Title: KPC - 3 Klebsiella pneumoniae ST258 clone infection in postoperative abdominal surgery patients in an intensive care setting: analysis of a case series of 30 patients.

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

Manuscript Paper number MS:5973459038450946

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Authors: Paola Di Carlo, Gulotta Gaspare, Casuccio Alessandra, Gianni Pantuso, Maurizio Raineri, Clizia Airò Farulla, Sebastiano Bonventre, Giuliana Guadagnino, Daniela Ingrassia, Gianfranco Cocorullo, Caterina Mammina and Antonino Giarratano

The Authors thank the reviewers for providing constructive comments and help in improving the contents of the paper.

As explained in the details below, the authors have made several changes in order to take into account the constructive remarks of both reviewers. All changes in the new version are highlighted in yellow.

The English writing has been revised and manuscript conforms to the journal style.
Reviewer's report

Title: KPC - 3 Klebsiella pneumoniae ST258 clone infection in postoperative abdominal surgery patients in an intensive care setting: analysis of a case series of 30 patients.

Version: 3 Date: 4 February 2013

Reviewer: Antonella Agodi

Reviewer's report:

The present paper is certainly of interest to the scientific community and the study design is appropriate to achieve the objectives, it describes a well-constructed study and provides interesting findings on clinical outcome of ICU patients with severe infections caused by Klebsiella pneumonia. The methodology is appropriate. However, the scientific message may be improved by the following corrections:

Minor Essential Revisions

Background section

• Please provide, if possible, other reference on the emergency of Kpc ST258 outbreaks especially in Italy.

In order to take into account this important suggestion of the reviewer, other reference was added as follows and cited in Background and Discussion section:


• The objective(s) of the study is not stringent and clear. The objective(s) of the study should be better stated, please clarify.

In order to take into account this important suggestion of the reviewer and the other suggestions of second referee, the authors have modified all sections of the Abstract (All changes in the new version are highlighted in yellow) and the Background paragraphs as follows:

Abstract.

Objective: We describe the clinical course of 30 Intensive Care Unit (ICU) patients who underwent abdominal surgery and showed severe infections caused by Klebsiella pneumoniae sequence type
(ST) 258 producing *K. pneumoniae* carbapenemase (KPC-Kp). The aim was to evaluate risk factors for mortality and the impact of a combination therapy of colistin plus recommended regimen (100 mg initially, followed by 50 mg every 12 hours) or higher dosage of tigecycline.

**Methods:** A prospective assessment of severe monomicrobial KPC-Kp infections occurring after open abdominal surgery carried out from August 2011 to August 2012 in the same hospital by different surgical teams is presented. Clinical and surgical characteristics, microbiological and surveillance data, factors associated with mortality and treatment regimens were analyzed. A combination regimen of colistin with tigecycline was used. A high dose of tigecycline was administered according to intra-abdominal abscess severity and MICs for tigecycline.

**Results:** The mean age of the patients was 56.6 ± 15 and their APACHE score on admission averaged 22.72. Twenty out of 30 patients came from the surgical emergency unit. Fifteen patients showed intra-abdominal abscess, eight anastomotic leakage, four surgical site infection (SSI) and three peritonitis. The **overall crude ICU mortality rate** was 40% (12 out of 30 patients). Twelve of the 30 patients were started on a combination treatment of high-dose tigecycline and intravenous colistin. A significantly lower mortality rate was observed among those patients compared to patients treated with recommended dosage of tigecycline plus colistin. No adverse events were reported with high doses of tigecycline.

**Discussion:** Critically-ill surgical patients are prone to severe post-surgical infectious complications caused by KPC-Kp. Timely microbiological diagnosis and optimizing antibiotic dosing regimens are essential to prevent worse outcomes. Further studies and well-controlled clinical trials are needed to define the optimal treatment of infections by KPC-Kp and, more generally, carbapenem-resistant bacteria.

**Background section.**

The aim of this study was to describe the clinical aspects of surgical KPC-Kp infections in patients who had undergone emergency or elective abdominal surgery. **Risk factors for mortality and the impact of a combination therapy of colistin plus recommended or higher dose of tigecycline on the patients’ clinical course were evaluated.**

- **Methods Section:** The Authors stated that “An active surveillance program including routine surveillance cultures and screening of high risk patients on admission is being carried out in the Surgical Emergency Unit since January 2010.” Please provide references if a standard international protocol was implemented.

During the studied period no standard international protocol was implemented but in order to take into account this important suggestion of the reviewer, the authors have modified the sentence in the Material and methods paragraphs to explain our surveillance program in surgical setting:

Page 4, Line 4-6
A 3-monthly serial surveillance program for multidrug resistant Gram negative bacilli, including active surveillance cultures, has been carried out in the Surgical Emergency Unit since January 2010. High-risk patients are routinely screened on admission.

- **Discussion Section**

  - Please refer to limits of the study such as the small size of the sample.

In order to take into account this important suggestion of the reviewer, the following sentence was modified as follows (page 10, line 20-22):

Our study is not without limitations. It includes a small number of patients with very complex and heterogeneous conditions, as well as the bias associated with non-random enrollment, as the choice of treatment was based on the patients’ medical conditions.
Reviewer's report

Title: KPC - 3 Klebsiella pneumoniae ST258 clone infection in postoperative abdominal surgery patients in an intensive care setting: analysis of a case series of 30 patients.

Version: 3 Date: 27 February 2013

Reviewer: David van Duin

Reviewer's report:

Major Compulsory Revisions

1. Methods should clarify which method of antimicrobial susceptibility testing was used for which antibiotic.

In order to take into account this important suggestion of the reviewer, the following sentence was added to the Microbiological methods section (All changes in the new version are highlighted in yellow):

Identification (ID) and antimicrobial susceptibility testing (AST) were routinely performed using a microdilution method (BD Phoenix™ Automated Microbiology System, Sparks, MD, US). Etest (bioMérieux) was used to determine susceptibility to colistin. Susceptibility and resistance categories were assigned following EUCAST guidelines.

Phenotypic confirmation of the presence of carbapenemases or overexpression of AmpC in combination with porin loss was obtained using a commercial synergy test (Rosco Diagnostica, Taastrup, Denmark). This test was carried out on putative carbapenemase-producing K. pneumoniae isolates based upon their screening cut-off values for meropenem, according to the recommended methods for detection of carbapenemases in Enterobacteriaceae [5-7, 10].

2. Tigecycline MIC values of less or equal to 1 mg/L are considered susceptible by EUCAST. Do the authors have support for treating 0.8-1 differently?

In order to take into account this important suggestion of the reviewer, the following sentence and reference were added to the Methods section:

Because tigecycline MICs between 0.8-1 µg/ml are close to the upper limit of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility range, they were considered suboptimal and taken into account when making this decision (http://www.eucast.org) [9].

3. In general, the organization of the results section is chaotic and should be improved.

We have accepted the suggestion and the section has been revised.

4. Mortality "due to infection" is listed in results, but the methods do not describe how this was defined.

In order to take into account this important suggestion of the reviewer, the following sentence was added:

In order to take into account this important suggestion of the reviewer, the section of statistical analysis have been revised and the following sentences were added in the results section.
Statistical analysis
Frequency analysis was performed with chi-square test or Fisher's exact tests, as needed. Univariate analysis of variance (ANOVA) was used for parametric variables. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated for associations with demographic and clinical variables. The continuous variables found to be independent predictors of mortality in the ICU were assessed using a linear regression model, and slope coefficients with their standard error were reported. Kaplan-Meier curve (log rank test) was plotted to estimate the cumulative incidence of mortality.

Results were expressed as mean ± standard deviation (SD) or median [interquartile range (IR)] for continuous variables or as percentages for categorical variables. Two-tailed tests were used and P values less than 0.05 were considered to be statistically significant. Data were analyzed using Epi Info software, version 3.2.2, (Centers for Disease Control and Prevention) and SPSS software (version 14.0; SPSS Inc., Chicago, IL, USA).

Result section (page 7 line 23-25 and page 8 line 1-2)
Risk factors for mortality in the ICU were assessed (Table 2). Using univariate analysis, a better outcome was associated with the presence of a surgical drainage, and a worse one with a higher APACHE II score and VAP. Treatment with high doses of tigecycline was associated with lower mortality (P=0.005). Kaplan-Meier curve showed that patients treated with high doses of tigecycline had a significant favorable outcome (log-rank test, p=0.0035) (Figure 2).

6. "Treatment with high doses [...] abscess". what does "favorable outcome" mean here? Please, see the point 5.
7. The last paragraph of the results are not results of the study and should be removed or moved to the discussion.
In order to take into account this important suggestion of the reviewer, the paragraph has moved to the discussion (page 11, line 7-13)

Minor Essential Revisions

- Please include units where applicable. For example age in years (first page of results)
- This has been done.
- The sentence "half of these patient […] 2 years." is unclear and should be rephrased or removed.

The sentence has been rephrased as follows:

Fifty percent of these patients had been hospitalized in the previous two years.

- Include space between "out of"
- This has been done

The English writing has been revised.