Heterogeneity of colistin resistance mechanism in clonal populations of carbapenem-resistant *Klebsiella pneumoniae* in Vietnam

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In Vietnam, limited access to novel antibiotics and a lack of alternative antimicrobial agents have led to the frequent use of colistin (despite its toxic side effects) to treat infections caused by carbapenemase-producing Enterobacterales. Vietnam stands out as a notable hotspot for carbapenem-resistant Enterobacterales (CRE) within Southeast Asia.[1–3] High prevalence of CRE in Vietnam may be related to extensive antimicrobial use in both human and animal sectors.[4] A study conducted in 76 countries between 2000 and 2015 found that Vietnam ranked 11th in antibiotic consumption, with 32.0 daily defined doses (DDD) per 100 inhabitants per day. This rate is significantly higher than the prescription rate in most EU countries (7 DDD per 100 inhabitants per day).[5] To elucidate the prevalence and mechanisms of colistin resistance, we performed a single-centre cross-sectional genomic investigation of carbapenem-resistant *Klebsiella pneumoniae* causing human infections in Vietnam.

Between January 1st and December 31st, 2021, we randomly collected 105 non-duplicate clinical isolates of carbapenem-resistant (CR)-*K. pneumoniae* strains from 101 patients (four patients had two morphologically different isolates). These isolates were characterised using a hybrid assembly from short- and long-read whole genome sequencing (accession numbers are provided in the Supplementary Appendix, NCBI Genbank Bioproject PRJNA1043438). ST16 was the predominant multi-locus sequence type (MLST), accounting for 62% (65/105) of CR-*K. pneumoniae* in our study. Other abundant STs were ST11 (9/105, 8.6%), ST15 (8/105, 7.6%), and ST231 (4/105, 3.8%), ST656 (4/105, 3.8%). Of the 105 CR-*K. pneumoniae* isolates, 98% (103/105) were carbapenemase producers, with New-Delhi metallo-β-lactamase (NDM) being the most prevalent (47% (48/103)), followed by oxacillinase (OXA-48-like) (43/103, 42%), and *Klebsiella pneumoniae* carbapenemase (KPC-2) (43/103, 41.7%) producers. Of the carbapenemase producers, 31/103 (30.1%) were found to harbour two various carbapenemase-encoding genes and 72/103 (69.9%) were found to harbour one carbapenemase-encoding gene. The most common NDM variant was $bla_{\text{NDM-4}}$ (32/48, 66.7%), followed by $bla_{\text{NDM-1}}$ (14/48, 29.2%), and $bla_{\text{NDM-5}}$ (2/48, 4.2%) (Fig. 1).

Thirty-nine isolates (39/105, 37%; 29–47% 95% CI) were phenotypically resistant to colistin (Methodology, see Supplementary Appendix). Only three of the 39 (8%) colistin-resistant *K. pneumoniae* were mediated by an IncX4_1 mobile plasmid harbouring the *mcr*-1.1 gene clustering phylogenetically with other published IncX4_1 plasmid isolated from *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella enterica* (ANI >99.95%, Supplementary Appendix). The majority of colistin resistance is associated with mutations in the *mgrB* gene (25/39, 64%, 48–77% 95% CI) (Fig. 1). The most common *mgrB* mutation (12/25, 48%) had an insertion of an ISL3-like transposase (ISAeme19) (Fig. 1, Supplementary Appendix). Two isolates carried the *mcr*-1.1 plasmid and F35L *mgrB* mutation. In 13 isolates (13/39, 33%) with phenotypic resistance to colistin,

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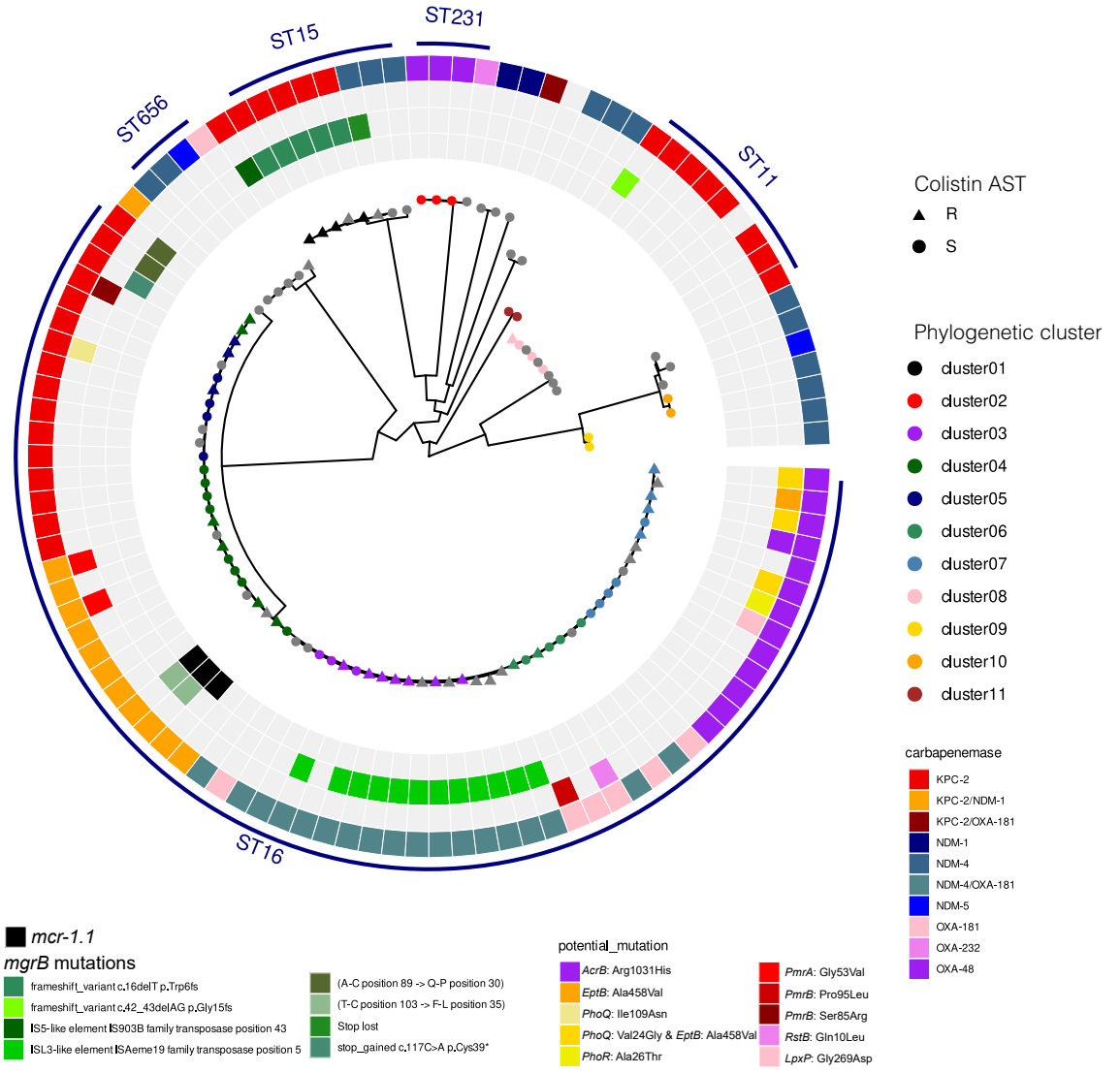

Fig. 1: **Phylogeny of carbapenem-resistant *Klebsiella pneumoniae* in Vietnam.** Phylogeny was calculated based on the core-genome (3878 genes present in all isolates) and corrected for recombination using Gubbins. Isolate phenotypically presenting colistin resistance (MIC ≥4 mg/L) are symbolised by triangle while sensitive isolates are symbolised by dots. The circles represent from inner to outside: 1) the presence of *mcr* genes, 2) mutations within *mgrB* gene, 3) mutations in other genes associated with colistin resistance, 4) Carbapenemase genes present in the isolates. Abbreviation: AST, antibiotic susceptibility testing.

mutations in genes *phoQ*, *phoR*, *pmrA*, *pmrB*, *lpxP*, *acrB*, *eptB* and *rstB* were identified.

The acquisition of colistin resistance appears to be independent of a SNP-based phylogeny and a generalised linear regression analysis showed no association with clinical parameters (age, gender, usage/duration of colistin therapy). A phylogenetic analysis over the core genome to identify potential transmissions and outbreak clusters with a cut-off value of 22 SNPs for defining the clusters using the algorithm proposed by Duval et al.[6] identified 11 clusters and 42 singletons (Fig. 1). In six of the 11 SNP clusters, isolates exhibited heterogeneity in phenotypic susceptibility to colistin, consisting of both colistin-susceptible and colistin-resistant isolates within the same SNP cluster. The heterogeneity of the colistin resistance determinants suggests that the acquisition of colistin resistance may have also been driven by colistin use and not only by transmission events (Fig. 1).

Our study revealed that over one-third of CR-*K. pneumoniae* exhibited co-resistance to colistin. The emergence and development of colistin resistance may be facilitated by the extensive use of this antibiotic in both the pharmaceutical sector and animal husbandry.[4,7] Unlike colistin resistance in animal isolates, which is largely mediated by *mcr* genes,[8] resistance in human

isolates is primarily driven by chromosomal mutations and appears to have followed a different evolutionary trajectory, as suggested by our findings and other published data.[9,10]

Our study has limitations. First, only a single isolate was sequenced per patient with the assumption of clonal homogeneity within a given sample, which may partly explain the variable colistin resistance determinants within the same SNP cluster. Second, our study focused on randomly selected clinical isolates, so we could not draw any conclusions about the colonisation burden in the community setting.

Nonetheless, our data suggest that the emergence of colistin resistance in CR-*K. pneumoniae* in Vietnam is not caused by clonal spread or horizontal gene transfer but rather due to mutations of genes associated with the biosynthesis of lipid A and lipopolysaccharide (LPS). Our findings underscore the necessity of continuing active surveillance and infection control measures to address the rising prevalence of co-resistance towards colistin in carbapenem-resistant *K. pneumoniae*. While reducing colistin use in animal husbandry and food production is legally enforced, restrictive prescribing and antimicrobial/diagnostic stewardship programs in human medicine should also be enforced to prevent the emergence and spread of colistin- and carbapenem-resistant *K. pneumoniae* in Vietnam.

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Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2024.101204.

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