Author’s response to reviews

Title: Carbapenemase-producing Pseudomonas aeruginosa from Central Greece: molecular epidemiology and genetic analysis of class I integrons

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your comments about our manuscript entitled: "Carbapenemase-producing Pseudomonas aeruginosa from Central Greece: molecular epidemiology and genetic analysis of class I integrons".

In this revised version the suggestions of the reviewers were taken under consideration.

Sincerely yours,

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**Reviewer’s report**

**Reviewer:** Szilvia Melegh

Thank you very much for your suggestions. In more details:

**Major Compulsory Revisions**

1. **Background – Paragraph 3:**
   The baseline was around 33% for several years and a sudden increase to 50% happened in 2011, as stated in the manuscript. Paragraph 3 has been revised, as suggested. Unfortunately, there was not an adequate sample of isolates in stock in 2010, so as to compare them with the isolates in 2011 and investigate the reason of the increase of carbapenem-resistant *P. aeruginosa*.

2. **Results and Discussion – Paragraph 1:**
   In the Methods section, the usage of CLSI breakpoints is stated which refers to imipenem $R \geq 16$ mg/L and not $R \geq 4$ mg/L. All carbapenem-resistant *Pseudomonas aeruginosa* isolates showed MICs of imipenem $\geq 16$ mg/L, as stated in the Results and Discussion section, Paragraph 1.

3. **Results and Discussion – Paragraph 1:**
   Indeed, we have identified 80 VIM-producing isolates out of 284 carbapenem-resistant isolates. The remaining 204 were negative for carbapenemase production and the beta-lactamase genes investigated. The numbers have been changed accordingly (204 and not 200 isolates).

**Minor Essential Revisions**

4. **Abstract – Background – Line 28:**
   The missing word in the sentence “Multidrug resistant *Pseudomonas aeruginosa*” was added, as suggested.

5. **Abstract – Results – Line 43:**
   The missing word in the sentence: “revealed high prevalence of” was added, as suggested.

6. **Background – Paragraph 2 – Line 72:**
   Reference [4] has been replaced by a more appropriate article referring to metallo-beta-lactamases.

7. **Background – Paragraph 2 – Line 76:**
   Reference [11] has been replaced by a more appropriate article referring to MLST of *P. aeruginosa*.

8. **Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 88:**
The other two hospitals are mentioned in the text, as suggested.

9. Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 94:
Disks and media used for DDST in this study are mentioned, as suggested.

10. Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 96:
Reference [5] has been replaced by a more appropriate article and disks and media used for the modified Hodge-test and imipenem-EDTA double-disk synergy test are stated.

11. Methods – Detection of beta-lactamase genes – Line 100:
The misspelling was corrected as ‘carbapenem-resistant’.

12. Methods – Detection of beta-lactamase genes – Line 104:
The sentence which describes the primers used for the detection of beta-lactamase genes has been rephrased and additional references were designated, as suggested.

The PCR products obtained from all VIM-positive isolates (n=80) were sequenced. This information has been added in the text.

14. Methods – PCR mapping of class I integrons – Line 127:
The primers used for sequencing can be found in references [19], [20], which are designated in the Methods section.

15. Results and Discussion – Paragraph 8 – Lines 203-206:
The two sentences have been rephrased to improve the readability.

16. Results and Discussion – Paragraph 8 – Lines 210-212:
This sentence was rephrased to make the message clearer.

17. Results and Discussion – Paragraph 9 – Lines 219-221:
We have not performed sampling from the environment and we have not screened the personnel for carriage. We have deleted this sentence so as to avoid confusion.

18. Conclusions – Line 244:
The misspelling of resistant (“carbapenem-resistance P. aeruginosa”) was corrected.

Discretionary Revisions

19. All these informations were added in the section Results-Discussion, lines 188-189
20. For submitting the novel integrons in the Integra ll database, additional sequencing should be performed at the flanking regions of the integrons. This could be done at a later stage, but we would prefer not to delay the publication of the paper.

Reviewer: Liang Chen

Reviewer's report:

Thank you very much for your suggestions. In more details:

1. Line 77-79: Unfortunately, there was not an adequate sample of isolates in stock in 2010, so as to compare them with the isolates in 2011 and investigate the reason of the increase of carbapenem-resistant *P. aeruginosa*.

2. Line 91: In our study, all carbapenem-resistant *Pseudomonas aeruginosa* isolates showed MICs of imipenem \( \geq 16 \text{ mg/L} \), as stated in the Results and Discussion section, Paragraph 1. Thus, the prevalence of carbapenem-resistant *P. aeruginosa* will remain the same even if the new breakpoint is applied.

3. Line 104: Which *bla*OXA genes were tested? We have tested for *bla*OXA-48 and *bla* OXA-50 genes. We have added this information, as suggested.

4. Among 80 vim-positive isolates 52 were collected from infections and the remaining from colonization. No association between them ((infections or colonizations) and *P. aeruginosa* clones or integron types) was observed. This information was added into the text lines 179-180.

5. Line 146: The numbers have been corrected (204 isolates that are VIM-negative), as suggested.

6. Line 147: As mentioned in the text, there were 204 carbapenem-resistant isolates “negative for *bla*VIM or other beta-lactamase genes tested”. We have added “including carbapenemases” for clarity.

7. Line 154: We have added “intermediate resistance” (16 mg/L) for ceftazidime, as suggested.

8. Line 163-170,215-223: The monthly distribution for VIM-4 producers was a tentative explanation and we have deleted this sentence from the text to avoid confusion.

9. Line 179: The high mortality rate (50%) is from this study. The sentence has been rephrased for clarity.

11. Page 10: A figure of the integron structures will be helpful for presenting the
results.
A figure of the integron structures has been added, as suggested.

12. Line 203-204.
The two sentences have been rephrased to improve the readability.

Line 219-221: We have not screened for carriage of VIM-4 producers from nursing staff or equipments, thus we have deleted this sentence.