Author’s response to reviews

Title: Molecular Characterization and Epidemiology of Carbapenem Non-susceptible Enterobacteriaceae Isolated from the Eastern Region of Heilongjiang Province, China

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Author’s response to reviews:

Dear Iris Spiliopoulou, Ph.D

I am very happy to receive your email. Thank you all for giving me advice, especially Myrto Christofidou. I think this is an encouragement and affirmation for me. I have modified it according to the editor's opinion. I hope you will get a positive response. The following is my reply to the editorial comments one by one. The revised manuscript has been marked in blue.

Technical Comments:
- Please remove duplicate tables and figures.

Reply: Duplicate tables and data have been removed.

Editor Comments: The manuscript can be accepted for publication, after a second minor revision concerning the language. Please, check all comments and correct this article again from a native speaker of English language.
Reply: The article has been revised.

Reviewer reports:

Myrto Christofidou (Reviewer 1):

The revised manuscript "Molecular characterization and epidemiology…” by Gong Xue et al, is significantly improve now, but some more revisions are still needed.

Please see below:

- Discussion must be read again with caution. Some sentences from "Results" are repeated identically in 'Discussion'

Reply: Some sentences have been modified and deleted.

- Genus and species of bacteria should be mention complete only the first time, for example Klebsiella pneumoniae, K. pneumoniae, throughout the text

Reply: It has been revised.

- Numbers 1-10 should be written in words, throughout the text

Reply: It has been revised.

Minor revisions

PAGE 5

Please write

Line 42 "Gentamicin and tobramycin…”

Reply: It has been revised.

Line 45 "Less than 30% resistance was detected…”

Reply: It has been revised.

Line 48 "Among CNSE 52(98.1%) and 48(90.6%) …”

Reply: It has been revised.

Line 59 "…was transferred…”
Reply: It has been revised.

PAGE 6

Please write…

Line 6 "A new one ST 2946 was identity by this study"

Reply: It has been revised.

Line 20 "of the multi drug-resistant bacteria (MDR)"

Reply: It has been revised.

Line 23 "Epidemiologic typing"

Reply: It has been revised.

Line 34 "MDR bacteria"

Reply: It has been revised.

Line 39 "MDR gram negative bacteria" and in the end of line add ",(CRE)"

Reply: It has been added.

Line 42 …instead of "has caused" write "is responsible for …"

Reply: It has been revised.

Near to reference 1, it will be useful to add another one (JAC, 67(12):2976-2981, 2012), and if needed, make the corrections of new numbers of references, throughout the text

Reply: References have been added and the order of references has been adjusted.

…instead of "to carbapenems in Enterobacteriaceae" write "of Enterobacteriaceae to carbapenems"

Reply: It has been revised.

Line 56 "…that accompanied more rarely with loss of outer membrane proteins"

Reply: It has been revised.

Line 59 please delete "the mobile"

Reply: It has been revised.
Please write…

Line 9 "epidemiologic characteristics" and delete "of CNSE"
Reply: It has been revised.

Line 25 please delete "with over" and write ", a 1600 beds hospital"
Reply: It has been revised.

Line 28 please delete "to be" and write "isolates confirmed as Carbapenems-nonsusceptible"
Reply: It has been revised.

Lines 28-37 please replace the sentence by… "As CNSE were considered only those isolates that confirmed as Carbapenems-nonsusceptible (either of ertapenem, imipenem or meropenem) according to Clinical and Laboratory Standard Institutes (CLSI-2016) criteria. "
Reply: It has been revised.

Line 39 please replace "testing" by "test"
Reply: It has been revised.

Line 45-53 please delete "strains separated in our hospital" and write "…CNSE isolates were evaluated using modified carbapenem inactivation test(mCIM). (please write more about this test? Reference?) The modified Hodge Test was used to screen isolates for the production of carbapenemases, according to CLSI 2016 guidelines"

Line 59 please delete line 59 and write: "five colonies of each CNSE isolate from overnight culture plates, suspended….."
Reply: The steps of the mCIM experiment are briefly described and references added.

Please write…

Line 3-6 "and after 100 μl of...Eppendorf tube. " ,please delete "which" and "EP"
Reply: It has been revised.

Line 31 For 'Conjugation experiments' add a reference, an article or a book of Bacterial Genetics
References have been added and the order of references has been adjusted.

PAGE 9

Please write…

Line 25-26 "From October 2015 to July 2017 a total of 53 non-duplicated CNSE", please delete "From this research" and "isolates of"

Reply: It has been revised.

Line 27-29 please delete "The CNSE strains isolated" and write "were isolated one from each patient, with age range (5 months - 89 years, median 60.5 years). Among 53 patients 34(64.2% were male)".

Reply: It has been revised.

Line 32-39 "The mortality of patients with CRE infections was 28.3%. Three patients were excluded; one outpatient who had no hospital records and two nosocomial patients who had no longer contact. The majority….

Reply: It has been revised.

Line 42 while (instead of "and") C. freundii

Reply: It has been revised.Since Citrobacter Freundii is the first time it appears, it is not abbreviated as C. freundii.

Line 44 "specimen with positive culture for CNSE, included sputum…….."

Reply: It has been revised.

Line 46 "urine and pus" delete "cultures"

Reply: It has been revised.
Line 48-52 "The CNSE isolates emerged from Neurosurgery..., Hematology 9.4% (n=5) and other Departments."

Reply: It has been revised.

PAGE 10

Please write...

36-38 "...all CNSE isolates were MDR (Table 1). The CNSE isolates were resistant to ampicillin.........and cefepime (100%), to amikacin (7.5%), to ciprofloxacin(39.2%) and levofloxacin (24.5%), to aztreonam (96.2%) and to gentamicin and tobramycin (88.7 and 83% respectively)

Reply: It has been revised.

PAGE 12

Please write...

Line 37 "All of 53 isolates.....genes, 42(79, 2%)", please delete "in this study: of the total 53 isolates"

Reply: It has been revised.

Line 39-51 please delete 'all of 40 K. pneumoniae produced KPC-2 enzymes except for three' and write"Six isolates (11.3%) including three E. cloacae, two K. pneumoniae and one K. oxytoca... Seven isolates (..%) produced NDM carbapenemase. " Delete "were identified" and write, "one .....and two.......two....., one.... one...")"

Reply: It has been revised.

Line 51 'ESBL genes were found in 43(81.1%)'...

Reply: It has been revised.

Line 57 'Isolates carrying at least two.....'

Reply: It has been revised.
Line 58 "most isolates (%) carried simultaneously the……genes"
Reply: It has been revised.

PAGE 13
Please write…
Line 7 "Forty seven….", delete "The 47"
Reply: It has been revised.
Line 9 "The conjugation experiments showed" delete "results of"
Reply: It has been revised.
Line 11 "from ten CNSE isolates" delete "from CNSE"
Reply: It has been revised.

PAGE 14
Please write…
Line 1 please delete "from both inpatients outpatients"
Reply: It has been revised.
Line 47 please delete "results"
Reply: It has been revised.
Line 53 please delete "bacteria"
Reply: It has been revised.
Line 53 please write "As the mortality in present study was found as high as 22% (11/50), it was necessary…."
Reply: It has been revised.
As it is well known, you can include one more reference, (Spyropoulou A et al, j. of Chemotherapy, Jan 2015).

In our study four E. coli, two E. cloacae, and one K. pneumoniae were the seven isolates carrying blaNDM. Among them, three were blaNDM1 positive, three others blaNDM 5 and one bla NDM7.

In the present study, CNSE......different types (delete "other").....beta-lactamase (delete "lactase")

bla kpc-2 gene (79.2%) was the most common. (Please delete your sentence)

Delete "the"

This and "false positive results"

Delete "the"
Line 56-58 "…carbapenemase positive isolates 48 (90.6%). Among them only four isolates were bla NDM positive and six others were bla IMP"

Reply: It has been revised.

PAGE 18
Please write…
Line 1 Delete "…respectively. Numerous of " and write "Many studies"
Reply: It has been revised.
Line 37-39 Delete "that" and "had been"
Reply: It has been revised.

PAGE 19
Please write…
Line 31 "Infraction"
Reply: It has been revised.

PAGE 20
Please write…
Line 3 "As it is well known"
Reply: It has been revised.
Line 6 delete "an"
Reply: It has been revised.

Matthaios Papadimitriou-Olivgeris (Reviewer 2): Authors properly responded to my remarks
Needs some language corrections
Reply: The manuscript has been revised.

Best wishes,

Xiaoli Zhang