

Supplementary methods

Genomic and molecular characterisation of a *Klebsiella pneumoniae* clinical isolate resistant to meropenem-vaborbactam, imipenem-relebactam, and ceftazidime-avibactam

Yu Wan, Joshua L. C. Wong, Julia Sanchez-Garrido, Wen Wen Low, Jane F. Turton, Fabio Morecchiato, Ilaria Baccani, Kirsty Dodgson, Gian Maria Rossolini, Neil Woodford, Gad Frankel, Elita Jauneikaite, Danièle Meunier, and Katie L. Hopkins

August 2025

Comparative structural analysis of TraN

Amino acid sequences of TraN_{pKpMVS1_1}, TraN_{pKpMVR1_1}, and TraN_{pKpMVS2_1} were extracted from the sequence annotations of plasmids pKpMVS1_1 (locus tag: WAS92_RS00545), pKpMVR1_1 (ACNQKT_RS26595), and pKpMVS2_1 (ACNQKS_RS28350), respectively. To contextualise these three proteins, the previously described TraN variants [1] TraN_{pKpQI} (NCBI protein accession: ARQ19727.1), TraN_{MV2} (BAS44060.1), TraN_{R100-1} (ABD60034.1), TraN_{pSLT} (AAL23498.1), TraN_F (WP_000821835.1), TraN_{MV1} (ANZ89826.1), TraN_{MV3} (WP_001398575.1) were downloaded from the NCBI Protein database (www.ncbi.nlm.nih.gov/protein). These 10 amino acid sequences were aligned with the ClustalW algorithm [2], and subsequently, a neighbour-joining phylogenetic tree was generated from the multi-sequence alignment, with the Poisson correction method as implemented in MEGA11 [3]. The phylogenetic tree was visualised using iTOL v7.2 [4].

Three-dimensional structures of TraN_{pKpMVS1_1}, TraN_{pKpMVR1_1} (identical to TraN_{pKpMVS2_1}), and TraN_{pKpQI} were predicted using AlphaFold 3 [5] with its default parameters on AlphaFold Server (alphafoldserver.com). The top-ranked (model 0) structural models of TraN proteins were visualised using UCSF ChimeraX v1.9 [6]. Superimposition analysis of these models was performed with ChimeraX's Matchmaker tool using default settings, including the use of the “best-aligning” or “bb” chain-pairing method, the Needleman-Wunsch alignment algorithm, and the BLOSUM-62 similarity matrix.

References

1. **Low Wen Wen, Seddon Chloe, Beis Konstantinos, Frankel Gad.** The Interaction of the F-Like Plasmid-Encoded TraN Isoforms with Their Cognate Outer Membrane Receptors. *Journal of Bacteriology* 2023;205:e00061-23.
2. **Thompson JD, Gibson TobyJ, Higgins DG.** Multiple Sequence Alignment Using ClustalW and ClustalX. *Current Protocols in Bioinformatics* 2003;00:2.3.1-2.3.22.
3. **Tamura K, Stecher G, Kumar S.** MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 2021;38:3022–3027.
4. **Letunic I, Bork P.** Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 2007;23:127–128.
5. **Abramson J, Adler J, Dunger J, Evans R, Green T, et al.** Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 2024;630:493–500.
6. **Meng EC, Goddard TD, Pettersen EF, Couch GS, Pearson ZJ, et al.** UCSF ChimeraX: Tools for structure building and analysis. *Protein Science* 2023;32:e4792.