Author’s response to reviews

Title: Fecal carriage and molecular epidemiology of carbapenem-resistant Enterobacteriaceae from outpatient children in Shanghai

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Author’s response to reviews:

Dear Prof. Cecilia Devoto,

Thank you for your letter. We have acknowledged your and the reviewer’s comments and constructive suggestions, which are valuable for improving the quality of our manuscript. Based on your helpful comments and request, we have revised the manuscript raised by the reviewers, and the amendments are made in the revised manuscript. Then some explanations regarding the revisions of our manuscript are as follows.

Reviewer reports:

Paul Lephart (Reviewer 2): I appreciate the improvements that have been made in response to the reviewers' comments. However, despite finding value in the data contained within this manuscript, I still do not find this manuscript to be of an acceptable quality for this journal at this stage and must recommend that major revisions are still required. As a reviewer with no stake in the authorship of this manuscript, I do not wish to rewrite this manuscript for you line by line but will try point out in detail below the key instances where as a native English speaker I find the writing and key points to be difficult to understand.
Outstanding issues:

* Page 3, line 4: A closer review of your first reference begs the question as why you used a pharmacology technical manuscript on carbapenems from 2003 as your reference for the statement that "carbapenems ..... are often recommended as antimicrobials of last resort especially in cases where extended-spectrum β-Lactamase (ESBL) producing organisms involved". A quick literature search uncovers many other more recent and clinically relevant manuscripts that better make that point.

Answer: Thank you your advice. We have cited the recent reference for this statement. Changes have been made in the revised version (Page 3, Line 4).

* Page 3, line 29: Epidemiological information including patient demographics, prior hospitalization, and previous receipt of antibiotic therapy, and invasive operation during hospitalization was obtained from the medical records of each patient

* Page 6, line 3: By analyzing the patient data, the CRE strains were isolated from in 32 individual children (19 male and 13 female) whose mean age was 1.3±1.5 years (include range)

* Page 6, lines 8-11. "Significant difference (P<0.05) was observed in the era about the prior hospitalization and previous receipt of antibiotic therapy between CRE carriers (28.1%, 9/32) and non-carriers (4.5%, 38/848)."

This statement is hard to comprehend and is an oversimplification of the presentation of some very interesting data! I believe you are trying to state here that out of your 880 patients, only 47 had been hospitalized and received antibiotics within the past 3 months. And of those 47 children, 9 were ultimately found to be CRE carriers and 38 were non-carriers. This should be stated then that of the 32 CRE carriers and 848 non-CRE carriers identified, 9 (28.1%) and 38 (4.5%) children, respectively, had been hospitalized and received antibiotics within the past 3 months (P<0.05).

Answer: Thank you your advice. Maybe this statement is hard to comprehend and do not reach your request. So we have changed this statement and changes have been made in the revised version (Page 6, Line 7-9).

Furthermore, as requested by reviewer #1 in his initial review and included in your response but not in the text of the manuscript, you should include the data regarding the min, max and mean time from last admission of those children with recent hospitalization. Whether or not there is a difference between those mean times between the CRE carrier group and non-CRE carrier group with hospital exposure is interesting information!
Answer: Thank you your advice. As requested by reviewer #1, we have provided an explanation about this suggestion. Among 9 children with CRE positive in fecal had a medical past history of hospitalization and the min, max, mean duration between last admission was 3 days, 7 days, and 4 days, respectively. Furthermore, the min, max, mean times of non-CRE carrier group with hospital exposure are 2 days, 10 days, and 5.1 days and there is no significant difference between the CRE carrier group and non-CRE carrier group. But we still consider that a history of prior hospitalization is a risk factor for children to acquire the CRE strains colonized in gastrointestinal tract. Thank you.

* Page 6, line 10: Among the nine previously hospitalized CRE carriers children, only two children had received an invasive operation during their hospitalization.

* Page 6, line 14: The results of the antimicrobial susceptibility testing of the 32 CRE strains are were shown in Table 1.

* Page 6, lines 16-29: Use of the word "susceptible" should be changed to "susceptibility".

* Page 6, line 17: Tigecycline remained retained excellent activity.…

Answer: Thank you your advice. Changes have been made in revised manuscript (Page 6, Line 10, 14, 16, 17-19).

* Page 7, line 17: To the best of our knowledge, knowing about investigation of the fecal carriage prevalence of CRE among outpatients from the community setting can help us to better understand the origin of CRE isolates responsible for outbreak events and contribute to control CRE dissemination.

* Page 7, line 20: Our study displayed that the rate of colonization with CRE in fecal samples collected from children in outpatient visits was 3.6%, which was lower than what had been reported in other countries (from what populations in the other countries??) [10, 19].

* Page 7, line 21-22: Several reasons contribute to this phenomenon of the colonization of CRE isolates in rectal gastrointestinal tract.

Answer: Thank you your advice. Changes have been made in revised manuscript (Page 7, Line 18, 19, 22, 23, and 25).

* Page 7, line 25: Previous studies reported that exposure to hospital setting or/and antimicrobial agents might increase the risk of colonization and it would be easier to acquire
CRE isolates, which may also explain also make an explanations of the occurrence in community-onset cases

* Page 7, line 30: Empirical use of antimicrobial agents? always be result in exposure to antibiotics, which ? may increase the risk rates of colonization of CRE. (Reference?)

Answer: Thank you your advice. It is incorrect to describe this sentence and we have deleted it. (Page 8, Line 2)

* Page 8, line 2: Last but only not least, CRE strains could spread via physical contact with other people and have the propensity to acquire genetic materials mostly in the form of plasmids and transposons, through horizontal gene transfer

o What kinds of "genetic materials" specifically? And why does that matter to this paper? Person to person spread from patients who are bringing CRE to the community from hospital exposure is a concern indeed, but be specific if you are talking on the potential spread of resistance genes from these community CREs to other susceptible organisms in the community via horizontal gene transfer.

Answer: Thank you your advice. The genetic materials such as plasmids and transposon maybe play important roles in in transmission of organisms. As the reference 22 mentioned that KPC-2 K. pneumoniae strains readily spread via physical contact as well as contaminated food and water and have the propensity to acquire genetic materials, mostly in the form of plasmids and transposons, through horizontal gene transfer.Recently, several reports indicated that intestinal carriage of KPC-2-KP plays an important role in transmission of this organism. K. pneumoniae is a ubiquitous colonizer of the human intestinal tract, which may serve as a reservoir from which KPC-2-KP strains are disseminated in health care settings. Therefore, we make a assumption that whether this this phenomonon could happen in community setting. And this assumption will be conducted for further analysis. Changes have been made in the revised version (Page 8, Line 6-8).

* Page 8, line 4: Also travelling in high endemic areas has been closely associated with acquisition of CRE, which are always result in this phenomenon of colonisation colonization in fecal sample.

o After stating "last but not least", do not include another reason below that in the text…. integrate this statement above this in the text, perhaps with the statement that CRE can spread person to person
"which are always result in this phenomenon of colonization in fecal sample" - this language is unclear and I do not know how to reword it

Include a reference here attesting to how travelling in high endemic areas is associated with acquisition of CRE colonization

Answer: Thank you your advice. Associated sentence has been removed (Page 8, Line 8-10).

Page 8, line 7: The surveillance definition of a for CRE are designed that is defined as an Enterobacteriaceae are that is resistant to imipenem, meropenem, doripenem, or ertapenem or is documented that the isolate to possess a carbapenemase. and CRE are reported to be most commonly identified in K.pneumoniae, E.coli, and E.cloacae.

Page 8, line 10: The major resistant resistance mechanism of CRE is…

Page 8, line 12: The NDM type, including NDM-1 and NDM-5, is found to be the key carbapenemase responsible for mediating development of the carbapenem resistance phenotypes in children

Requires a reference for this statement

Answer: Thank you your advice. A reference for this part has been added in this manuscript (Page 8, Line 18).

Page 8, line 14: NDM-1 and its minor variants, as a class B carbapenemase first clinically isolated from a patient at a hospital in New Delhi, India, have has since been identified all over the world and always only detected in E.coli and K.pneumoniae

Page 8, line 16: Consistent with the above findings, most of the E.coli and K.pneumoniae colonized CREs found in the fecal samples in the present study harbored the blaNDM gene and a previous study in our hospital also has reported the an outbreak of CRE strains caused by NDM-1 producing K.pneumoniae among neonates.

Page 8, line 20: As is known to all, NDM-1 producing K.pneumoniae are highly resistant pathogens with no effective beta-lactams, including recent ones such as ceftolozane tazobactam and ceftazidime avibactam and the only one that works are is aztreonam

This statement is near exact wording to a suggestion provided by Dr Davido in his initial review, is this ok with him?
Answer: Thank you your advice. Changes have been made in the revised version (Page 8, Line 20, 22). This statement is recommended by Dr Davido and he suggests us to add this statement into this manuscript.

* Page 9, line 1: ….several STs were clearly responsible for specific strains, which were prevalence all over the world.

o Unclear what you mean by this statement.

Answer: Thank you your advice. We want to state that several STs were clearly detected in specific bacteria. For example, ST11 were major in K.pneumoniae not in E.coli. Changes have been made in the revised version (Page 9, Line 5).

* Page 9, line 5: Additionally, another carbapenem resistant K.pneumoniae harboring blaNDM-1 belonged to ST37 and this ST had been identified previously during an outbreak of this phenotype in our hospital.

o Was this associated with one of the children who had recent hospital/antibiotic exposure?

Answer: Thank you your advice. Two carbapenem resistant K.pneumoniae harboring blaNDM-1 belonged to ST37 were detected in study. Only one child had a history of prior hospitalization or receive therapy with antibiotics in recent three months. But the original of CRE still be unknown. Therefore, we do not know whether this associated with one of the children who had recent hospital/antibiotic exposure or not.

* Page 9, line 9: The ST101 type of E.coli produced NDM-1 carbapenemase, a phenotype that has been linked to nosocomial transmission in Korea

o Again, were these strains hospital/antibiotic exposure linked?

Answer: Thank you your advice. Maybe it is a good point that we should pay more attention to. In China, the resistant rate of carbapenem in E.coli is still low (<5%). And we have not investigated the molecular epidemiology of carbapenem resistant E.coli in our hospital and do not know that the ST101 type of E.coli produced NDM-1 carbapenemase was detected. Then this child did not have a history of prior hospitalization or receive therapy with antibiotics in recent three months. Therefore, we don’t know that whether these strains were linked to hospital/antibiotic exposure.
Deeply and Importantly, children with CRE strains in fecal samples are considered as a high risk group, which can spread CRE by intimate contact and travel.

Considered by who as a high risk group and high risk for what? Are they more likely to spread CRE by intimate contact and travel and if so, reference that data.

Answer: Thank you your advice. Children with CRE strains in fecal samples are considered as a high risk group by WHO, and CDC of USA. And intimate contact and travel may contribute to the spread of CRE strains. Reference has been added in revised manuscript (Page 9, Line 17).

The origin of CRE isolates in these children remains unknown and it is the main drawback limitation of this study that we don't know if the the source of the described CRE carriage of in these children is not the result of an adult transmission in the community or in the hospital.

This hypothesis must be confirmed over time with the screening of the close family

What hypothesis? If you are stating that you think their carriage is coming from exposure to adult carriers, is this happening in the hospital or in the community? Your data seems to provide evidence that a significant percentage of carriers have a history or recent hospital/antibiotic exposure. You provide no data regarding adult family exposure as a risk factor for CRE acquisition so why mention that as a hypothesis at this stage of the manuscript?

Answer: Thank you your advice. Changes have been made in the revised version (Page 9, Line 19-21).

Also we know that children (are burden for multi-drug organism carriages?) and further studies should be carried out to (figure out evaluate the phenomenon?).

Reference?

I do not understand this sentence

Answer: Thank you your advice. Children are easy to acquire multi-drug organisms and become the multi-drug organism carriages. Therefore, further studies should be carried out to figure out evaluate the phenomenon of the original source of CRE strains. And there is no reference about it. Changes have been made in the revised version (Page 9, Line 24).
Page 9, line 21: In summary, the current data reveal the prevalence of CRE colonization in fecal sample from pediatric outpatients and strategies to control the dissemination of antimicrobial resistant isolates from stool to other sterilized sites should be developed.

Page 9, line 23: Furthermore, the origin of CRE isolates in these children remains unknown.

Do not repeat text you already wrote above in the discussion (page 9, line 13) a few lines later in the conclusion.

Answer: Thank you your advice. Changes have been made in the revised version (Page 9, Line 30).

Page 9, line 24: Such data must be confirmed over time with the screening of the close family considering the risk of transmission from and to adults.

This statement is near exact wording to a suggestion provided by Dr Davido in his initial review, is this ok with him?

Who else could be screened? As hospitalization/antibiotic exposure is a risk factor, perhaps children should be screened at discharge? Or are they more susceptible to picking CRE up in the community from after hospital/antibiotic exposure?

Answer: Thank you your advice. This statement has been removed from this manuscript. Children who had hospitalization exposure should be screened for CRE at discharge, which may decrease the occurrence and transmission of CRE in children group. Changes have been made in the revised version (Page 9, Line 30 and Page 10, Line 1-2).

We hope that the revised version of the manuscript will meet the publication standard of BMC Infectious Diseases. We are looking forward to hearing from you soon.

Thank you and all the reviewers for the helpful advice.

Best wishes,

Sincerely yours,

Hong Zhang.

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