

Limited historical distribution of *mcr-1* and the pHNSHP45 plasmid in a global collection of human clinical and carriage strains of *Escherichia coli* and *Klebsiella pneumoniae*

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Dear Editor,

Liu et al[1] recently reported the first plasmid-mediated polymixin resistance gene, *mcr-1*, in Enterobacteriaceae isolated from human clinical isolates, retail meat and food animals in China, 2011-14. Of 16 *mcr-1* positive isolates from hospital in-patients in two geographic regions in 2014, 13 were *Escherichia coli* and 3 *Klebsiella pneumoniae*, suggesting inter-species transfer of *mcr-1* occurs. The identification of *mcr-1* in a Danish clinical *E. coli* isolate from 2014[2] supports the hypothesis that *mcr-1* has disseminated internationally to a degree.

In response to these findings, we screened our database of >1,100 *E. coli* and 550 *K. pneumoniae* sequences collected from several Southeast Asian, European and North American locations, 1967-2012 (primarily 2008-2012). We identified the presence of *mcr-I* in a single *E. coli* ST354 strain (1/1738 [0.06%]), isolated April 2012, in faeces from a hospitalised child in Cambodia (isolate IHD86_4, SRA: PRJNA274331; BioSample: SAMN0332599). As with the Danish strain, this isolate also contained *bla*_{CTX-M-55} and other resistance genes.

The *mcr-I*-associated plasmid in Liu et al, pHNSHP45, is a ~64kb IncI plasmid with significant sequence homology ($\geq 80\%$ sequence identity over $\geq 80\%$ of the query) to only four other plasmid sequences in GenBank (Accessions: JN983044.1, KJ460501.1, KM202012.2, CP009581.1). Using BLASTn to probe our dataset for pHNSHP45, we found only 25/1738 (1%) isolates with significant matches (16 *E. coli*, 9 *K. pneumoniae*), suggesting this plasmid structure has not historically been widespread in these species. These 25 isolates were however geographically distributed (Laos, Cambodia, Bangladesh, USA), and from as early as 2003. Interestingly, our *mcr-I*-positive isolate did not contain this plasmid signature.

In our case, the investigation of the genetic context surrounding *mcr-I* was limited by the size of the contig in which it was assembled, but it was adjacent to *IS1294*, flanked by signatures consistent with it being nested within an *ISApII* composite transposon (Fig.1). *IS1294* employs a one-ended, rolling circle transposition mechanism capable of mobilising adjacent sequences[3]. We postulate that *mcr-I* was originally introduced into the *ISApII* element by *IS1294*, which was subsequently lost.

We have identified *mcr-1* in a human faecal *E. coli* isolate in Cambodia two years prior to human isolates in the Liu study, and associated with different genetic structures. IS1294-mediated transposition of the gene into an IS*AplI* composite transposon may have represented the original import mechanism of *mcr-1* into Enterobacteriaceae. Large, established databases of whole genome sequences represent a rich repository to investigate the historical presence of novel resistance mechanisms.

References

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Figure 1. Genetic context of *mcr-1* in IHD86_4 and pHNSHP45