



E.R. Verhagen¹, G.J. Boland(†)¹, D.D. de Vries¹, A.C. Fluit¹, M.S.M. van Mourik¹, E. Carrara², A. Hristea³, G. Daikos⁴, P. Retamar⁵, A. Sonpar⁶, W. Zingg⁶, J.A.J.W. Kluytmans¹



H2020-SC-1-2020-Single-Stage RTD
No 965265

¹-UMC Utrecht - Utrecht (Netherlands), ²-University Verona - Verona (Italy), ³-University of Medicine and Pharmacy "Carol Davila" - Bucharest (Romania), ⁴-University of Athens - Athens (Greece), ⁵-University of Sevilla - Sevilla (Spain), ⁶-University Hospital Zurich - Zurich (Switzerland)

Summary of most important findings

1. Analysis of 384 carbapenemase-producing *Klebsiella pneumoniae* isolates collected from hospitalized patients in 24 European hospitals revealed geographical clustering of both Sequence Types (STs) and carbapenemase genes.
2. High-risk clone ST258 predominated in Greece and, to a lesser extent, in Romania, whereas ST307 was relatively more common in the lower-prevalence settings of Spain and Italy. Both clones showed country-specific carbapenemase profiles.
3. A minimum spanning tree based on Multi Locus Sequence Typing (MLST) showed limited cross-country admixture.
4. Carbapenemase co-production was very common among isolates from Greece (particularly ST512, ST258) and Romania (mainly ST383) and uncommon in Italy and Spain.

Introduction

REVERSE is a Horizon 2020 project, studying the effect of **infection prevention** and **antibiotic stewardship** on reducing hospital acquired infections with **multidrug resistant organisms (MDRO)**. This poster shows results of genetic analysis of *K. pneumoniae* isolates, retrieved in the point prevalence screening conducted in 24 European hospitals, at baseline of the trial.

Methodology

Point Prevalence Survey for MDRO colonization

Rectal screening for MDRO was conducted in 24 European hospitals in Greece, Italy, Romania and Spain. Rectal swabs were cultured on selective CRE-agar. Isolates were phenotypically tested for carbapenemase activity using the Carbapenem Inactivation Method (CIM)¹.

Whole Genome Sequencing

After phenotypic confirmation of carbapenemase activity, *K. pneumoniae* isolates were incubated 24h on a blood agar at 37°C to obtain single colonies. A single colony from each isolate was subsequently inoculated into liquid culture medium and incubated for another 24h at 37°C. DNA was extracted using the QIAcube automated extraction platform (Qiagen). Sequencing was performed on the GridION platform (Oxford Nanopore Technologies). Sequencing output was analysed using Kleborate (version 3.0.0)².

Data analysis

Data was analysed using R (version 2026.01.1+403). A minimum spanning was constructed using MLST-derived STs.

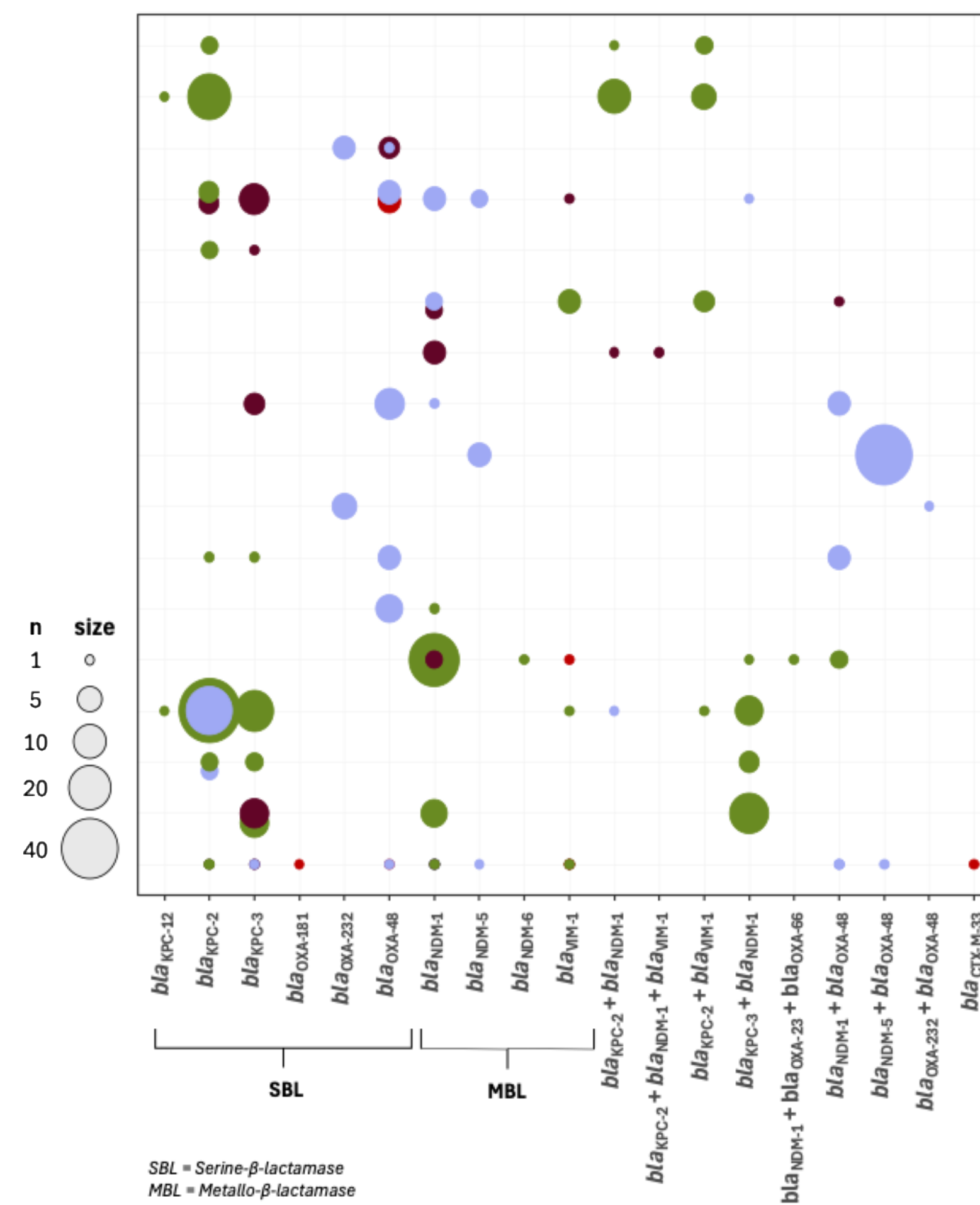
References

1. Van Der Zwaluw K, De Haan A, Pluister GN, Bootsma HJ, De Neeling AJ, Schouls LM. The Carbapenem Inactivation Method (CIM), a simple and low-cost alternative for the carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. *PLoS One*. 2015;10(3).
2. Lam, MMC. et al. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex, *Nature Communications* (2021). <https://doi.org/10.1038/s41467-021-24448-3>

Results

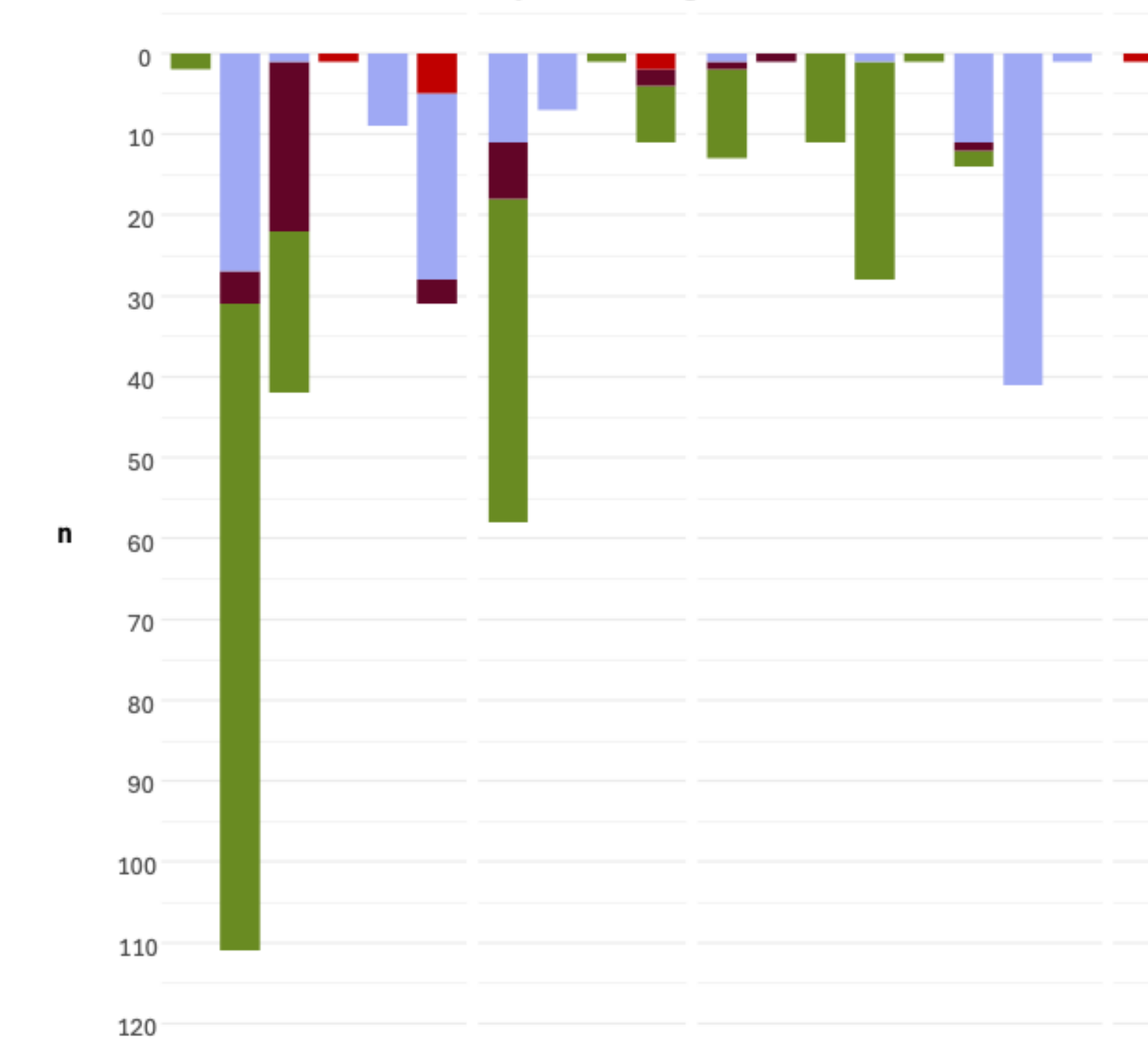
A. Sequence Type-specific carbapenemase genes

Sequence Types sorted by structure of Minimum Spanning Tree, bubble size corresponds with count.



SBL = Serine-β-lactamase
MBL = Metallo-β-lactamase

C. Distribution of carbapenemase genes

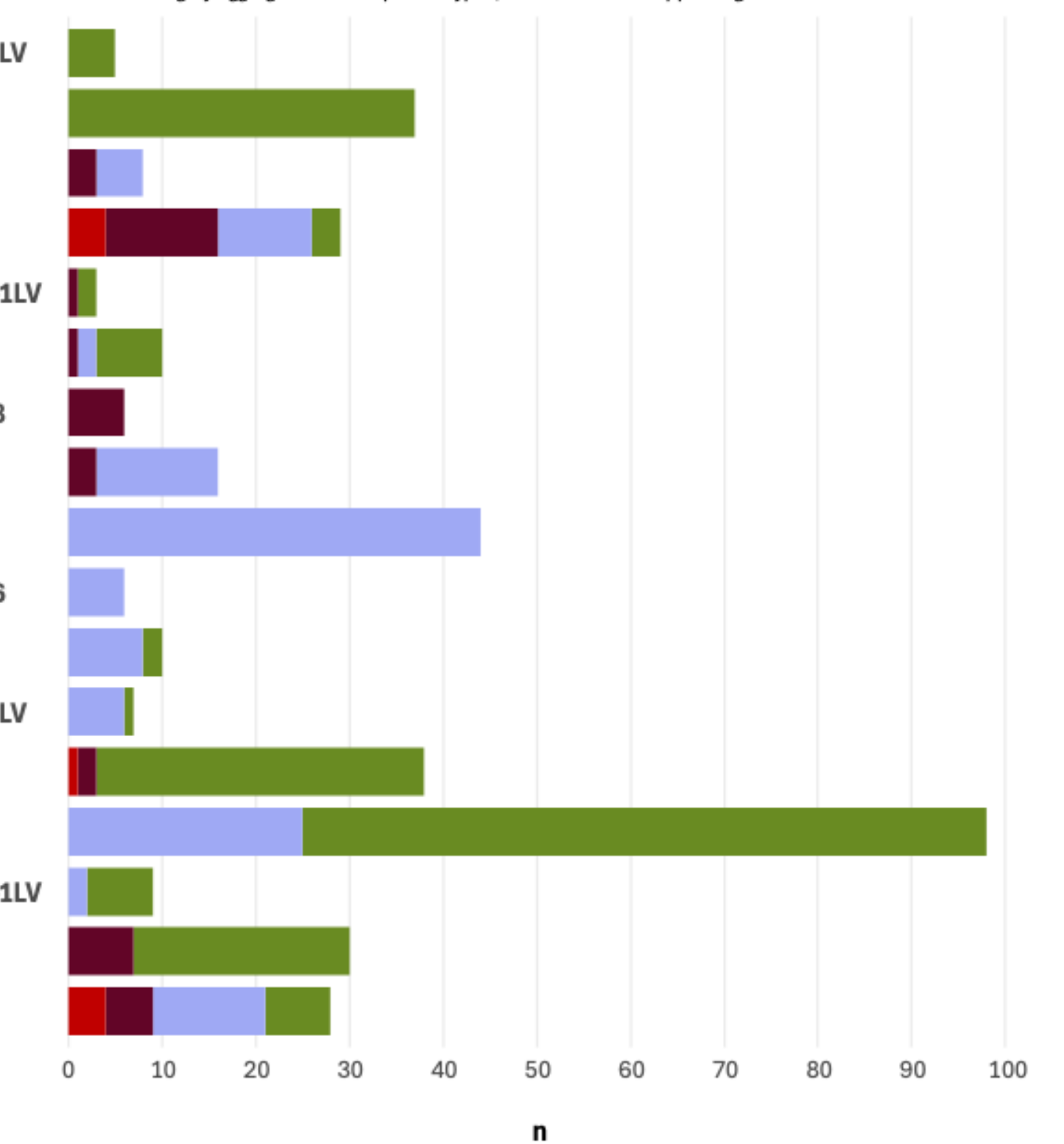


Legend

- Greece (n = 202)
- Romania (n = 133)
- Italy (n = 40)
- Spain (n = 9)

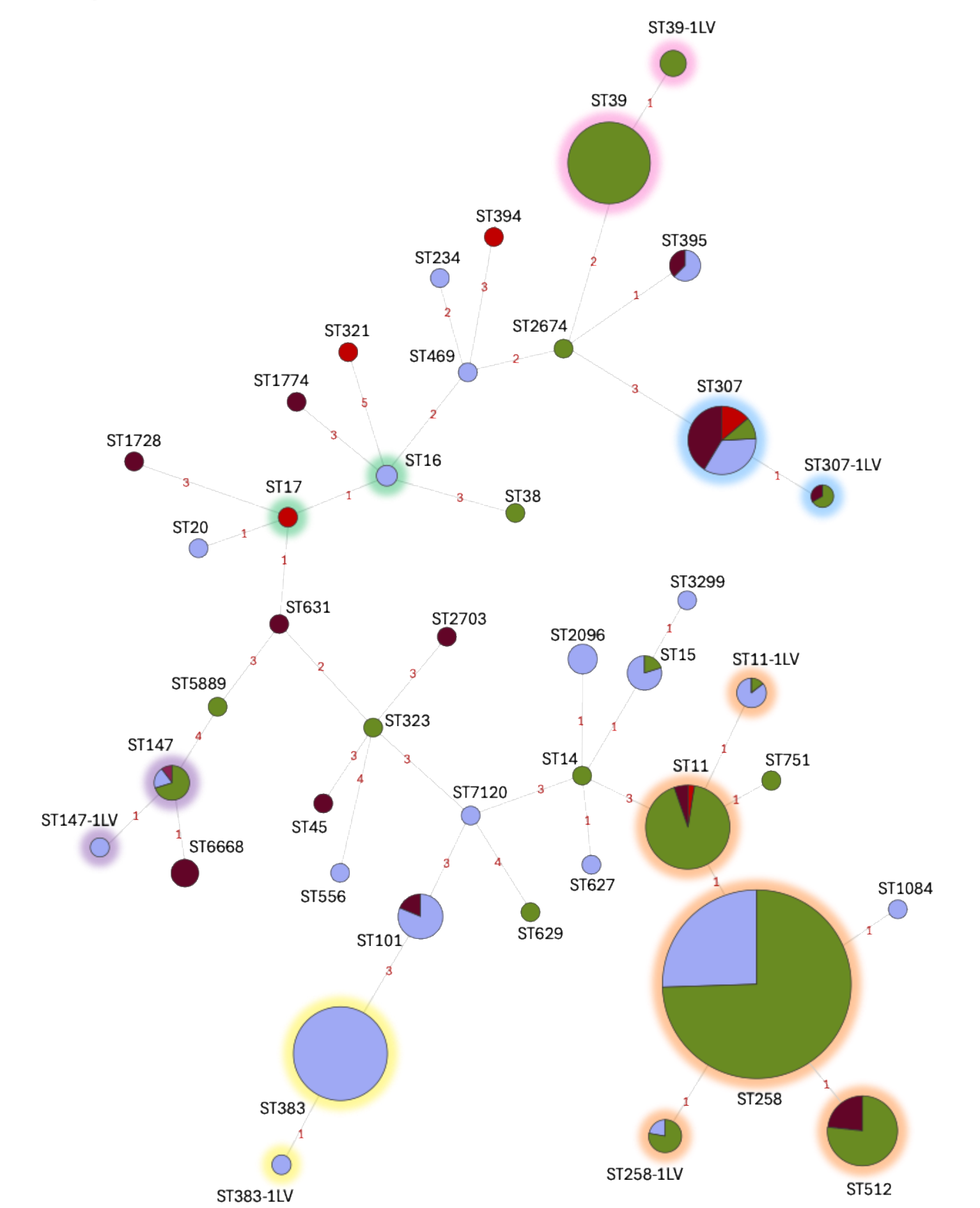
B. Distribution of Sequence Types (n = 384)

Other category aggregates rare sequence types, defined as those appearing <= 2 times across all countries.



D. Minimum Spanning Tree based on MLST

Edge lengths are non-proportional to genetic distance; allelic distances are indicated by red numbers on the branches. Node size corresponds to the number of isolates. Clonal Complexes are highlighted with coloured shading.



Acknowledgements

REVERSE has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965265.

A special thanks to all REVERSE partners .

REVERSE Consortium



Follow REVERSE H2020 on



www.reverseproject.eu

Contact the presenter

Eline Verhagen

e.r.verhagen-4@umcutrecht.nl