Author’s response to reviews

Title: Prospective study on human fecal carriage of Enterobacteriaceae possessing mcr-1 and mcr-2 genes in a regional hospital in Hong Kong

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We would like to thank Dr. Habip Gedik and Dr. Shetty Ravi Dyavar for their invaluable comments on our work. Our response to their comments are as follows:

Reviewer 1: Dr. Habip Gedik

1. The selection of persons who underwent fecal analysis was not described in the study. We have added information regarding sample selection in both ‘Abstract’ (Line 29-31) and ‘Methods’ (Line 81-90). Briefly, selection of experimental subjects was in blinded fashion, with 672 consecutive fecal specimens collected from 616 individuals between October 31 and November 25, 2016. These specimens were submitted to our hospital for routine testing, and the leftover samples after testing were used for surveillance screening. There were 3 main types of experimental subjects, including inpatients, outpatients and asymptomatic clients coming for health assessment. We routinely perform vancomycin-resistant enterococci (VRE) screen for inpatients who have been hospitalized in/ outside Hong Kong, underwent VRE screening in other hospitals, undergone surgical operation overseas or staying in a nursing home within past 6 months. In this cohort of 144 inpatients, 40 have been subjected to VRE screen. We have described the available clinical/ epidemiological information of the mcr-1 carriers in Results & Discussion (Line 187-194). For occupation, the clients/ patients of our hospital, including the 14 mcr-1 carriers, were not farmers or animal breeding people.
2. MIC values of the isolates were found with VITEK and Etest, but those methods are not reference methods for colistin. We have determined the colistin MICs using reference method (broth microdilution, BMD), ranging from 2 to 4 g/mL. The results are summarized in Table 1. We have also compared and discussed BMD and Vitek 2 AST results (Line 230-235).

3. The time when this study was performed was not described. We have provided the time in ‘Abstract’ and ‘Methods’ (Line 30-31, 82-83).

4. Those results should have been compared with colistin-resistant isolates that caused infections. None of the 14 mcr-1 positive E. coli has caused clinical infection. The range of colistin MICs determined in this study was 2 to 4 g/mL, which appeared to be narrower than that of mcr-1-positive E. coli isolates causing human infection (4 to 16 g/mL) reported by various research groups (Line 227-229). We have further looked for the presence of plasmid replicons from WGS data of all mcr-1-positive isolates in this study. The plasmid incompatibility groups present in these isolates were diversified. With reference to a recent study [5], significant geographical clustering was observed with regional spread of IncI2 and IncHI2 types of mcr-1 plasmids in Asia and Europe, respectively, while there was no significant correlation with sources of isolation (Line 284-296).

5. The title of study is not understandable, so might be more understandable. The title has been amended to ‘Prospective study on human fecal carriage of Enterobacteriaceae possessing mcr-1 and mcr-2 genes in a regional hospital in Hong Kong’.

6. The discussion part is very long and complicated, might be better designed. We have restructured ‘Results & Discussion’. Subheadings were added to enable clear presentation of contents.

Reviewer 2: Dr. Shetty Ravi Dyavar

1. Introduction needs more description of mcr-1 plasmid containing E. coli and their significance and geographical distribution and show the importance of the current study. We have added more information in ‘Introduction’ (Line 62-69) and ‘Results & Discussion’ (Line 201-205, 284-296). For human fecal carriage of mcr-1-possessing Enterobacteriaceae, prevalence estimated in this study was lower than that of mainland China while higher than several European countries. Regarding plasmid incompatibility groups, recent study [5] has unraveled significant geographical clustering with regional spread of IncI2 and IncHI2 types of mcr-1 plasmids in Asia and Europe, respectively. In our study, the plasmid replicon types
of the isolates were diversified and not confined to IncI2, yet further large-scale study is needed to determine the characteristics of local mcr-1-carrying E. coli isolates.

2. The structural maps of the IncP-1 and IncX4 types of mcr-1 plasmids should be presented as figures in the manuscript. We have constructed the structural maps for these plasmids, which are shown in Figure 1 and 2.

3. In the abstract and also when noted for the first time in the introduction, full length names of mcr-1 and mcr-2 genes, MIC, ESBL need to be written. We have corrected them accordingly.

Thank you for your kind attention and we are looking forward to your favorable reply.