Author's response to reviews

Title: Serratia marcescens outbreak in a neonatal intensive care unit: crucial role of implementing hand hygiene among external consultants.

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Author's response to reviews: see over
Florence, 20th November 2014

Dear Editors,

Enclosed is the revised manuscript entitled:


We thank the editor and the reviewers for their valuable comments, which we carefully considered, and addressed in the revised manuscript. Details of the major revisions and responses are described on the next page.

Ethical approval was not required because the manuscript is only a report and a critical description of the management of an outbreak, according criteria of good clinical practice.

We believe that the manuscript has been much improved as a result of the revisions and we hope it may be reconsidered for publication in the journal.

Sincerely yours,

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Reviewer: Bulent Bozdogan

Reviewer's report:
Authors report an *S. marcescens* outbreak in a neonatal ICU. During outbreak 6, 4 and 2 samples of ocular secretion, blood and intestinal stoma from babies were positives for *S. marcescens*.

Major points
1. During outbreak a 2nd clone was disseminated. Which means that happened after the measures were already taken and a new clone spreads? How the Authors explain development of this second outbreak? It seems that the measures taken were not enough. What kind of hand hygiene is under use in NICU?
   
   *We agree with the reviewer, this is a critical point. Clone B has been indentified during the third surveillance procedure and was not associated with symptomatic cases. We postulated that clone B was less virulent and ubiquitous in our NICU and started to replace the clone A, as reported by David et al. Unfortunately we were not able to identify the clone from three of four of initial strains. Therefore, we can not be sure about our hypothesis (lines 205-210). Seventy percent ethyl alcohol hand sanitizer or 4% chlorhexidine gluconate solution were under use in our NICU. We added this clarification in the text (line 103-104).*

2. It is well known that contaminated parenteral nutrition may be a source of infection. Were PN samples tested for presence of *S. marcescens*?
   
   *Reviewer is right. However, most of our patients presented with ocular symptoms and we thought to look for Serratia contamination on common used items or ophthalmic medical devices.*

3. Antibiotic susceptibilities were studied but little information was given. We know that none of the isolates was resistant to carbapenem and of 34 isolates 32 were resistant to gentamycin. However no information is given about beta lactam
resistance. Authors blame the molecular methods that they used for their inefficacy but if they look for gentamycin resistance genes using primers specific for beta lactamase genes, it is sure that no positive results can be obtained. *We added details about antimicrobial susceptibility and molecular methods in the text (139-151).*

4. Authors also blame health workers from other services. They carried their hypothesis to the title. Is there any evidence (isolated S. marcescens from these health workers) for that? If there is clonality, contamination should be from one patient to another. I believe that workers in the ward are much more important for contamination than the outside workers who comes usually to see only one patient. *Referee is right. However, during our outbreak NICU staff was strictly involved in surveillance procedures. Hand sampling was performed, with negative results, and NICU staff was quickly pushed into implementing effective hand hygiene and gloves use, but we did not experiment outbreak control. Our NICU admitted mainly surgical patients, that are often visited by external consultant. We were not able to perform hand sampling from external consultants, but when they were also pushed into implementing hygiene procedures, outbreak was controlled. Hence, we empirically conclude that external consultants were involved in S. marcescens transmission (188-195).*

**Reviewer**: Raffaele Zarrilli

**Reviewer's report:**
The manuscript by Montagnani et al. aims to investigate a *Serratia marcescens* outbreak into a neonatal intensive care unit. The works should be important to the field and potentially of interest for the readers of the Journal, but suffers from an incomplete experimental design and technical inconsistencies. I am listing below specific comments that should be addressed.
- Major Compulsory Revisions
1. The design of the study should be better delineate and isolates should be described according to date, source and patients from which they were isolated. In particular, it is important to know if rectal swabs were isolated before ocular swabs in the patients enrolled in the study.

   Ok. We added table 2 describing patients, date of screening and type of samples resulted positive.

2. The results of environmental sampling, even if S. marcescens was not isolated, should be provided. It is impossible that no microbial species were isolated.

   Sampling results were reported at lines 107-109 and commented at lines 182-184.

3. To argue that hand hygiene may have played a crucial role in controlling the outbreak, the compliance of hand hygiene procedures should have been measured.

   Reviewer is right. We documented increased consumption alcohol-based sanitizer and chlorhexidine solution (lines 188-189).

4. Antimicrobial susceptibilities of the two ERIC-PCR clone identified need to be presented in detail. Are the S. marcescens isolates ESBL producers?

   Ok. We added details about antimicrobial susceptibility and molecular methods in the text (lines 139-151).

- Minor Essential Revisions

1. The study period should be detailed in the Abstract and Results section.

   Ok. We added the study period.

2. Methods section: The expression “We used the rapid and accurate PCR assay by Colom and colleagues to test the presence of three bla genes” should be
replaced by “We used the rapid and 89 accurate PCR assay by Colom and colleagues to test the presence of blaTEM, blaSHV and blaOXA genes”.

Ok. We replaced the expression.

- Discretionary Revisions

Ok. We added the reference.