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Staphylococcus aureus plasmids of animal origin, a vector of antibiotic resistance



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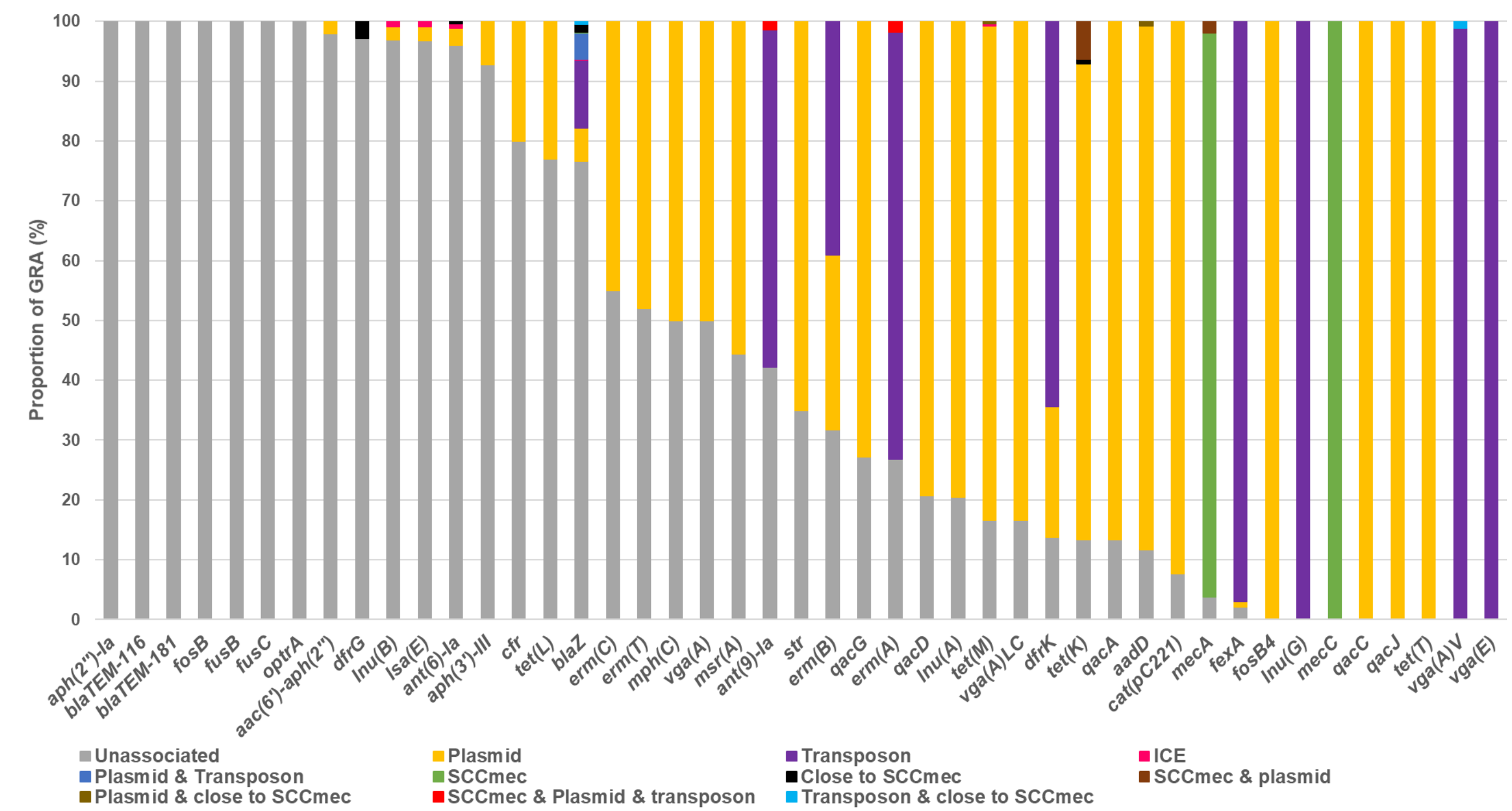
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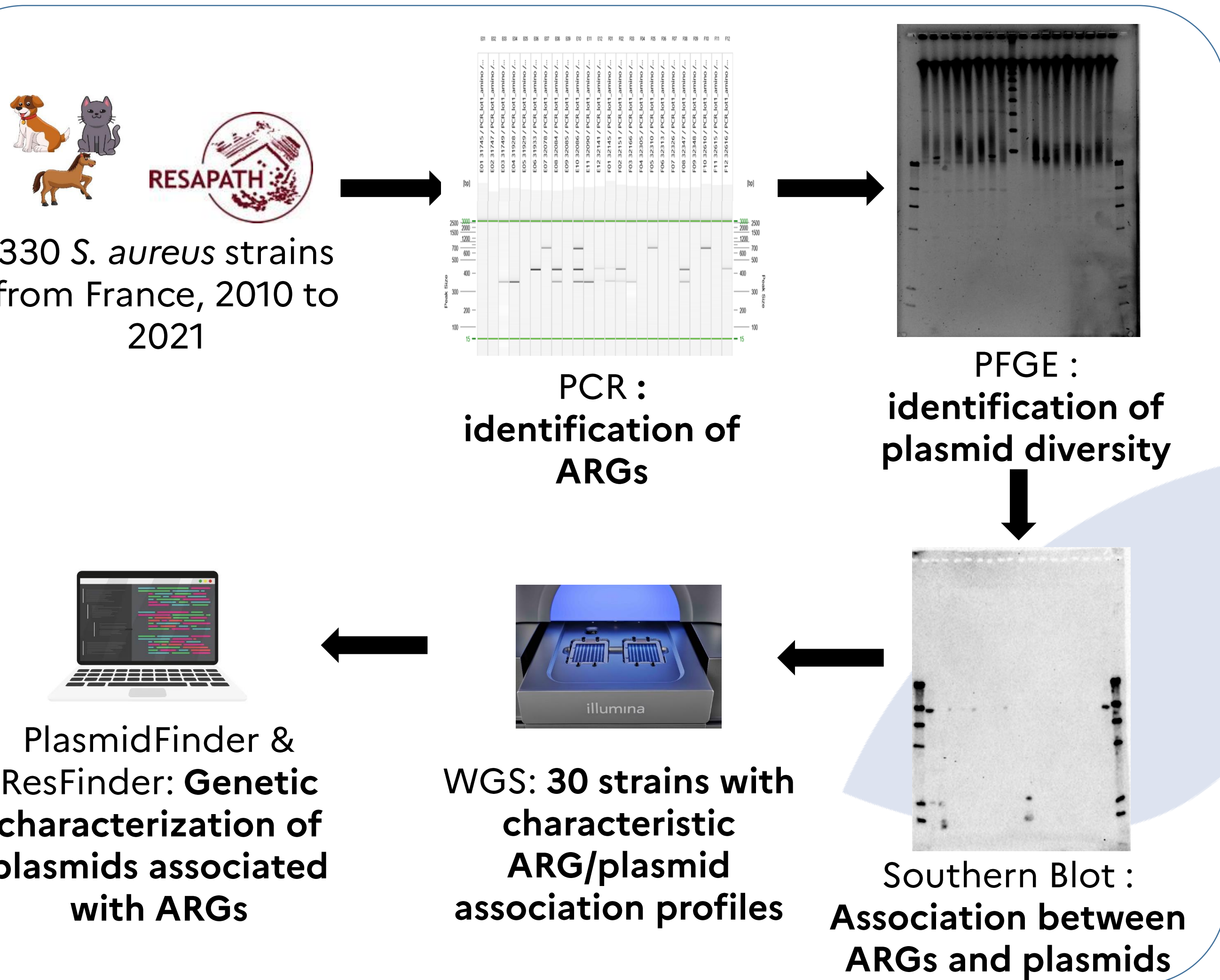


Staphylococcus aureus is a human and animal opportunistic pathogen that has demonstrated the capacity to develop resistance to a wide range of antibiotics. Its ability to acquire antibiotic resistance genes (ARGs) is closely associated with the large proportion of mobile genetic elements (MGEs) present in its genome (between 15 to 20%) and its capacity to exchange genetic material with its environment. We recently conducted an *in silico* analysis of MGEs and their associated ARGs on a dataset of 1257 *S. aureus* genomes isolated from animals, obtained from the NCBI database. We demonstrated that ARGs are predominantly associated with plasmids. Given the central role of MGE in gene exchange, we focused our studies on plasmids from field strains collected by the Resapath network. This passive network monitors AMR in bacteria responsible of infection in animals through the antibiograms generated by veterinary laboratories using disc diffusion¹.

Proportion of ARGs associated with MGEs in *S. aureus* of animal origin

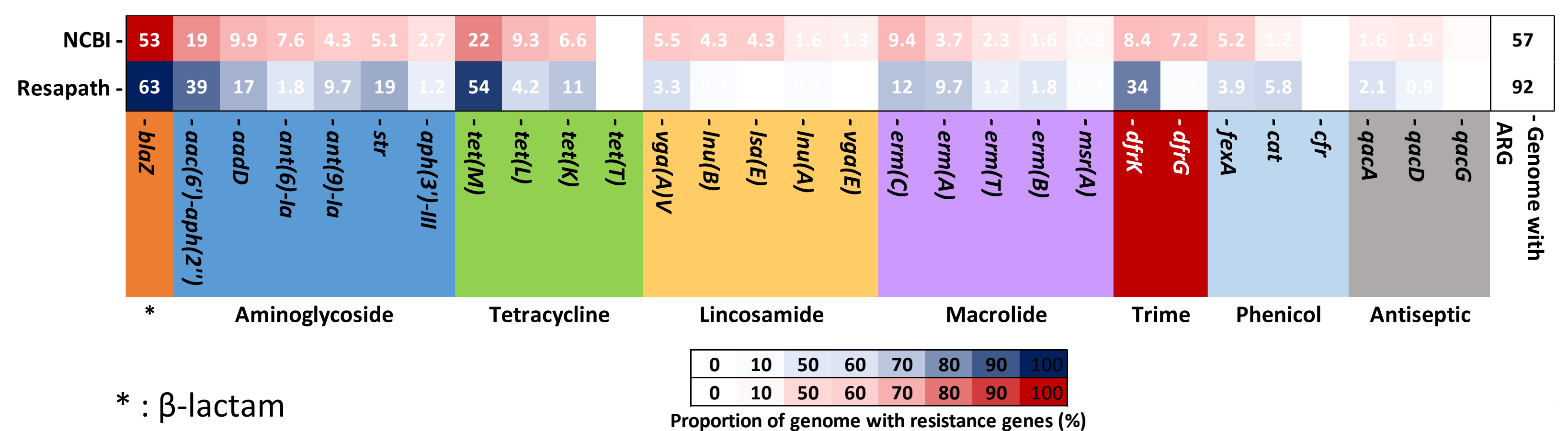


Materials & Methods



S. aureus from Resapath are multi-resistant strains

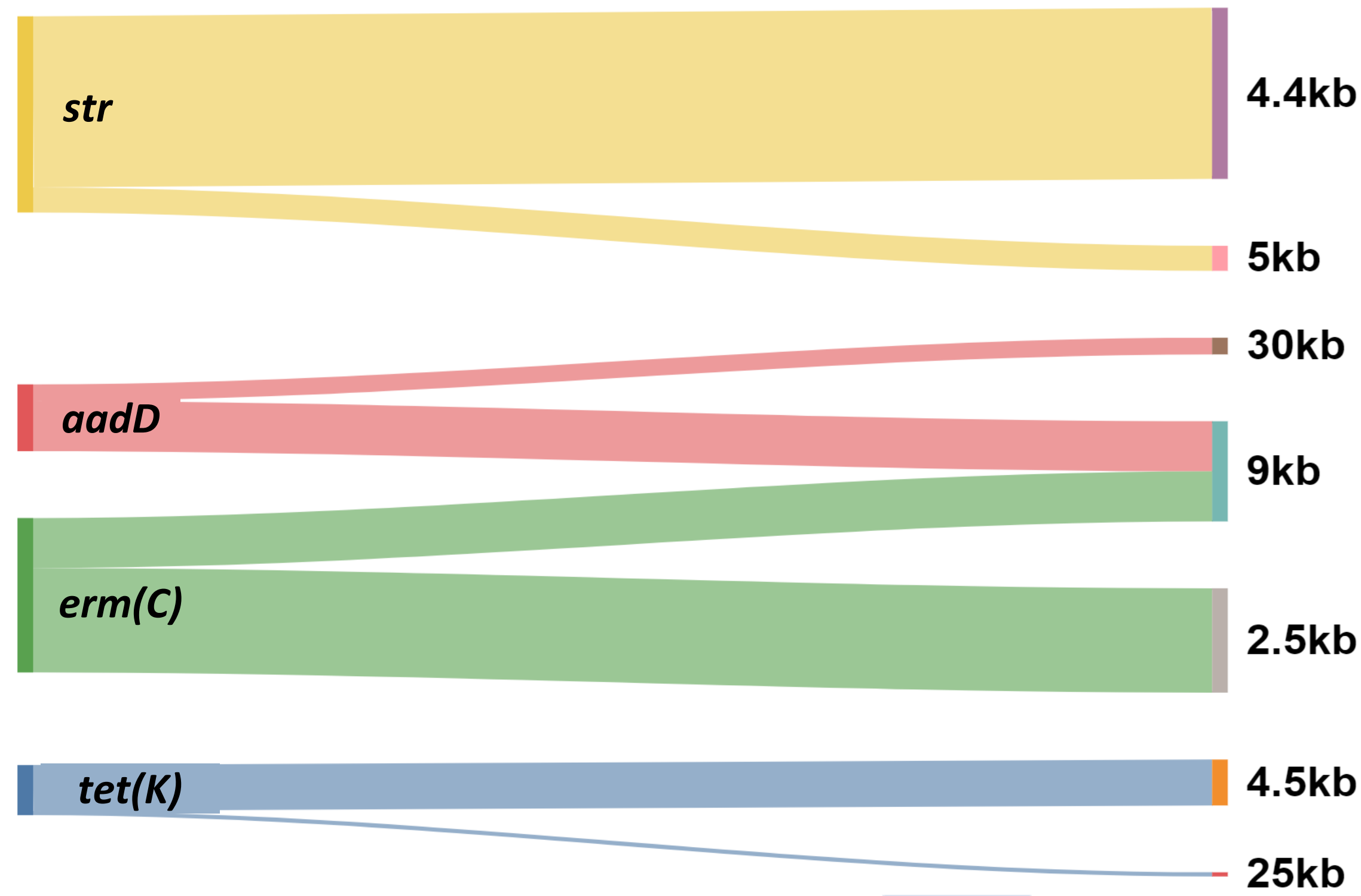
Repartition of resistance genes in *S. aureus* from animals



92% of strains carry at least one resistance gene. The pool of strains analyzed is relatively representative of NCBI data, the differences observed being due to a different animal host distribution.

Each ARG is associated with one type of plasmids in *S. aureus*

Sankey diagram depicting ARGs/plasmids associations identified by PFGE and Southern Blot, in *S. aureus* from the Resapath network

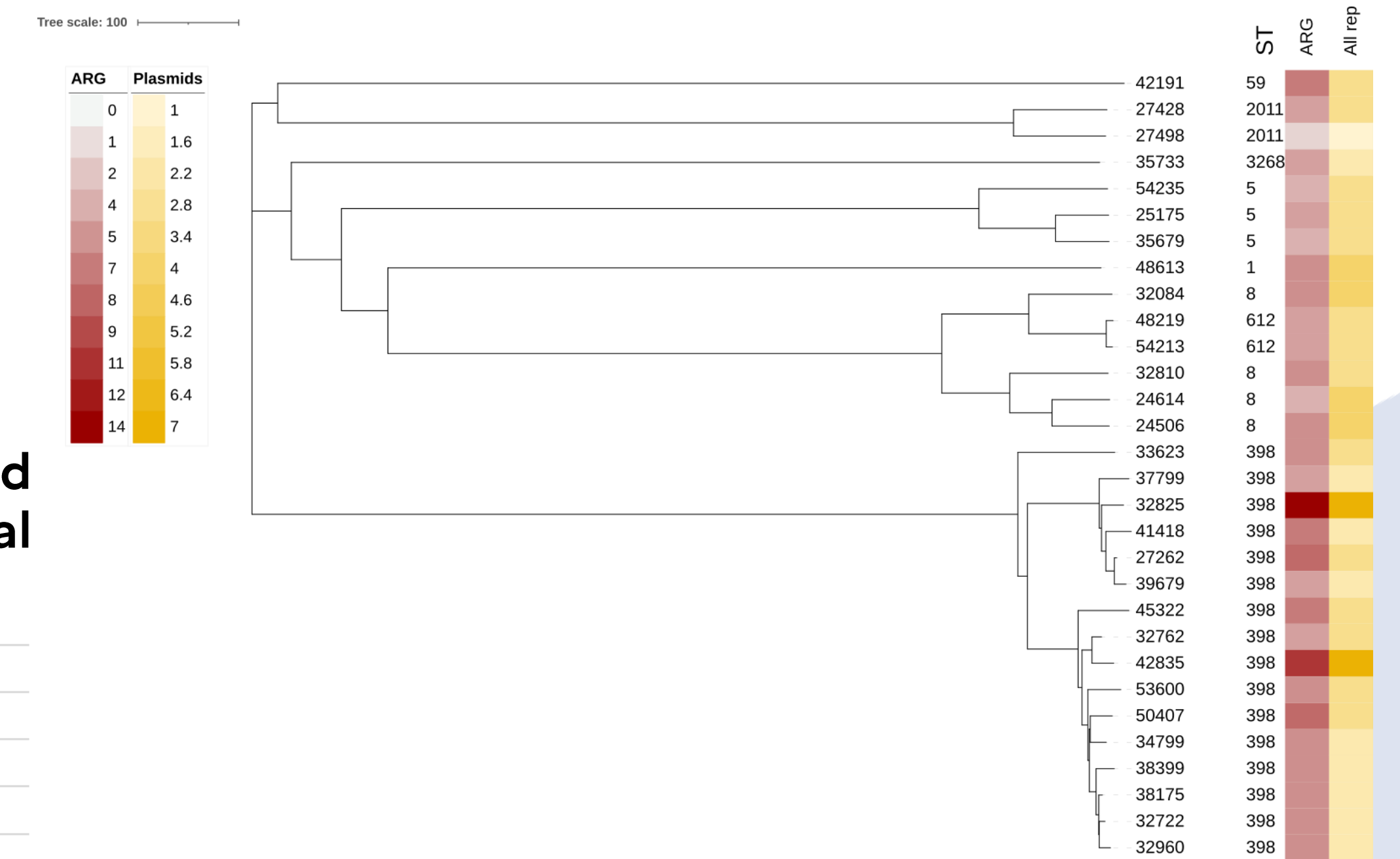


55% of strains carried at least one plasmid. The *tet(M)* gene was found only on the chromosome. *In silico* data showed that this gene is often associated with an ICE (Integrative and Conjugative Element) carrying a Rep protein, and therefore identified as a "plasmid" by *in silico* analysis.

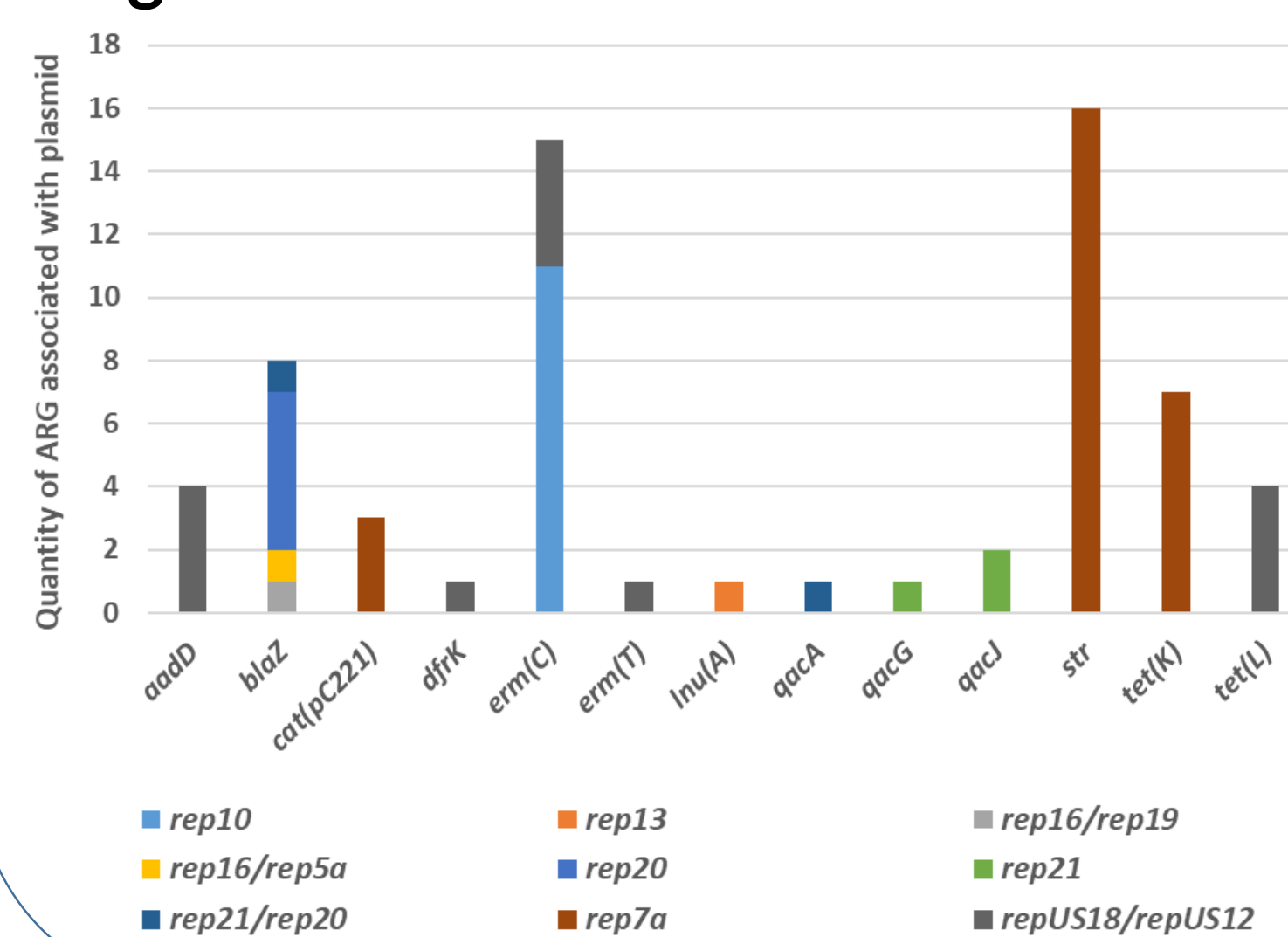
ARGs/plasmids associations of *S. aureus* strains from Resapath are in line with *in silico* data

The phylogenetic tree was built using pyMLST which compared the distance between 1861 core genes. It shows that Resapath *S. aureus* sequenced strains are quite diverse from a core genome point of view. A positive correlation between plasmids and ARGs was observed (Pearson r: 0,73 ; p-value <0,001)

Neighbor-joining phylogenetic tree based on *S. aureus* core genome showing ST, plasmid and ARG quantity per *S. aureus* strain



Associations between plasmids and resistance genes in *S. aureus* of animal origin



As found during Southern Blot analyses, each ARG is associated with a Rep protein family. The *aadD* and *erm(C)* genes are found associated on the same *repUS18/repUS12* plasmid. Interestingly, Rep proteins (mainly *repUS43* and *rep22*) were identified on chromosomal contigs, which could correspond to ICEs.

This study highlights the diversity of ARG/plasmid associations and confirms our first *in silico* analyses on *S. aureus* NCBI genomes. It suggests that *S. aureus* plasmids of animal origin play a key role in the spread of multidrug resistance, reinforcing the interest to deepen our knowledge and understanding of the dispersal mechanisms of this type of MGE. Indeed, plasmids are well characterized in Enterobacteriales but poorly studied in Staphylococcaceae despite their major interest. Studies will be carried out on the evolution of these ARG-carrying plasmids within a bacterial population and under different conditions (biofilm, antibiotic pressure, *in vivo*).