Onward clonal transmission of *Escherichia coli* co-carrying bla_{KPC-2} and *mcr-1* in a hospital environment: Analysis of whole genome sequences and shoe-leather epidemiology

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Background

Optimal infection prevention strategy for control of *mcr-1* harbouring Enterobacteriaceae is still unknown, contributed in part by the lack of understanding of the transmission patterns of these organisms in the healthcare environment. In this study, we investigate the horizontal transmission of Enterobacteriaceae harbouring *mcr-1* in Singapore acute care hospitals, using whole genome sequencing (WGS) and shoe-leather epidemiology

Method

Enterobacteriaceae isolates used in the study were obtained via two approaches: 1) prospective PCR screening for *mcr-1* from January – May 2016 at NUH (*n*=350); 2) retrospective analysis of whole genome sequence data of CPE which included, New Delhi Metallo-β-lactamase (NDM) isolates (*n*=91) and, Klebsiella pneumoniae carbapenemase (KPC)– producers (*n*=210). **Genomic and clinical relatedness analysis:** We investigated the patient-to-patient transmission of *mcr-1* by analysing overlaps in ward stays, admitting disciplines, endoscopic procedures, and place of residence in the community. These data were collected from hospital electronic records and analysed using R statistical package. Relatedness between isolates was investigated using plasmid identity (*mcr-1*, bla_{NDM}, bla_{KPC}), bacterial chromosomal core genome cluster assignment, and single nucleotide polymorphism (SNP).

Results

Results: We identified six patients carrying *mcr-1* Enterobacteriaceae (5 *E. coli* and 1 *K. pneumoniae*). Considerable plasmid diversity was noted (table 1), with 4 of the six isolates bearing different *mcr-1* plasmids. Three out of the five *E. coli* isolates co-carried plasmid-borne bla_{KPC-2}. Two *E. coli* isolates (ENT 563 and ENT 564) harbouring both *mcr-1* and bla_{KPC-2} fulfilled clinical and genomics criteria for horizontal transmission (Figure 1). The host patients had temporal overlap in the same ward under the same clinical discipline. They stayed at different residential areas in Singapore suggesting nosocomial transmission. Based on core chromosomal analysis, the two *E. coli* isolates, met the criteria for phylogenetic transmission clustering, with identical core chromosome (pairwise SNP = 10) and plasmids carrying *mcr-1* (FAP1 plasmid) and bla_{KPC-2} (pHS102707 plasmid).

Table 1. Characteristics of *mcr-1* positive clinical Enterobacteriaceae

Isolate name/ Hospital	Species	Source	ST	Date of isolation	Replicon of *mcr-1* plasmid	Reference *mcr-1* plasmid	Reference bla_{KPC-2} plasmid
ECO247MCR/X	*E. coli*	Urine	156	14/01/2016	IncI2	pHNSHP45	-
ECO589MCR/X	*E. coli*	Urine	1843	11/01/2016	Unknown	Unknown	-
ENT563/Y	*E. coli*	Rectal swab	2006	07/08/2013	IncI2	FAP1 plasmid unnamed 3	pHS102707
ENT564/Y	*E. coli*	Rectal swab	2006	07/08/2013	IncI2	FAP1 plasmid unnamed 3	pHS102707
ENT577/Y	*E. coli*	Sacral ulcer	224	13/08/2013	Unknown	Unknown	Unknown
KPN069MCR/X	*K. pneumoniae*	Urine	New ST	13/01/2016	IncX4	pmcr1_IncX4	-

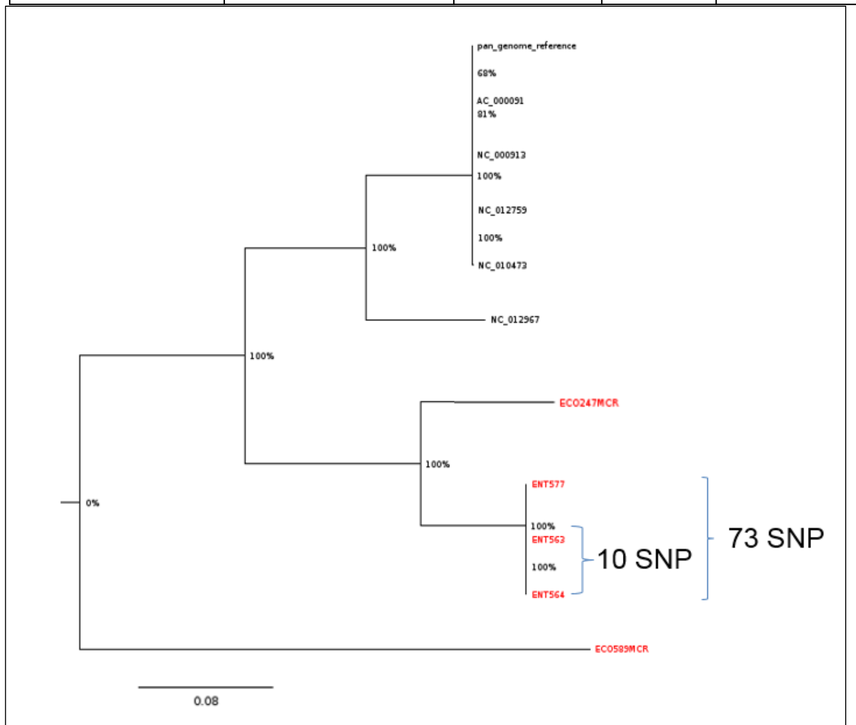

FIG. 1. Maximum likelihood phylogenetic tree based on the core genome of *E. coli* isolates. Brackets denote a transmission cluster as was defined as isolates which (i) shared the same *mcr-1* positive plasmid, (ii) had the same ST and (iii) had ≥99% bootstrap support at all nodes in the chromosomal core genome phylogeny tree.

Conclusion

Our results show that it is possible for *mcr-1* and KPC-plasmids to be co-transferred among hospitalised patients. Hence, screening of CPE carriers for co-carriage of *mcr-1* should be considered. Transmission-based precaution for CPE carriers as recommended by the current guidelines remain relevant.

References:

1. Doi Y, Paterson DL. 2015. Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med. 36(1):74-84.
2. Liu YY, Wang Y, Walsh TR et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis.18. pii: S1473-3099(15)00424-7.
3. Zhang et al. 2016. Possible Transmission of mcr-1-Harboring Escherichia coli between Companion Animals and Human. Emerg Infect Dis. 2016 Sep 15;22(9).
4. Veldman et al. Location of colistin resistance gene mcr-1 in Enterobacteriaceae from livestock and meat. J Antimicrob Chemother. 71(8):2340-2.
5. Yu et al. 2016. Detection of mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae (CRE) from different hospitals in China. Antimicrob Agents Chemother. 60(8):5033-35.
6. Inouye et al. 2014. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. Genome Med. 20;6(11):90.