Author's response to reviews

Title: Successful control of a neonatal outbreak caused mainly by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece

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Author's response to reviews: see over
Larissa, 17 March 2014

Dear Editor,

The corresponding author Dr Efthymia Petinaki and the co-authors would be grateful, if you would consider the submitted manuscript entitled << Successful control of a neonatal outbreak caused mainly by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece >> as research article in BMC paediatrics.

We confirm that the manuscript has not been submitted elsewhere for publication.

This manuscript was previously submitted in your journal (reference number 5427017301166257) and according your decision we could resubmit a corrected version.

Here are the comments and the changes according the suggestions of the reviewers.

Reviewer's report
Title: Successful control of a neonatal outbreak caused by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece

Version: Date: 20 January 2014

Reviewer: Gil Klinger

Reviewer's report:
I read with interest the paper entitled "Successful control of a neonatal outbreak caused by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece". The paper describes in detail the microbial characteristics of a neonatal outbreak caused by ESBL bacteria. The paper is well written and provides extensive microbial data.

My comments are as follows:

Major revisions
1. The paper focuses on the microbiology of an ESBL NICU outbreak. A myriad of bacteriology details is provided. The manuscript would be more balanced if it had less microbiology details and more clinical data relevant to the readers of the journal. Also some of the genetic workup uses terms that may require explanation for the pediatrician reading the journal (eg "The most widespread ESBLs belong to the TEM, SHV and CTX-M…"). The term ST is used without explaining what it means. Please add "sequence type" to the place where it is first used. Possibly add a short explanation of the importance of various ST's.
Authors’ reply:
More clinical data (e.g., details of the surveillance period, infection control measures and clinical characteristics of colonized infants by ST1114) are provided and discussed in the next sections of the article, as suggested.

In the “Background” section (lines 71–85), more information on the genetic workup is provided for clarity, as suggested. The following terms were also explained, as suggested: extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* (ESBL-Kp)–lines 76-77, beta-lactamase (*bla*) genes–line 116, sequence types (STs)–line 127.

A short explanation of the importance of the various STs is provided in the text (lines 213 and 218-221).

2. Over the last 2 years there have been a number of reports of NICU outbreaks by ESBL bacteria. These should be cited and discussed specifically regarding what differentiates the present report from former reports.

Previous reports include:

Authors’ reply:
The reports of NICU outbreaks by ESBL bacteria are cited (references 24-27) and discussed in the “Results and Discussion” section (lines 258-273), as suggested.

3. The Discussion and Conclusions segments should give more insight. Did the reported infants have any special characteristics, were they similar or different from previous reports, what standard preventive measures do the authors recommend, should NICUs be continuously surveyed for ESBL bacteria, are there any risk factors for ESBL infection and how do they compare to other reports, etc

Authors’ reply:
The neonates affected by ESBL-Kp showed low gestational age, low birth weight and use of invasive devices have been reported previously among the risk factors for acquiring ESBL-producing Enterobacteriaceae in NICUs [please see references 6, 24-27]. These comments have been added in the manuscript (lines 258-273), as suggested.

4. Have any changes been made to the NICU routines as a result of the
outbreak? What was the antibiotic policy of the NICU at the time of the study and has it changed as a result of the study?

**Authors’ reply:**
Changes made to the NICU routines, such as strict cohorting, designation of groups of nurses caring for different cohorts of infants and changes in the antibiotic policy of the NICU have been added in the manuscript, lines 170-185, as suggested.

5. For how long did surveillance continue and why was it discontinued. Benenson et al (Neonatology. 2013;103(2):155-60, Continuous surveillance to reduce extended-spectrum #-lactamase Klebsiella pneumoniae colonization in the neonatal intensive care unit.) showed that prolonged surveillance is needed and that even after 4 years cultures may be positive. Please discuss this point.

**Authors’ reply:**
Surveillance was implemented from September to December 2012. This information is added (lines 117-118) and further discussed (lines 274-285) in the manuscript, as suggested.

6. Previous reports have stated that only a small percentage of ESBL carriers develop sepsis (Rettedal et al – 1 of 51, Giuffre et al – 0 of 15). In the present report the rate of sepsis was 50% (13/26). Do the authors have insight as to why the difference in sepsis rates? Also, Table 2 shows that 13/16 infants with ST20 SHV-5 developed an infection. As 13/26 infants with ESBL bacteria had an infection, I understand that 0/10 infants with ST1114 bacteria developed infection. Does the different ST mean difference virulence of the bacteria or possibly were the populations different (maybe infants with ST1114 were of a greater birth weight, were healthier, became carriers at an older age, etc). Could this be related to the age at which ESBL bacteria was identified?

**Authors’ reply:**
Indeed 13/25 neonates affected by ESBL-Kp and 13/16 neonates affected by ST20 ESBL-Kp developed an infection. No differences in the virulence gene content among ESBL-Kp belonging to ST20 and ST1114 were observed, but several virulence characteristics (e.g. the mucoid phenotype, aerobactin production, capsular serotype) are associated with the type of infection in *K. pneumoniae*, as reported previously (please see Reference -28) . As shown in Table 2, no differences in the clinical characteristics of the neonates affected by ST20 and ST1114 ESBL-Kp were observed. These comments have been added in the manuscript (lines 258-273), as suggested.

**Minor Revisions**
1. Table 2 gives information on dates of infection that is repetitive and appears in Figure 2. Please delete dates from Table 2. Instead add day of life that ESBL was first isolated.

**Authors’ reply:** We have deleted the dates of infection from Table 2 and we have added the day of life of the infants that ESBL was first isolated, as suggested.
Discretionary Revisions
1. From the Title it is understood that a single strain caused the outbreak, but 2
different bacteria were isolated, so possible rephrase and say "Successful
control of a neonatal outbreak caused mainly by ST20 multidrug-resistant
SHV-5-producing Klebsiella pneumoniae, Greece"

Authors' reply:
Indeed, two different clones of ESBL-Kp were detected in the NICU and the title has been
changed, as suggested.

2. 2 different NICU antibiotic susceptibility patterns are described in Table 1. This
suggests that the outbreak described is not a single outbreak but possibly 2
outbreaks. Comparison to other hospital wards shows 2 other ESBL bacteria,
different from those in the NICU. So it seems that multiple ESBL bacteria exist in
the Greek hospital. The authors state that this "indicate the ongoing evolution of
ESBL-producing K. pneumoniae in our area." Do the authors know of any
reference regarding the prevalence of ESBL bacterial carriage in their area.
Could it be that some of the mothers were carriers of ESBL bacteria and that
they were the source for some of the infants and that possibly the second
outbreak originated from one of the infants mothers?

Authors' reply:
Indeed, the antibiotic susceptibility patterns in the NICU described in Table 1, corresponded to
two different bacterial clones (BOX-PCR profiles P1 and P2 and MLST STs 20 and 1114,
respectively), which were not genetically related to each other or other ESBL-Kp isolated from
other wards of the hospital. Furthermore, surveillance cultures from the hands of the nursing staff
and environment were negative for ESBL-Kp, and therefore, we were not able to identify the
source of the outbreak. Unfortunately, there are no reports for the prevalence of ESBL bacterial
carriage in our area, so as we can provide such data. It could be possible that some of the mothers
were carriers of ESBL bacteria and that they were the source for some of the infants and that possibly the second
outbreak originated from one of the infants mothers. We have added these
insightful remarks in the discussion section –lines 227-230 and 234-246), as suggested.

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's report
Title: Successful control of a neonatal outbreak caused by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece

Version: Date: 14 February 2014
Reviewer: Samuel Zangen

Reviewer's report:
What was the frequency of the screening during the outbreak period in the NICU?
how many new cases were acquired each week/month for infections and carriers? what was the percentage of new carriers out of the total amount that were screened during the outbreak period?
It is not possible to understand the outbreak!
not enough information about the measures that were implemented (Audits, breast milk kitchen, cohorting policy ...)

Authors' reply:
The frequency of screening (in a weekly basis during September-December 2012). This information is added (lines 117-118) and further discussed (lines 274-285) in the manuscript, as suggested.
Information about the measures that were implemented, such as strict cohorting, designation of groups of nurses caring for different cohorts of infants and changes in the antibiotic policy of the NICU, have been added in the manuscript (lines 170-185), as suggested.

The new cases acquired each week/month for infections and carriers are provided in Table 2, where we have also added the cases and the clinical data of colonized infants by ST1114 ESBL-Kp.

The percentage of new carriers out of the total amount that were screened during the outbreak period was 19.35%, and it was added in the text as suggested (lines 277)

Level of interest: An article of limited interest

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.

Authors' reply:
We did not perform a case-control study, but a descriptive analysis and molecular characterization of the outbreak by microbiological and molecular methods. Nevertheless, the range and the mean values for the gestational age, age at first isolation of ESBL-Kp (days), length of stay (days, weight at birth (g) are provided in Table 2.
Declaration of competing interests:
'I declare that I have no competing interests'
Reviewer's report
Title: Successful control of a neonatal outbreak caused by ST20 multidrug-resistant SHV-5-producing Klebsiella pneumoniae, Greece

Version: 1 Date: 1 February 2014
Reviewer: Imad R Makhoul

Reviewer's report:
Minor Essential Revisions

Level of interest: An exceptional article

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'

When assessing the work, please consider the following points:
1. The aim posed by the authors is well defined, i.e., to describe how they were able to contain an outbreak of virulent ESBL-Kp in the NICU.
2. The methods are highly appropriate and well described. The meticulous molecular characterization of the infecting ESBL-Kp strain and its virulence gene is highly appreciated.
3. The data are rock sound and indicate high professional work by their bacteriology laboratory and the infectious disease and neonatology staff.
4. The manuscript adheres to the relevant standards for reporting and data deposition.
5. The discussion and conclusions are well balanced and adequately supported by the data. The conclusion statement can be shortened.
6. The authors clearly acknowledge any work upon which they are building, both published and unpublished.
7. The title and abstract accurately convey what has been found.
8. The writing is acceptable.
9. Tables and figures are appropriate.
Sincerely yours,

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