Reviewer's report

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

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Reviewer: Szilvia Melegh

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Summary

The authors investigated the genetic background of carbapenem resistance in Pseudomonas aeruginosa strains isolated in Central Greece during an 8 months period in 2011. Molecular typing, integron mapping and assessment of clinical background were performed for the carbapenemase producing ones. Besides less frequent clones, the presence of two international VIM producing clones (ST111, ST235) was confirmed. Both of these latter clones were identified earlier in Greece.

General comment

To me the most interesting thing was the diversity of clones and integrons detected in them. It indicates a complex epidemiology of carbapenemase producing P. aeruginosa in Greece. Unfortunately the question, whether what attributed to the increase in carbapenem resistance in 2011, could not be clearly answered on the bases of the data found in the text. The two most prevalent clones observed in this study have already been identified in Greece. No data can be found in the manuscript about the clones indentified and the rate of carbapenemase producers among carbapenem resistant isolates in the preceding years. The novel integron gene cassette arrays identified marks the ongoing evolution of these multidrug resistant pathogens.

Major Compulsory Revisions

1. Background – Paragraph 3:

The rate of carbapenem resistance among P. aeruginosa isolates are presented only for two years (2010 and the study period, 2011). A longer period of time would help to come to a better estimate of the real trends of carbapenem resistance. If just the data of this two years are considered, one can think: (1) the baseline was around 33% for several years and a sudden increase happened in 2011 as stated in the manuscript; or (2) the rate had been continuously increasing for a longer period of time, and the increase observed in 2011 wasn’t such a sudden increase, rather it was a part or an acceleration of a trend which had started much earlier. In order to better elucidate this question, it would be useful to present the rate of carbapenem resistance for a minimum of 5 years period.
2. Results and Discussion – Paragraph 1:
According to this paragraph 284 isolates were identified as resistant to carbapenems (MIC values of imipenem $\geq 16$ mg/L). I find this sentence a bit confusing, because in the Methods section the usage of CLSI breakpoints is stated which refers to imipenem $R \geq 4$ mg/L. Which criteria (imipenem MIC $\geq 16$mg/L or $\geq 4$ mg/L) was used to define the carbapenem resistant isolates for this study? How many isolates had imipenem MIC values of 4-8 mg/L? Were these isolates also tested for carbapenemase production and beta-lactamase gene content?

3. Results and Discussion – Paragraph 1:
284 isolates were resistant to carbapenems. Of these 80 proved to be VIM producers and 200 were negative for carbapenemase production and the beta-lactamase genes investigated. So the VIM producing ones and the beta-lactamase negative ones make a total of 280 isolates. What kind of beta-lactamase genes were identified in the remaining 4 isolates?

Minor Essential Revisions

4. Abstract – Background – Line 28:
Missing word: “Multidrug resistant Pseudomonas aeruginosa”

5. Abstract – Results – Line 43:
Missing word: “revealed high prevalence of”

6. Background – Paragraph 2 – Line 72:
Reference [4] is about structure and nomenclature of integrons. A reference about metallo-beta-lactamases would be more appropriate.

7. Background – Paragraph 2 – Line 76:
Reference [11] does not refer to MLST of P. aeruginosa. Please remove it from the list of references.

8. Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 88:
Only one hospital is named exactly as the source of isolates. Please name the other two hospitals as well.

9. Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 94:
Various substrate, inhibitor and medium combinations can be used for DDST. Please name the disks and media used in this study.

10. Methods – Bacterial isolates and antimicrobial susceptibility testing – Line 96:
Reference [5] does not refer to modified Hodge-test and imipenem-EDTA double-disk synergy test. State what disks and media were used for the modified Hodge-test.
11. Methods – Detection of beta-lactamase genes – Line 100:
There is a misspelling: “carbapenemase-resistant”. Which isolates, the carbapenemase producer or the carbapenem resistant ones, were tested for beta-lactamase gene content?

12. Methods – Detection of beta-lactamase genes – Line 104:
Reference [16] relates to PCR of MBL genes. Please designate or cite the methods used for detection of the other genes. BlaGES can be considered as ESBL, but blaOXA can show non-ESBL phenotype.

How many PCR products of how many isolates were sequenced exactly?

14. Methods – PCR mapping of class I integrons – Line 127:
What primers where used for sequencing?

15. Results and Discussion – Paragraph 8 – Lines 203-206:
The coherence of these two sentences with each other and with the whole text should be enhanced in order to improve the readability. The first sentence about mortality is lacking a reference.

16. Results and Discussion – Paragraph 8 – Lines 210-212:
I have some difficulties in understanding the last sentence of this paragraph. It was complicated to figure out what “that” was referring to. This sentence should be rewritten to make the message clearer.

17. Results and Discussion – Paragraph 9 – Lines 219-221:
The epidemiological connection between the occurrences of strains of the same Pseudomonas aeruginosa clone (ST235) in two different departments (internal medicine and urology) could not be confirmed without proving that the shared equipments and/or the members of the staff were colonised or infected with strains of the clone in question. Without sampling the environment and screening the personnel for carriage one can not state downright that the shared equipments and staff are responsible for the spread of this clone. You should word this hypothesis more carefully.

18. Conclusions – Line 244:
Misspelling of resistant (“carbapenem-resistance P. aeruginosa”).

Discretionary Revisions

19. It would be interesting to see how many carbapenemase producing isolates were responsible for infections and how many were considered to be coloniser only. What sort of treatment did the infected patients get and what was the outcome?

20. The novel integrons could be deposited in the Integrall database.
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: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests