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The dissemination of bla_{NDM-5} was mediated by both vertical (*Escherichia coli* ST-167) and horizontal (an IncX3 plasmid) transfer

Yu Feng^{*1}, Zhiyong Zong¹, Xiaoxia Zhang¹

¹West China Hospital, Sichuan University

Background: A 3-year surveillance for carbapenem-resistant Enterobacteriaceae (CRE) in a hospital during 2014 to 2016 has revealed a potentially persistent dissemination of bla_{NDM-5} among the in-patients, involving multiple clones and an outbreak of *Escherichia coli* (*E. coli*) ST-167.

Material/methods: Ten bla_{NDM-5}-carrying *E. coli* strains were isolated from rectal swabs during routine CRE-screening of ICU patients. Investigation started with rapid methods, such as pulsed-field gel electrophoresis (PFGE) and multiple locus sequence typing (MLST), along with minimal inhibitory concentration (MIC) tests for multiple antimicrobial agents, and followed by whole genome sequencing. A comprehensive phylogenetic analysis was performed by identifying the core genome using *E. coli* strain Sanji (CP011061), a closely related and complete genome of ST-167 as the reference, followed by single nucleotide polymorphism (SNP) calling with recombination filtration and finally reconstructing a phylogenetic tree using maximum-likelihood (ML) method.

Results: Multiple patterns were observed in PFGE and all isolates were found to highly resistant to carbapenem (e.g. MIC of meropenem ranged from 64 to 256 mg/L). Isolates were separated into ST-167 (n=7), ST-617 (n=2) and ST-448 (n=1) according to MLST scheme. An IncX3 plasmid carrying bla_{NDM-5} was identified in all isolates from the whole genome sequencing. The 7 ST-167 isolates were closely related to the previously described *E. coli* strain 0215, a bla_{NDM-5}-carrying ST-167, which was recovered in 2013 in the same hospital. SNP calling showed that all ST-167 isolates differed from each other by the number of sites ranging from 4 to 109. Only 7 SNPs were observed between the two ST-617 isolates, but around 300 SNPs were found comparing to the strains from ST-167. ST-448, differing from ST-167 and ST-617 by all MLST alleles, was found to contain 31280 SNPs using 0215 as the reference strain. In the phylogenetic tree, three clades were observed among 10 isolates.

Conclusions: The same plasmid was identified among all isolates suggesting a horizontal transfer of bla_{NDM-5}. Comparatively fewer SNPs found within the group of ST-167 showed an existence of an outbreak for a common clone. Approximately 200 more SNPs were noticed when compared the two isolates of ST-617, differing from ST-167 by one allele, to the strain 0215 indicating that they

potentially came from a different origin. The isolate from ST-448, all 7 alleles of which varies from the other two strain types, harboring 31280 SNPs implying a third origin of dissemination. Consistent with the results above, three clades were observed among 10 isolates in the phylogenetic tree, which confirmed that there were three origins of transmission of *bla*_{NDM-5}-carrying *E. coli*. Hence, it becomes evident that both vertical and horizontal dissemination were responsible for the persistence of *bla*_{NDM-5} in our local settings.