drop “an” from sentence: “Various antibiotics have an antimicrobial activity against C. difficile.”
- We did so.

Line 115 – Should be “repeat” and not “repeated” - “There were no repeated stool specimens from patients with recurrent CDI”
- We corrected it.

Line 204 – „Inpatients were hospitalized during 17. 2 days in” Does not make sense. Do you mean inpatients were hospitalized for 17.2 days on average?
- We appreciate the critical eye of the reviewer and have corrected the sentence.

Line 206-9 - Thirty patients (32.6%) underwent one or several complications: 20 patients (21.7%) suffered from infectious complications, and further complications spanned the entire spectrum of medicine (Suppl. Table 12). Seven patients (7.4%) died shortly thereafter, of causes unrelated to CDI. Review English as does not make sense: You do not undergo a complication. You undergo surgery. Also should be „one or more“ instead of „several“.
- We corrected it.
Line 210 – “Before the first positive culture, 83.3% of the patients were prescribed antimicrobials, 37.8% antifungal medication…” Clarify this: Do you mean that in the preceding 8 weeks prior to 1st positive culture, as you have mentioned in your methods?

- We changed the sentence as suggested:

  **Line 208-211:** In the 8 weeks preceding the first positive culture, 83.3% of the patients (i.e., in- and outpatients) were prescribed antimicrobials, 37.8% antifungal medication, 73.3% gastrointestinal medication, 37.8% immunosuppressive medication, and 20.0% cytostatic medication (Suppl. Table 1).

Line 224 - We believe that the observed resistance is a laboratory artifact. What evidence do you have to say this?

- The referee is asking a very critical question. Considering the data from EUCAST (see below), the resistance we got for this one strain is really amazing. Thus, the microbiologist in our study, Prof. Reinhard Zbinden, is rather convinced that we are confronted here with an erroneous measurement of the MIC, but formal proof for it we do not have.

Line 281 – review grammar
As mentioned above, an American native editor checked the entire manuscript for grammar and style.

Line 306 – „intermediate“ instead of „intermediates“
- We corrected the word.

Line 317-321 - Review this entire sentence as does not read well/make sense: eg: „high activity“, spelling of „suffered“, „under CDI“ does not make sense
- We corrected the wording.

Line 327 – „but for“ does not make sense: should be „apart from“
- We corrected the wording.

Line 329 – review „rather“
- We removed “rather.”

Line 331 – “and the absence of an outbreak in ZH” Do you mean absence of an 027 outbreak or outbreak in general? Suggest removing this as does not make sense in current form
- We removed this part of the sentence as suggested.

Line 339 – Review and/or remove: „reported in a seminal work“
- We removed as suggested

Line 348-51 – „acknowledge that interpretations must be done cautiously and put in a bigger context in concert with published data. While we made a major effort to get hands on a homogenous cohort, the changes in microbiological assays over the time may be regarded as a confounder for diagnosing CDI. Irrespective of this confounder, we are pretty convinced that the retrospective identification of the“ Remove colloquialisms such as “bigger”, “hands on” and “pretty convinced”. These are not scientific terms and should be removed.
- We changed/removed wording:

- Line 349-357: Limitations of the study are the number of cases and the retrospective nature of this work. The number of cases we analyzed is in the range of cases presented in published work; nonetheless, we acknowledge that interpretations must be done cautiously due to the limited number of cases and the changes in microbiological assays over time. Our study was further limited by the lack of individual treatment information
for the control patients. We therefore had to rely on hospital-wide prescribed DDDs of
the different antimicrobials as a comparison group without information on which drugs
were given simultaneously. This and the limited sample size precluded multivariable
analyses including all antimicrobials which would have been preferable.

Supplementary table 1 - Confusion is not a systemic disease? Was there an underlying diagnosis
found?

- The referee is right; we want back to the chart of this patient, he suffered from acute
renal failure.

**Discretionary Revisions**

5. Line 187 - some evidence that antifungals may predispose to CDI. Worth commenting on. Evidence
Prevalence and clinical features of Clostridium difficile-associated diarrhea in a tertiary hospital in
northern Taiwan. Hsu MS1, Wang JT, Huang WK, Liu YC, Chang SC.

species overgrowth protect against Clostridium difficile infection? Manian FA, Bryant A.

- We thank the referee for making us aware of these reports. We added following lines to
the manuscript:

- Lines: 275-279: Notably, 34/90 (37.8%) of patients received antifungal drugs. Some
studies have reported that *Candida* in the GI-tract acts as protective factor against
CDI, and that the use of antifungals drugs is an independent risk factor for CDI [3, 4].
We do not know the extent to which antifungal drugs contributed to the CDI in our
cohort.

**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 have received funding for research and fees for public speaking from

AstraZeneca-
Reviewer’s report

**Title:** Antibiotic susceptibility of Clostridium difficile is unchanged despite widespread use of broad spectrum antibiotics

**Version:** 3  **Date:** 18 August 2014

**Reviewer:** Elvira Garza-Gonzalez

**Reviewer’s report:**

Major revision

Undoubtedly, there is valuable information in this manuscript. I still think that Authors cannot assume that antibiotic susceptibility of Clostridium difficile is unchanged despite widespread use of broad spectrum antibiotics if they did not analyzed the antibiotic susceptibility in the same population.

By all the analysis they made they can draw the conclusion that the antimicrobial susceptibility in the population studied is similar to observed in other populations.

I think this concept should be changed.

A better title could be:

“Antibiotic susceptibility of Clostridium difficile is similar to observed in other populations despite widespread use of broad-spectrum antibiotics at the University Hospital of Zurich”

- We appreciate the suggestion of Dr. Garza-Gonzales, and changed the title taking into account Dr. Garaz-Gonzels’ remarks:
  
  o Antibiotic susceptibility of Clostridium difficile is similar worldwide over two decades despite widespread use of broad-spectrum antibiotics: An analysis done at the University Hospital of Zurich

Others changes need to be done of this concept is changed:

Line 53: is similar to reported in other populations over the last two decades

- Done as suggested.
Line 59: Zurich is similar to the one reported by others since the 1980s. (delete “and appears to be mostly unchanged since the 1980s.”)
- Done as suggested.

Lines 244-246: Clindamycin has been considered the major catalyst for CDI. Here, we determined which antibiotics are associated with CDI in a tertiary hospital center in Switzerland and whether antibiotic susceptibility of C. difficile strains influenced the occurrence of CDI. Delete: “a change in the”
- Done as suggested.

Lines 339-340: In summary, antibiotic susceptibility of the collection of C. difficile from the University Hospital of Zurich is similar to the one reported by others since the 1980s. Delete: “and appears to be mostly unchanged since the 1980s.”
- Done as suggested.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests:
None to declare

Reviewer's report

Title: Antibiotic susceptibility of Clostridium difficile is unchanged despite widespread use of broad spectrum antibiotics

Version: 3 Date: 22 September 2014

Reviewer: Andrew M Morris

Reviewer's report:
I have been asked to review from a statistical perspective only. I found this paper interesting and thought-provoking however, my comments are limited to statistical methodology and conclusions:

Minor Essential Revisions
1. The rationale behind sampling is unclear, as the data set does not contain one continuous period, and therefore may result in bias.

- We added the reasons for the two distinct sampling periods.

Major Compulsory Revisions

1. The data set only contains 90 samples. The authors have drawn conclusions on 10 different classes of antimicrobials. Sample size precludes inferences from the number of variables they have considered. Additionally, reasonable conclusions cannot be made using the analysis they have used, as they use univariate methods. Because a majority of patients were on more than one antimicrobial prior to CDI diagnosis, multivariate methods are required. A regression model seems considerably more appropriate here, and would largely account for sample size issues.

- We agree with the reviewer that a multivariable analysis would have preferable in our study. As we described in the Methods Section (line 168-177), we did not have access to individual patient data for the comparison group but worked with hospital-wide drug use in terms of prescribed Defined Daily Doses (DDD's) for the different anti-microbials. Therefore, we do not have information about combination treatments in the comparison groups, and multivariable methods are not possible. We described this issue in the Limitations section (line 349-357).

- Lines 349-357 Limitations of the study are the number of cases and the retrospective nature of this work. The number of cases we analyzed is in the range of cases presented in published work; nonetheless, we acknowledge that interpretations must be done cautiously due to the limited number of cases and the changes in microbiological assays over time. Our study was further limited by the lack of individual treatment information for the control patients. We therefore had to rely on hospital-wide prescribed DDDs of the different antimicrobials as a comparison group without information on which drugs were given simultaneously. This and the limited sample size precluded multivariable analyses including all antimicrobials which would have been preferable.

2. It is unclear how many patients were on antecedent antimicrobials. From Supplementary Table 2, it appears that only 59 patients were on prior antimicrobials. Using n=94, this would make the percentage of patients receiving antecedent antimicrobials at 63%, even though they mention 83% in the manuscript. This requires clarification in the manuscript.
- In Suppl. Table 2, we only listed data from inpatients. In Suppl. Table 1, we listed the data for the in- and outpatients together. We clarified this issue by changing the title of Suppl. Table 2 (Number of inpatients with multiple classes of antibiotics prior to CDI) and Suppl. Table 1 (Patient characteristics of in- and outpatients before first *C. difficile*-positive culture or toxin). We also explained this in the text:

- Lines 208-211: In the 8 weeks preceding the first positive culture, 83.3% of the patients (i.e., in- and outpatients) were prescribed antimicrobials, 37.8% antifungal medication, 73.3% gastrointestinal medication, 37.8% immunosuppressive medication, and 20.0% cytostatic medication (Suppl. Table 1).

Penicillins (i.e., amoxicillin, amoxicillin/clavulanate and piperacillin/tazobactam) were the most commonly prescribed antibiotics (i.e., 38.5% of our cohort took penicillins with an average duration of 4.6 days (median 4 days), followed by cephalosporins (29.5%) and quinolones (26.9%) with an average duration of 6.8 days and 4.9 days, respectively (median 6 days and 2 days, respectively)) (Table 2). Additional antimicrobials were carbapenems (15.4%), glycopeptides (10.3%), nitromidazoles (5.1%), lincosamides (2.6%), aminoglycosides (3.8%) and others (11.5%). Among the hospitalized patients, 25 patients were given one class of antibiotics, and 34 were given more than one class before diagnosis of CDI (Suppl. Table 2).

3. Time on antimicrobials--especially in this kind of study--is relevant. A proper analysis exploring the role of each antimicrobial in contributing to risk should also explore the time each patient was on an antimicrobial (e.g. antimicrobials utilized for 1 day should not be considered in the same manner as antimicrobials utilized for 14 days).

- The point Dr. Morris makes is well taken. Antibiotics have a major effect on the gut microbiota and thus on CDI [5-7]. There is a complicated and intricate network/interaction between the gut microbiota and ecologic niche of *C. difficile* and thus CDI. As Dr. Morris suggested, we should explore the time each patient was on antimicrobial therapy. We know from various studies that even one dose of antibiotics can significantly affect the gut microbiota that can persist for years [6, 8]. We believe his suggestion deserves more study for a deeper understanding of antibiotic use and CDI. However, the experimental design of our study does not permit this. In such a study, we would also need to consider the time between the last dose of the antimicrobial used and the occurrence of CDI. In other terms, the concept of this study should take into account i) the duration of the antimicrobial drug, ii) the dose of the antimicrobial drug, iii) whether the incriminated drug was given together
with other drugs, and iv) the interval between the last dose of the incriminated drug and the occurrence of CDI. These questions are beyond the scope of this study and, in particular, the study design and the existing data set. We hope that Dr. Morris accepts our point of view.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.

**List of references:**