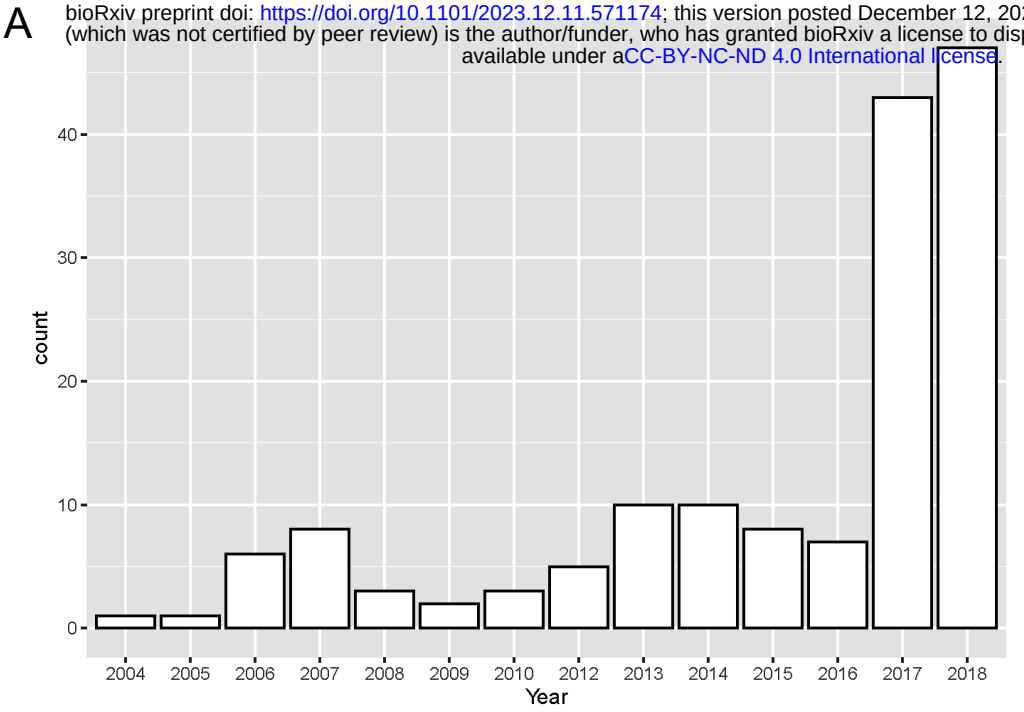

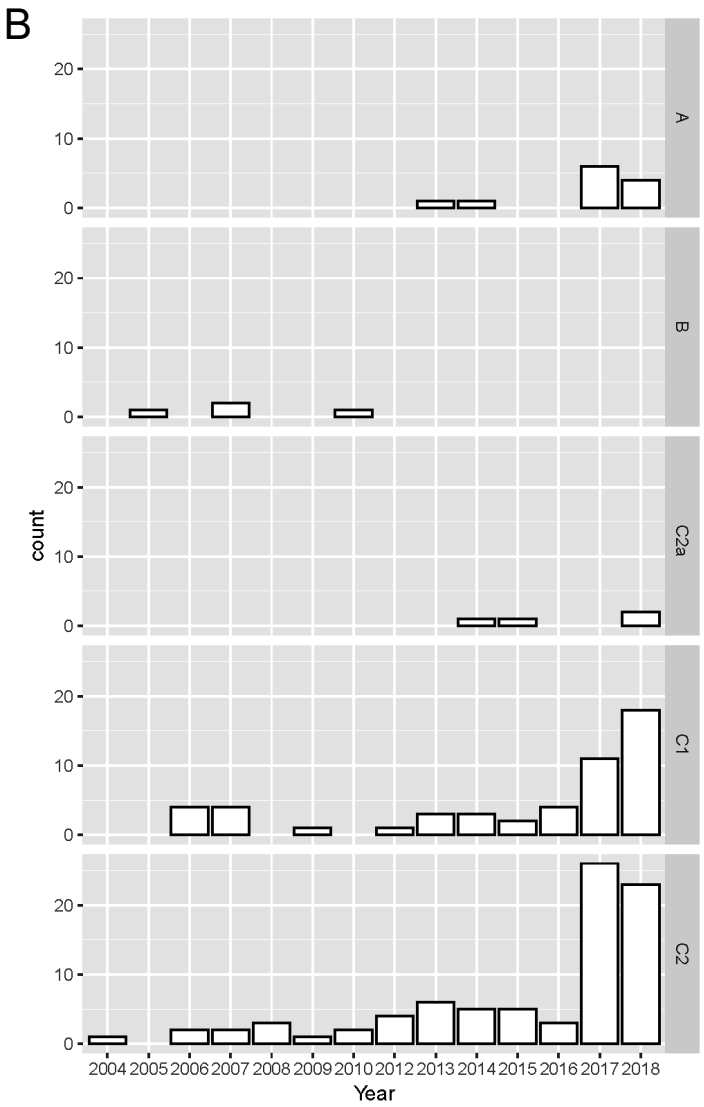

Figure S1. Timelines of ST131 isolate collection. (A) Total number of isolates collected each year. (B) Collection timelines for isolates belonging to each clade, subclade, and subgroup in the dataset.

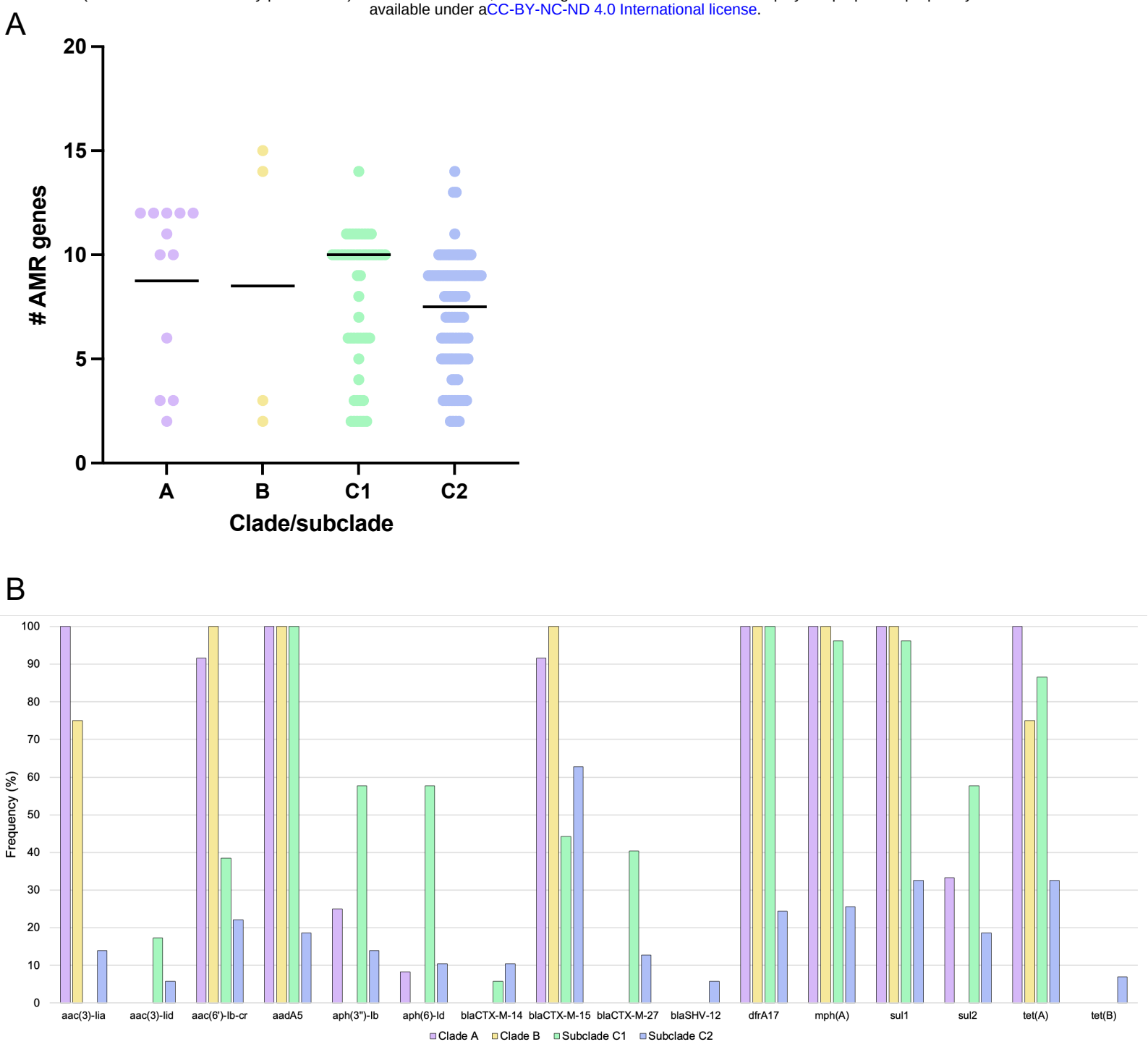

Figure S2. Differences in antibiotic resistance gene content between ST131 clades and subclades. (A) Antimicrobial resistance (AMR) gene abundance in isolates belonging to different ST131 clades and subclades. Horizontal lines show median values or AMR genes per genome in each isolate. AMR genes were identified by BLASTN to the ResFinder daabase. (B) Frequency of individual AMR genes among isolates in each clade or subclade. Genes with notable frequency differences between groups are shown. Complete data on AMR genes is provided in Table S2.

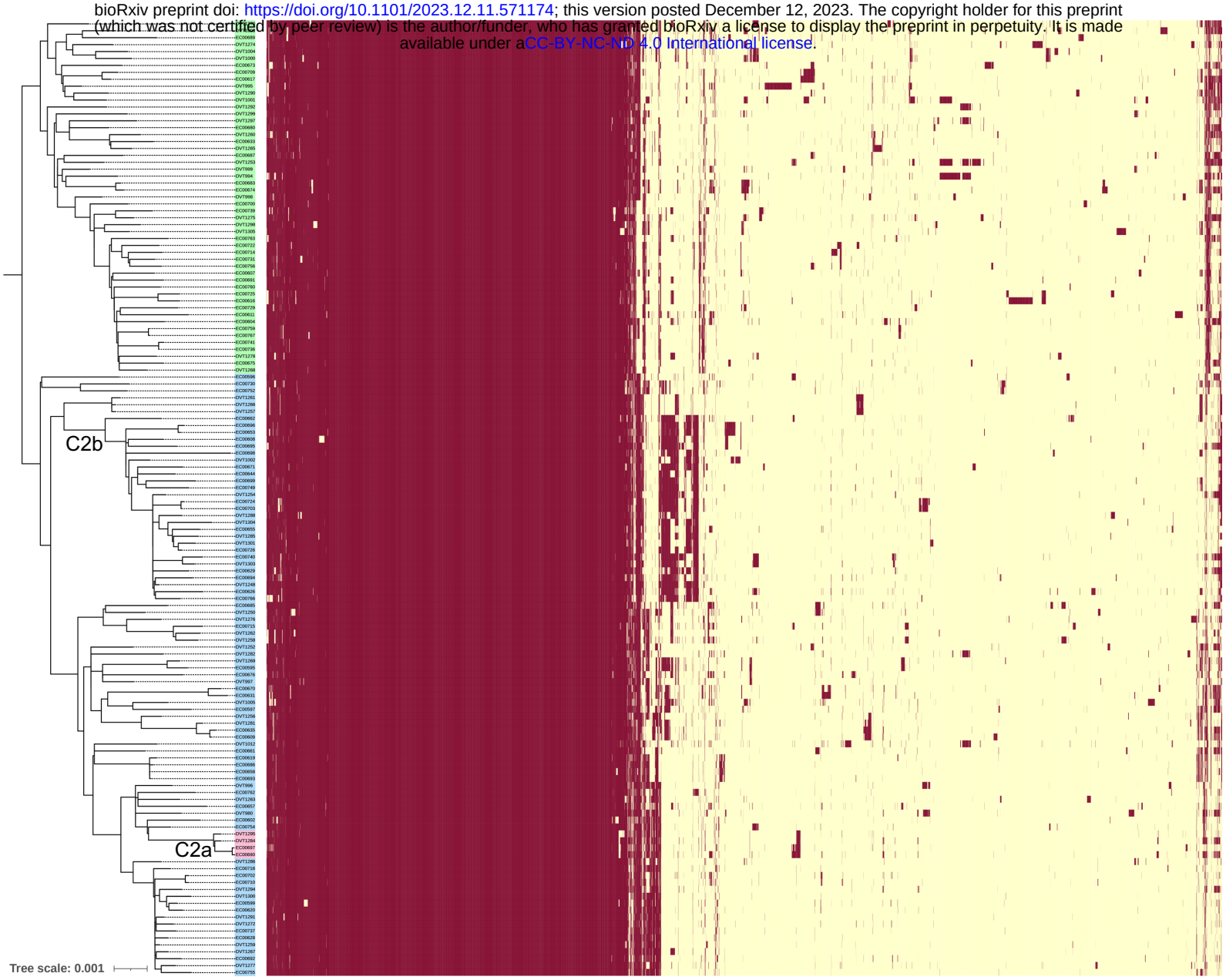

Figure S3. Pangenome analysis of 138 clade C ST131 *E. coli* isolates. Phylogenetic tree on the left was generated with RAxML using a core genome, post-ClonalFrameML SNP alignment. The tree was midpoint rooted to separate subclades C1 (green shaded) and C2 (blue shaded). The heatmap on the right shows the pangenome matrix generated by Roary. Each column represents one gene group, and each row represent one genome. The presence or absence of a gene in a given genome is shown as red or yellow, respectively. Subgroups C2a and C2b are labeled below the corresponding branches on the phylogenetic tree.

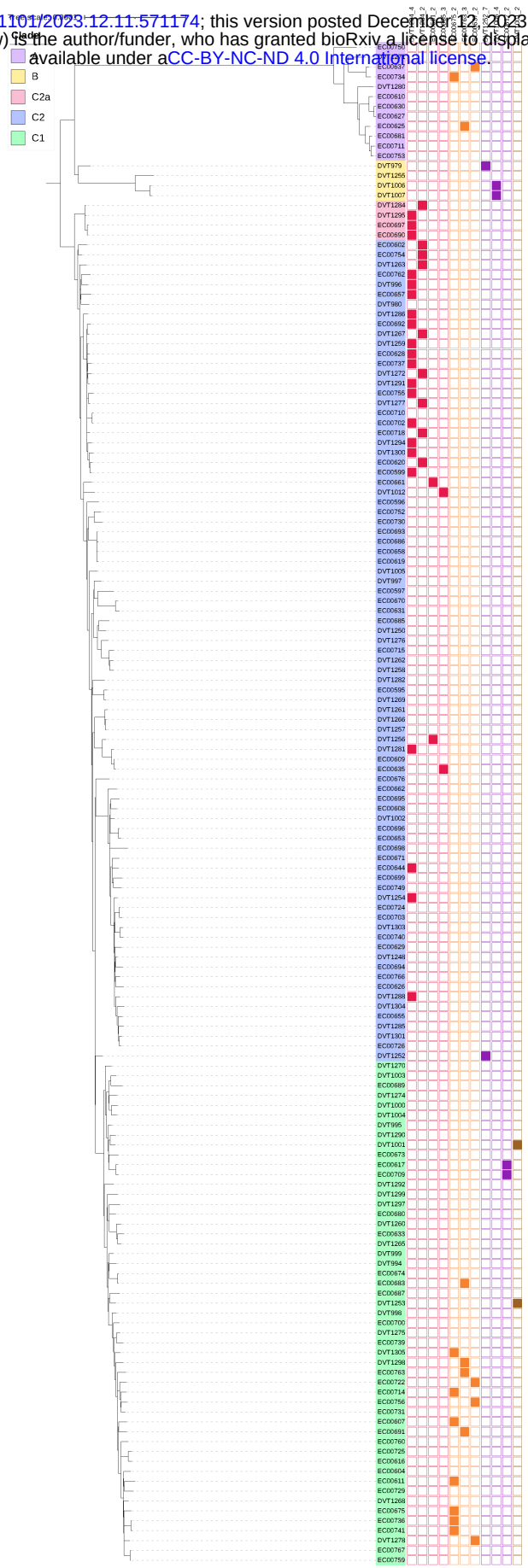

Figure S4. Distribution of ESBL-encoding plasmids among ST131 *E. coli* isolates. The core genome phylogeny is annotated with the presence of eleven ESBL-encoding reference plasmids that were detected in more than one genome in the dataset. Plasmids DVT1294_4, DVT1284_2, EC00661_2, and EC00635_3 harbor *bla*$_{\text{CTX-M-15}}$ (red); plasmids EC00675_2, EC00763_3, and EC00637_2 harbor *bla*$_{\text{CTX-M-27}}$ (orange); plasmids DVT1252_7, DVT1006_4, and EC00617_2 harbor *bla*$_{\text{SHV-12}}$ (purple); and plasmid DVT1001_2 harbors *bla*$_{\text{CTX-M-2}}$ (brown).

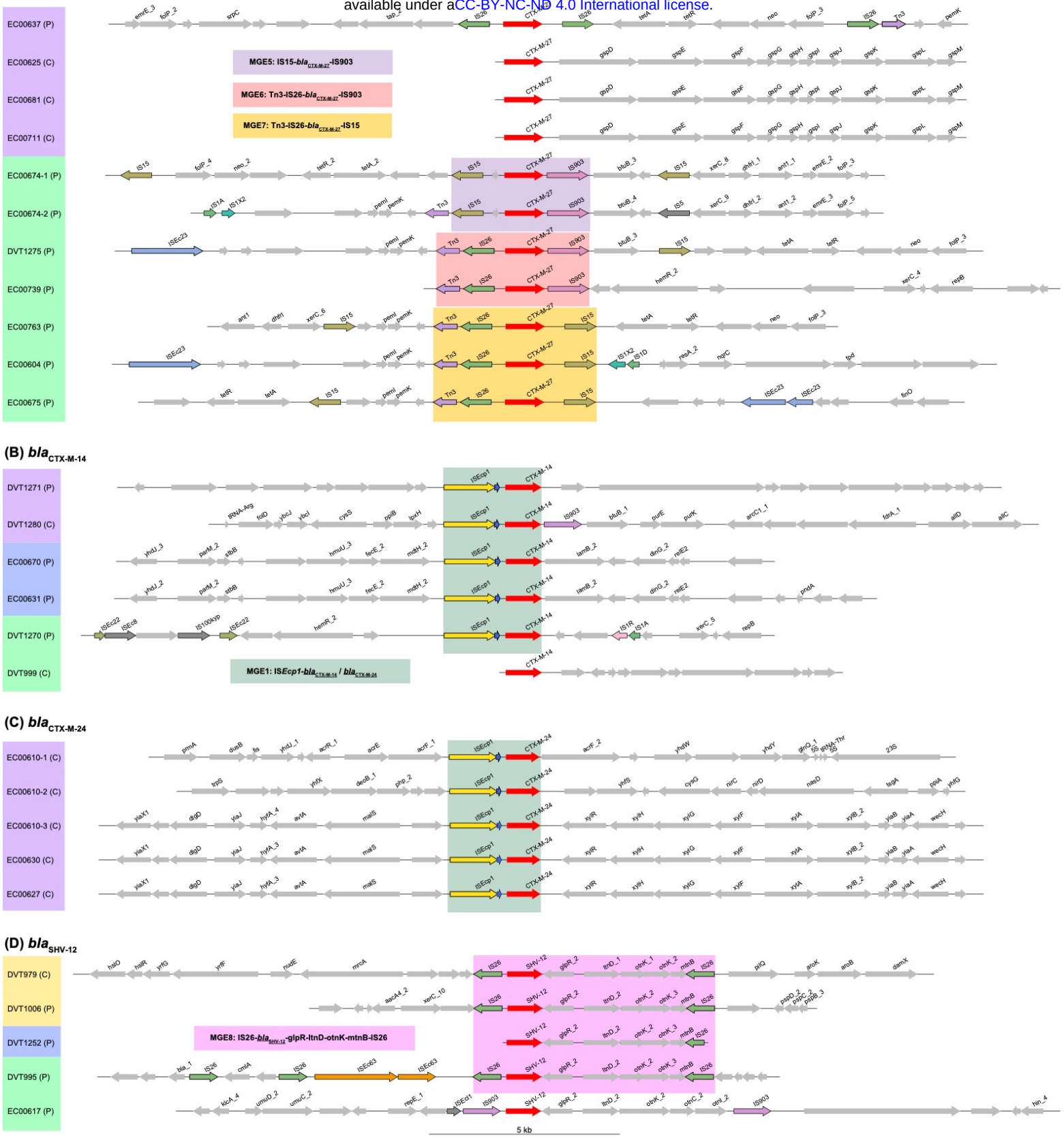

Figure S5. Regions flanking ESBL genes among ST131 *E. coli* isolates. (A-D) Genomic context of different ESBL-carrying MGEs is shown. Isolate names are shaded based on their phylogenetic clade assignments (clade A=purple; subclade C1=green; subclade C2=blue; clade B=yellow). The genomic context of each sequence is indicated (C=chromosome, P=plasmid) and ESBL genes are colored red. Genes were annotated with Prokka, and genes with predicted functions are labeled. Genes associated with MGEs and transposases are highlighted with black outlines, and are colored if found in more than one region. Regions that were used for MGE classification are shaded in each panel.