

Supplementary materials

Table S1. Galaxy Tool version and parameters.

Server	Step	Tool	Software version	Galaxy wrapper version	Database
usegalaxy.org	Annotation	Prokaryotic genome annotation / Prokka	1.14.6	1.14.6+galaxy2	Prokka default annotation databases
usegalaxy.org	Functional annotation	eggNOG Mapper	2.1.13 / emapper-2.1.13	2.1.13+galaxy0	eggNOG 5.0.2
usegalaxy.org	Pangenome	Roary	3.13.0	3.13.0+galaxy3	-
usegalaxy.eu	AMR and virulence screening	ABRicate	1.4.0	1.4.0	VFDB, CARD, ARG-ANNOT, NCBI Bacterial Antimicrobial Resistance Reference Gene Database, ResFinder
usegalaxy.eu	Insertion-sequence detection	ISEScan	1.7.3	1.7.3+galaxy0	ISEScan insertion-sequence profiles/database bundled with Galaxy tool
usegalaxy.eu	Phylogeny	IQ-TREE	2.4.0	2.4.0+galaxy2	Built-in IQ-TREE model set / ModelFinder
usegalaxy.eu	Plasmid/provirus prediction	geNomad	1.12.0	1.12.0+galaxy0	geNomad reference data 1.9
usegalaxy.eu	Recombination detection	Gubbins	3.2.1	3.2.1+galaxy0	-
usegalaxy.eu	SNP-site extraction	Finds SNP sites / SNP-sites	2.5.1	2.5.1+galaxy0	-
usegalaxy.eu	Reference-based variant calling	snippy	4.6.0	4.6.0+galaxy0	CD630 reference sequence AM180355/NC_009089.1 plus plasmid NC_008226.2
usegalaxy.eu	Core SNP alignment construction	snippy-core	4.6.0	4.6.0+galaxy0	-
usegalaxy.eu	Core alignment cleanup	snippy-clean_full_aln	4.6.0	4.6.0+galaxy0	-
usegalaxy.eu	MLST	MLST	2.22.0	2.22.0	PubMLST cdifficile scheme
usegalaxy.eu	AMR summary	AMRFinderPlus output summary	AMRFinderPlus 3.12.8	3.12.8+galaxy0	AMRFinderPlus database amrfinderplus_V3.12_2024-05-02.2; NCBI Reference Gene Database
usegalaxy.eu	harmonization	hAMRonization	1.1.4	1.1.14+galaxy0	-

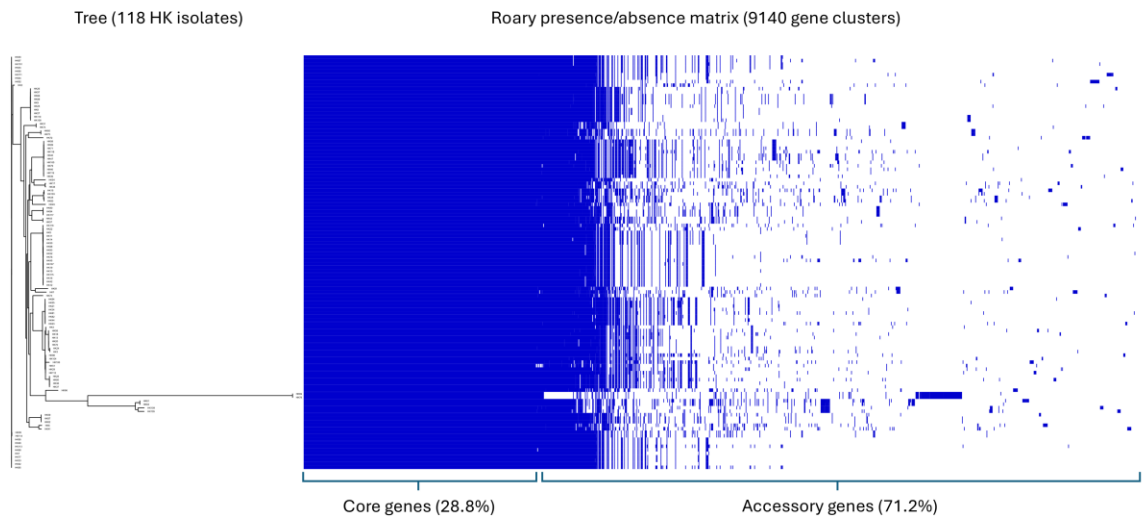


Figure S1. Pangenome alignment of 118 *Clostridioides difficile* isolates. Pangenome analysis was performed using Roary. The gene matrix shows the presence (blue blocks) and absence (gray areas) of core and accessory genes. In the matrix, genomes are shown as rows and gene clusters as columns. The dendrogram is based on the phylogenetic relatedness of the core genes and accessory gene clusters.

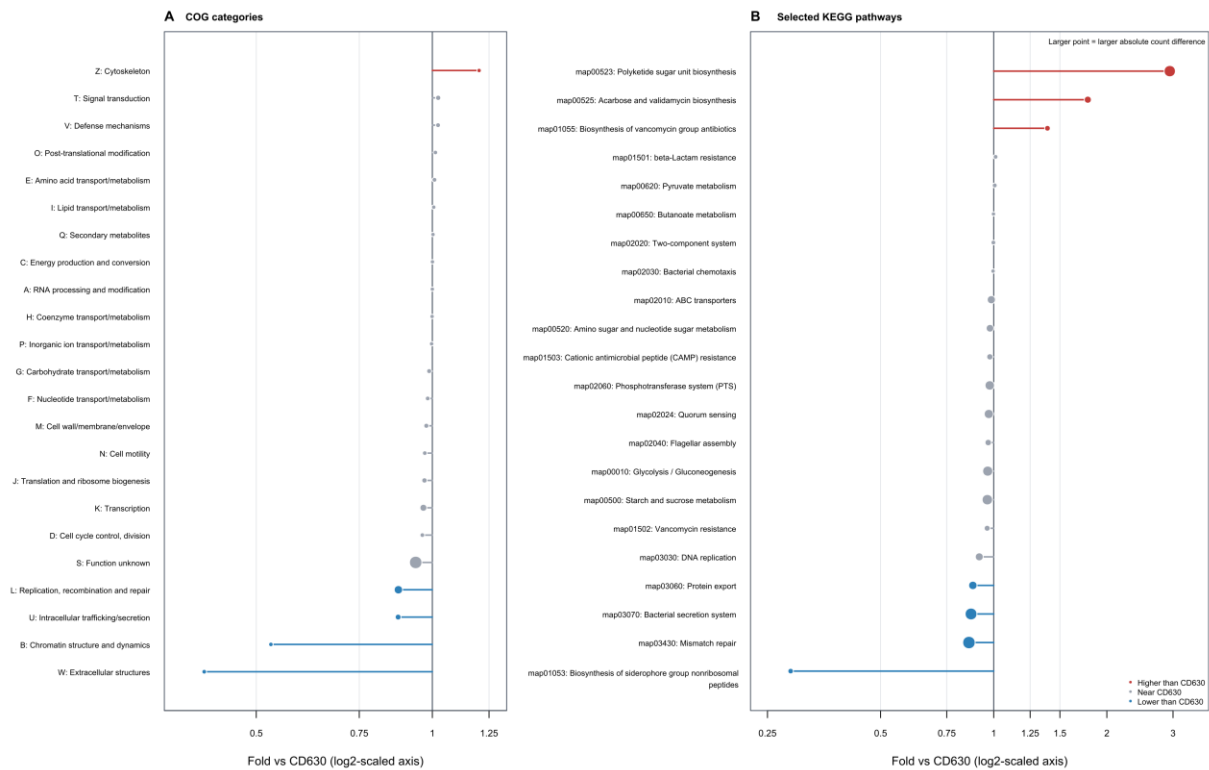


Figure S2. CD630 reference-normalised eggNOG functional comparison. KEGG pathway and COG category annotation burdens in the 118 *Clostridioides difficile* study isolates were compared with the CD630 reference genome using eggNOG-mapper outputs generated with comparable settings.