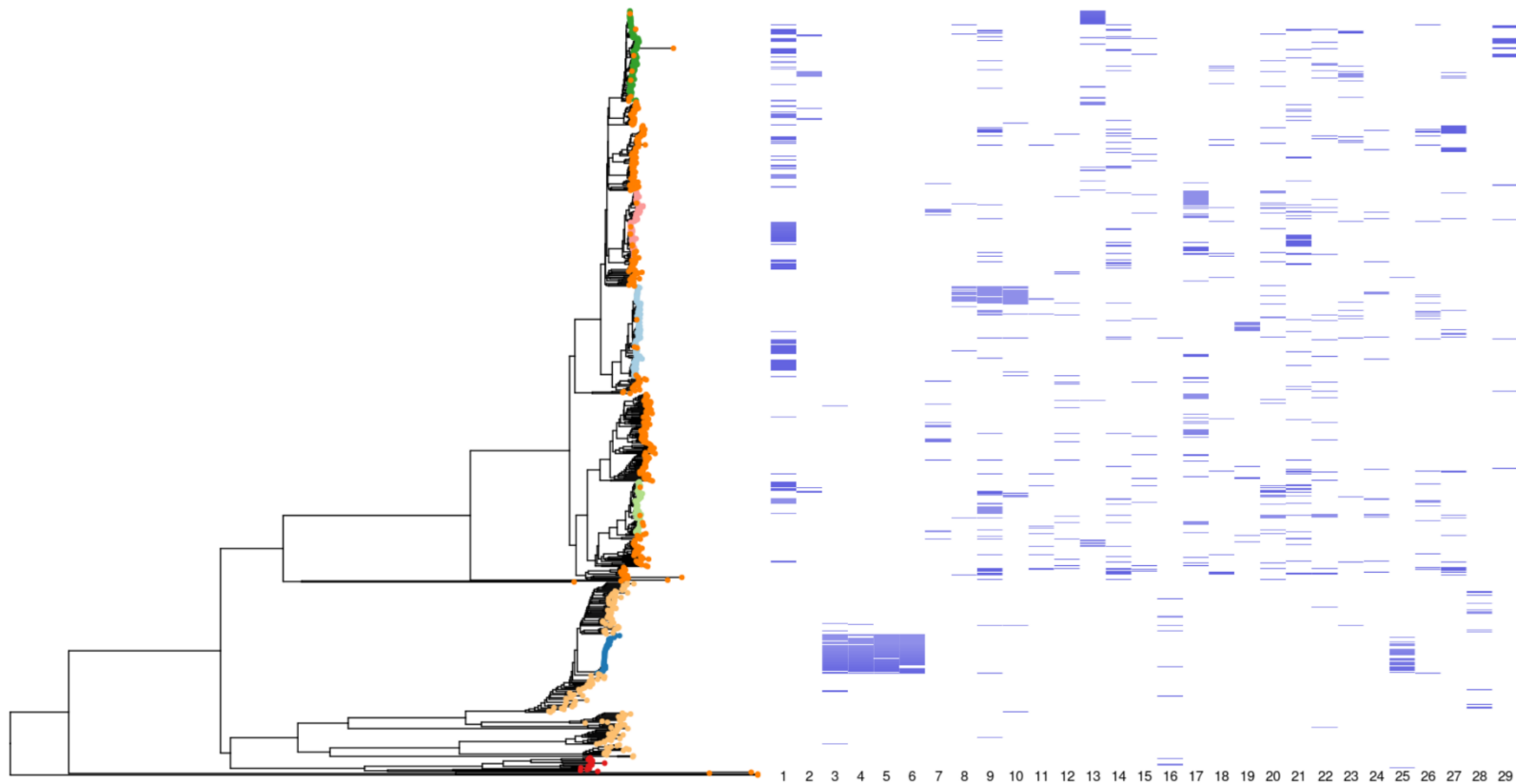


Introduction: Plasmids carried by *Enterobacteriales* contain clinically important genes and facilitate horizontal transfer of these within and between species. Most genomic studies of these to date have primarily utilised short-read sequencing which has substantial limitations.

Methods: We performed Illumina (short-read) and Oxford Nanopore (long-read) sequencing on all *E. coli*/K. pneumoniae bloodstream isolates from 2009 and 2018 as well as a selection from intervening years designed to represent the overall genomic diversity observed from short read assemblies as well as capturing clinically important local outbreaks. Hybrid assembly was performed with Unicycler and annotation with NCBI AMR-Finder. Clustering of plasmids was performed using graph based single-linkage of Mash distances, with a threshold of 0.01 (analogous to 99% average nucleotide identity).

Results: We identified 1091 clusters from 2598 assembled plasmids originating from 921 isolates. Plasmid clusters were generally genus-restricted, though there were eight clusters shared between-genus. Cluster-1 (figure) was of particular interest given its wide dissemination over phylogroup B2 and ST69 *E. coli* isolates, association with AMR genes and near-universal carriage (132/136) of the enterotoxin *senB*. Of the 466/840 isolates carrying at least one plasmid-borne AMR gene, most (372/466(80%)) carried all such genes on a single plasmid despite having median 3 (IQR 2-5) total plasmids. Larger clusters (≥ 10 plasmids) can be summarised as being neither being involved in virulence factor nor AMR gene carriage (18 clusters, 567 plasmids), associated with AMR genes but not virulence factors (4 clusters, 96 plasmids) and associated with both AMR genes and virulence factors (7 clusters, 368 plasmids). The total number of times an AMR gene was observed strongly correlated with the number of unique plasmid clusters it was found on (Pearson correlation-coefficient 0.98). Within AMR associated clusters, carriage of these genes was not universal; in only 2/11 AMR associated clusters did every isolate have at least one AMR gene. Finally we observed significant chromosomal integration of clinically relevant AMR genes (e.g. 40/130 (31%) *bla*CTX-M-15 genes)

Conclusion: Clinically relevant AMR genes are typically carried on a single large plasmid, disseminated over a diverse plasmid repertoire and often co-located with virulence factors. Plasmids carrying AMR genes are often highly similar to those with no AMR genes.



Species/MLST

- 131
- 490
- 69
- 73
- 95
- Enterobacter cloacae
- Klebsiella spp.
- Other

Present

- no
- yes