Author's response to reviews

**Title:** Clostridium difficile 027 infection in Central Italy.

**Authors:**
- Stefano Di Bella ([stefano932@gmail.com](mailto:stefano932@gmail.com))
- Maria G Paglia ([pagliamicromol@inmi.it](mailto:pagliamicromol@inmi.it))
- Emma Johnson ([emmas@hotmail.com](mailto:emmas@hotmail.com))
- Nicola Petrosillo ([petrosillo@inmi.it](mailto:petrosillo@inmi.it))

**Version:** 3  **Date:** 2 November 2012

**Author's response to reviews:** see over
To BMC Infectious Diseases

The Editor

RE: revised manuscript submission. ID 1691513407513288

November 2, 2012

Dear Editor,

Attached please find our revised manuscript ID 1691513407513288 “Clostridium difficile 027 infection in Central Italy” to be submitted to for publication.

We include the replies point by point to the reviewers.

Kind Regards

The corresponding author

Nicola Petrosillo, M.D.

2nd Infectious Diseases Division,
National Institute for Infectious Diseases “L. Spallanzani”,
Via Portuense, 292-00149 Rome, Italy.

e-mail: nicola.petrosillo@inmi.it
tel +390655170432  fax +390655170486
Reviewer #1: lei liu
Reviewer's report:
In current work, Stefano Di Bella et al. described some new cases of Clostridium difficile 027 (CD027) infection occurred in Italy. The author stated that CD027 is emerging in healthcare facility in Italy. However, there only 24 samples from 19 patients from central Italy and the author did not indicate whether these samples come from same or several different hospitals. Based on this data, it's difficult to get a justified epidemiological conclusion and the answers to the question "where are we now and where are we going" is perfunctory.

R: These samples came from 7 different hospitals. This information was added in the text. Moreover, we modified the title as follows: "Clostridium difficile 027 infection in Central Italy."

Other comments:
1. Is the 29 years old patient male or female? Whether this patient also get long term healthcare?

R: The 29 year old patient was a female. She had no history of long term healthcare stay. This was added in the text.

2. Besides CD associated infection, whether there are co-infection with CD?

R: Among the 027 infected group one patient had chronic hepatitis C; among the non-027 group there were: 1 patient with pulmonary tuberculosis, 1 AIDS patient with tuberculous meningitis and urinary tract infection, and 1 AIDS patient with extrapulmonary tuberculosis (lymph nodes) and esophageal candidiasis. No patients were diagnosed as having any bacterial, protozoan or elmintic enteric infection. It was added in the text.

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

Reviewer #2: MURAT AKOVA
Reviewer's report:
This is a well-written and an important report describing a small cohort of patients infected with hypervirulent C. difficile ribotype 027. The strength of the paper comes that it describes 10 new patients from different institutions in Italy over a two-year period whereas there had been only 8 cases described in a single center previously. My comments are as follows:

Major Compulsory Revisions
1) The title reads "C. difficile 027 infections in Italy..." which seems to be assertive. The cases came from the reporting institution or from local hospitals. Unless the authors confirm that these sampling represent the whole Italy (which I seriously doubt), this title does not seem very appropriate and should be changed.

R: we modified the title (see above).
2) It would be interesting if the authors can describe the treatment strategies applied and outcome of the C. difficile 027 infections in those 10 patients.

R: The management of the 10 patients with C. difficile 027 infections was as follows: 2 patients were treated with oral metronidazole alone; 1 was treated with vancomycin alone; 7 were treated with metronidazole and vancomycin in combination (4) or sequentially (3). No patients died. Both the 2 patients treated with metronidazole alone had a recurrence. The other 10 recurrences occurred in patients treated with vancomycin and metronidazole. It was clarified in the text.

Discretionary Revisions
1) In Results, the 1st paragraph, the authors mention 24 samples were received from 19 patients. Then 17 patients were found positive for the test organism. I assume that each of these patients had a single isolate. This is not clear from the text and may be clarified.

R: Stool cultures were not performed, as specified in the text. The number of samples was higher than the number of patients because we had more than one stool sample from 3 patients.

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.

Reviewer #3: Jesús Rodríguez-Baño
Reviewer's report:
This paper deals with CDi caused by C. difficile 027 in one referral center in Italy. This may be an important and emerging problem in many European countries after the outbreaks that occurred in Canada, USA and UK some months ago. The paper is well written.

Major compulsory revisions
1. Please include in the abstract where the cases came from (number of hospitals)

R: According to the reviewer’s suggestion, we included in the abstract the number of the hospitals.

2. It would be important to know if the emergence of 027 cases increased the incidence of episodes of CDI (at least in the Lazzaro Spallanzani hospital, because they may not know in the others), meaning that 027 was able to add to previous "endemic" cases.

R: We understand the point raised by the reviewer; even though the number of cases increased more than twice in our hospital from 2006 to 2010, no data are available on the impact of 027 CD on this increase because at that time PCR for 027 was not routinely performed on stool samples.

3. In the definition of healthcare-associated cases and community cases there is a gap for cases diagnosed >4 weeks and <3 months after a hospital admission.
How were these defined?

*R: We are aware of the existing gap. The definition that we used derives from the literature [Cohen SHEA-IDSA Guidelines 2010]. There is a grey zone, the so called “indeterminate cases”, that corresponds to this gap. We had no cases in this grey zone. In order to avoid misunderstanding, we added this in the definition of cases.

4. Please clarify that all other usual aetiologies of acute diarrhoea were excluded by conventional culture. This is particularly important in the community-acquired case, which is really interesting.

*R: according to the reviewer’s suggestions we clarified this in the text

5. The number of relapses is probably wrong; if there were 5 patients who relapsed, and 2 of them had 4 and 6 episodes, the sum should be at least 14, not 12.

*R: Five patients experienced relapsing episodes. Among them three had one relapsing episode each and the remaining two patients had 4 and 5 relapsing episodes each (total of 12 relapsing episodes). We corrected it in the text.

6. I think it would be necessary to provide more epidemiological data: from how many centers did the cases come from, and in which wards were they admitted? An epidemic curve would help. Was there a clear relationship between the cases?

*R: According to the reviewer’s comments, we added the number of hospitals and the wards in the text. In our opinion, an epidemic curve is not feasible, because there was no surveillance system for CD in the hospitals who sent the samples to our laboratory, and the samples that were submitted to our laboratory belonged to patients with severe diarrhea and clinical suspicion of a hypervirulent strain. For this reason, it is not possible to establish if there was a clear relationship between the cases.

7. How were the patients treated? Thus is key to understand the high rate of recurrences, beyond the fact that 027 is known for increasing the risk.

*R: we already replied to the reviewer 2, at point 2, on this issue.

8. The fact that CD was not cultured, identified and typed should be acknowledged as an additional limitation.

*R: according with the reviewer’s comment, we added this limitation in the text

Minor essential revisions
None

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.