# Genomic surveillance of multidrug-resistant bacteria - first results of the SLOSEQ project

P2145

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## Background

In the face of escalating antibiotic resistance, Slovenia has adopted the National "One Health" antimicrobial resistance strategy (2019-2024) with Action plan to combat antibiotic resistance across human and veterinary medicine, as well as partially addressing it in the environment (1). In alignment with the national Action plan, we are implementing genomic surveillance of antibiotic-resistant bacteria in human medicine through the EU-funded project SLOSEQ - Consolidation and integration of whole genome sequencing (WGS) into routine surveillance in Slovenia (2), prioritizing pathogens according to ECDC strategic frameworks (3), WHO priority list (4) and national needs.

In the first six months of the project duration, we have established definitions and logisticss for routine isolate collection, optimised protocols for bacterial whole genome sequencing on the NextSeq2000 platform (Illumina) and data analysis (i.e., MLST. core-genome MLST and identification of genes and chromosomal mutations mediating antimicrobial resistance).

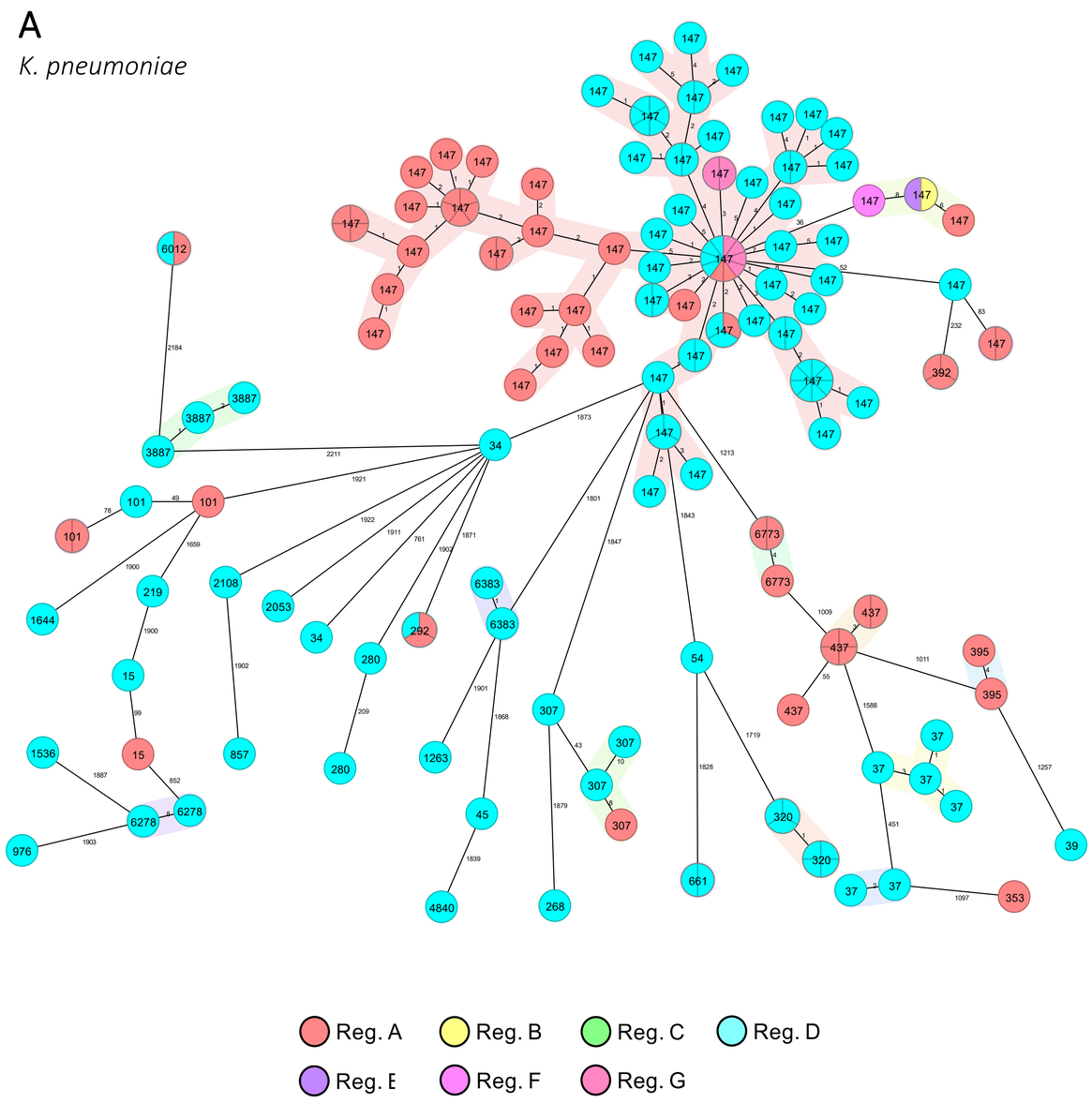


**Figure 1. Minimum spanning trees showing clustering of isolates, A) *K. pneumoniae*, B) *A. baumannii*, C) *P. aeruginosa* and D) *E. coli*, based on comparison of cgMLST allelic profiles.** Each circle represents one or more isolates (isolates with identical cgMLST allelic profile are grouped within the same circle). The numbers within the circles indicate MLST-ST. The number of different alleles between isolates is shown along the line connecting the isolates. MST clonal clusters are surrounded by colors. The MST cluster distance thresholds are A) 15 alleles for *K. pneumoniae*, B) 9 alleles for *A. baumannii*, C) 12 alleles for *P. aeruginosa*, and D) 10 alleles for *E. coli*. The colour of the circle represents the region.

## Methods

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| Isolates | Carbapenemase- and ESBL-producing bacteria, mainly *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* isolated in 2022 and 2023, were sequenced. |
| Genome sequencing and analysis | Genomes were sequenced on Illumina platform NextSeq2000. Paired-end libraries were generated with NEBNext Ultra II FS DNA library Prep Kit (NEB). Trimmomatic v0.39 was used for adapter and quality trimming and trimmed reads were *de novo* assembled into draft genomes using SPAdes |
| | Ridom SeqSphere+ 9.0.2 software was used to determine MLST sequence types (ST) and cgMLST (core genome MLST) cluster analysis. |
| | AMRFinderPlus (part of SeqSphere+) was used to detect acquired genes and point mutations conferring antibiotic resistance. |

## References

1. https://www.gov.si/novice/nov-vlada-sprejela-drzavno-strategijo-eno-zdravje-za-obvladovanje-odpornosti-mikrobov-2019-2024-z-akcijskim-nacrtom-za-obdobje-2019-2021/;
2. https://www.nlzoh.si/programi-in-projekti/sloseq/
3. https://www.ecdc.europa.eu/en/publications-data/ecdc-strategic-framework-integration-molecular-and-genomic-typing-european;
4. https://www.who.int/publications/i/item/WHO-EMP-IAU-2017.12

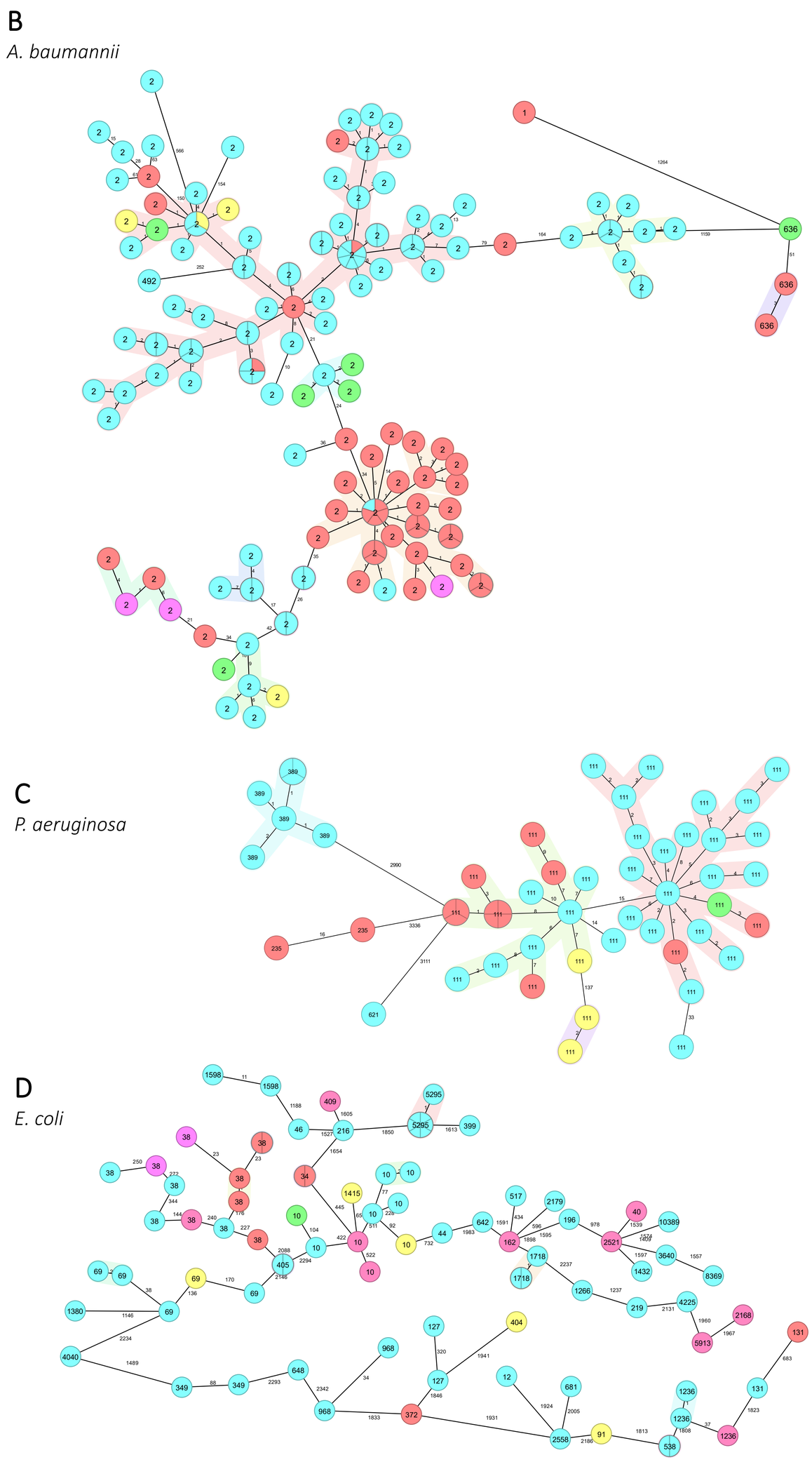
## Results and conclusions

Results of the project provided the first important insights into the prevalence and mechanisms of resistance in selected carbapenemase- and ESBL- producing bacteria in Slovenia.

Among the genes mediating carbapenem resistance the most common were *bla*NDM-5/*bla*OXA-48 in *K. pneumoniae*, blaOXA-48 in *E. coli*, *bla*VIM-2 in *P. aeruginosa,* and *bla*OXA-23 in *A. baumannii*. The most prevalent ESBL variants in *E. coli* included *bla*CTX-M-15, -14, -27, and *bla*SHV-12. In *K. pneumoniae*, *bla*CTX-M-14/*bla*CTX-M-15 were commonly found, while in *P. aeruginosa* and *A. baumannii*, the predominant variants were *bla*PDC-3 and *bla*ADC-73, respectively.

Clonal spread between regions (Reg. A to Reg. G) was observed for all species (Fig. 1-A, B, C), except for *E. coli* (Fig. 1-D).

The utilization of genome sequence analysis played a crucial role in resolving an outbreak of *K. pneumoniae* ST147 carrying *bla*NDM-5/*bla*OXA-48 in a regional hospital (located in Reg. A), which persisted from August 2022 (data not shown). This further underscores the pivotal role of genomic surveillance in in effectively managing outbreaks.

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