Supporting information for:

Characterisation of Plasmid-Associated Antimicrobial Resistance Genes in Coastal Marine *Enterobacterales* from the Central Adriatic Sea: De Novo Assembly and Bioinformatic Profiling

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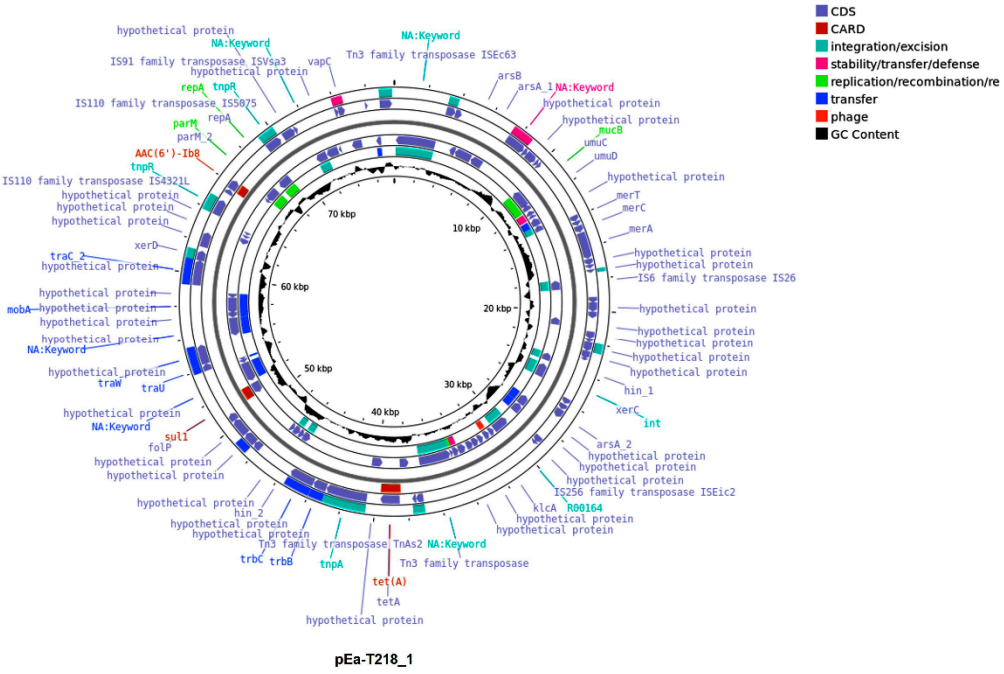
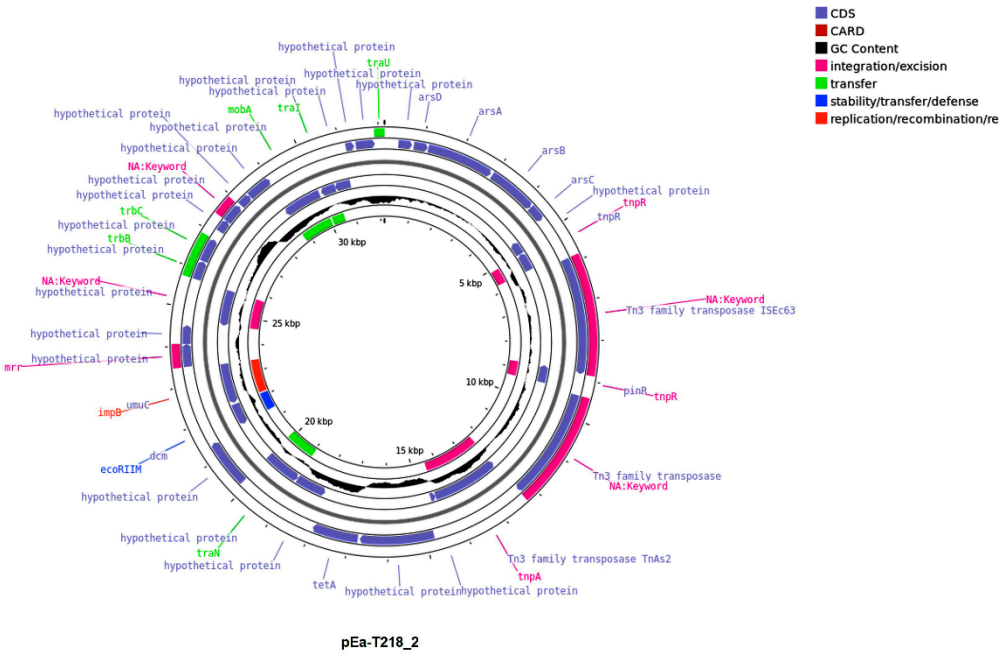
Supplementary Figures S1–S8. Annotated maps of putative plasmid-derived contig assemblies from marine *Enterobacterales*.

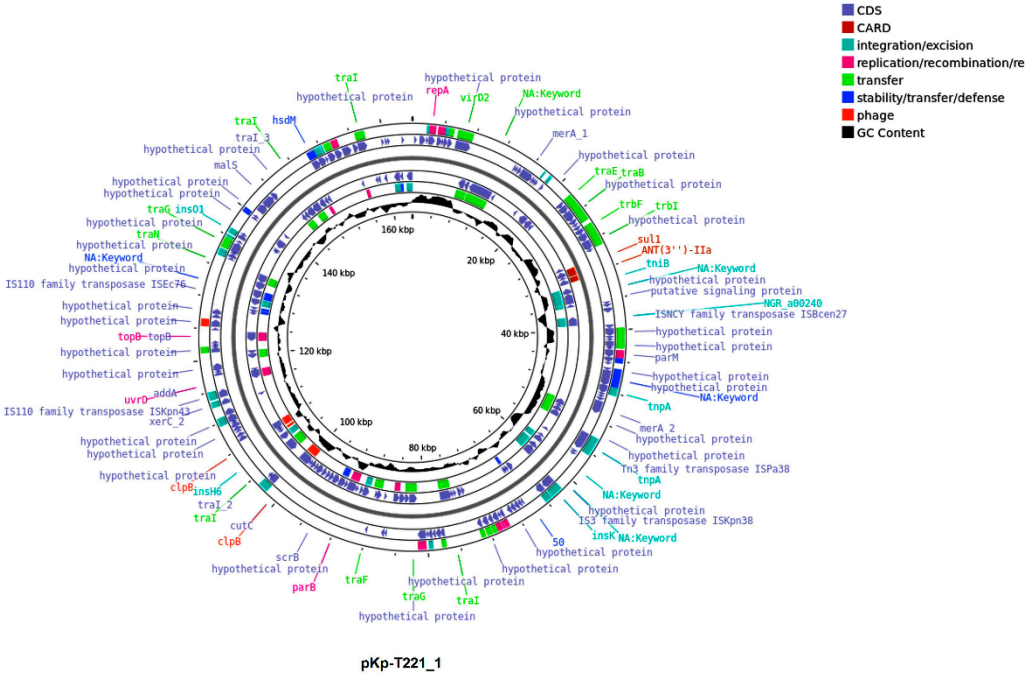
Circular genome visualisations of eight putative plasmid-derived contig assemblies reconstructed from short-read de novo assemblies of marine *Enterobacterales* isolates: pEa-T218_1, pEa-T218_2, pKp-T221_1, pEc-T224_1, pEc-T224_2, pEc-T227_1, pEb-T200_1, and pEc-T205_1. Assemblies were annotated and visualised using the Proksee (v1.0.0a6) platform (Grant et al., 2023), which integrates Prokka for gene prediction, CARD-RGI (Resistance Gene Identifier) for antimicrobial resistance gene (ARG) detection, mobileOG-db for mobile genetic element (MGE) identification, and a GC content track for local sequence composition. These contig assemblies, clustered by MOB-suite based on plasmid-associated features, are presented as circularised visualisations, although physical circularity and structural integrity were not experimentally confirmed. The maps support the functional annotations discussed in the main text and provide graphical insight into ARG and MGE distribution.



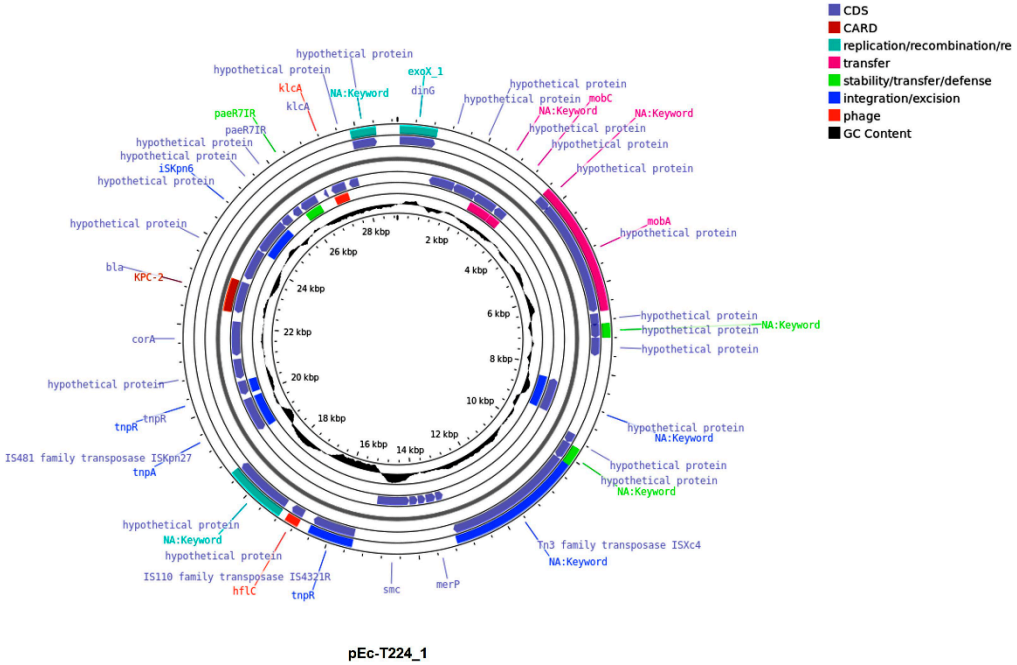
Supplementary Figure S1_Annotated Map of the Putative Plasmid-Derived Contig Assembly pEa-T218_1_phs



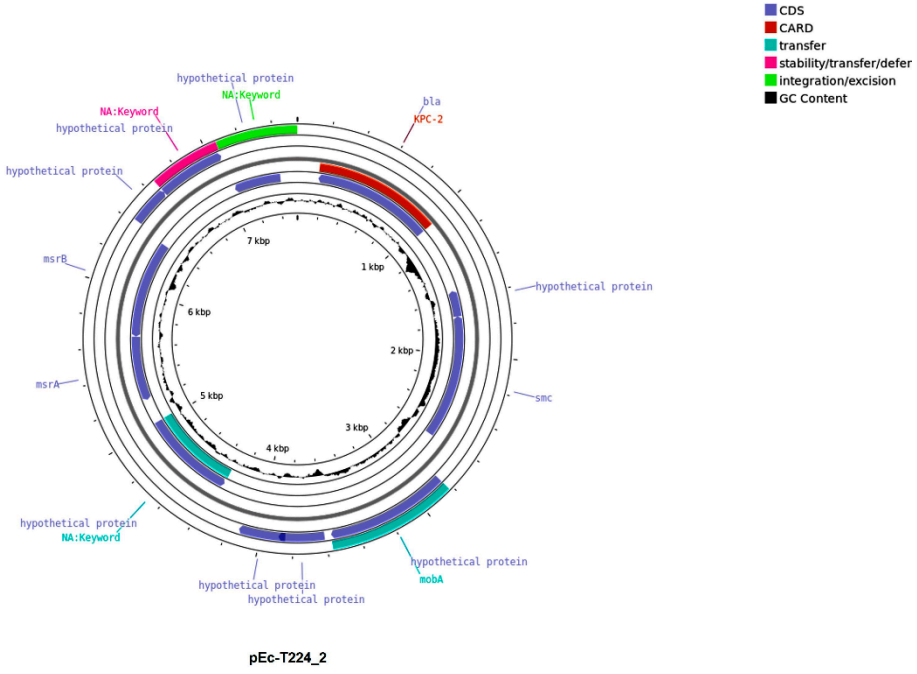
Supplementary Figure S2_Annotated Map of the Putative Plasmid-Derived Contig Assembly pEa-T218_2_phs



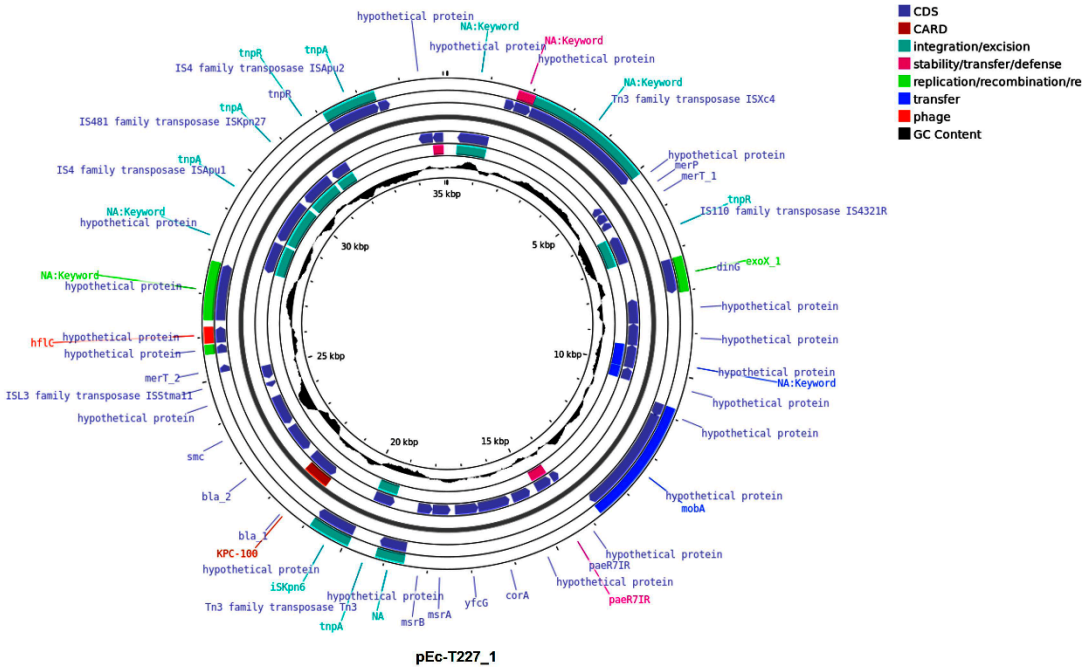
Supplementary Figure S3_Annotated Map of the Putative Plasmid-Derived Contig Assembly pKp T221_1_phs



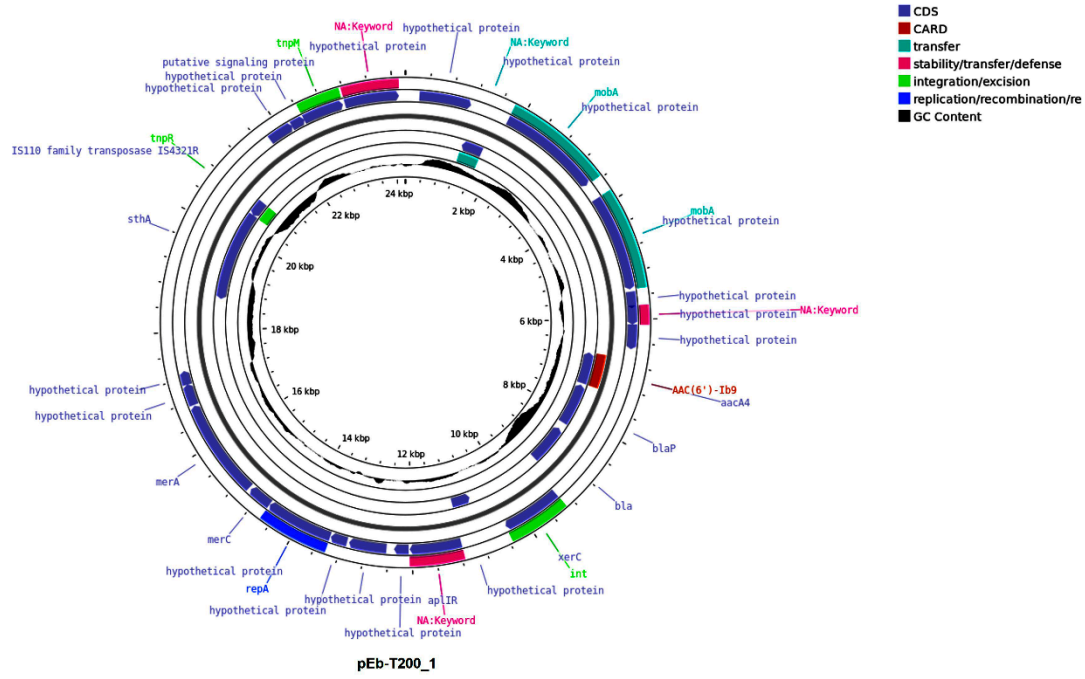
Supplementary Figure S4_Annotated Map of the Putative Plasmid-Derived Contig Assembly pEc-T224_1



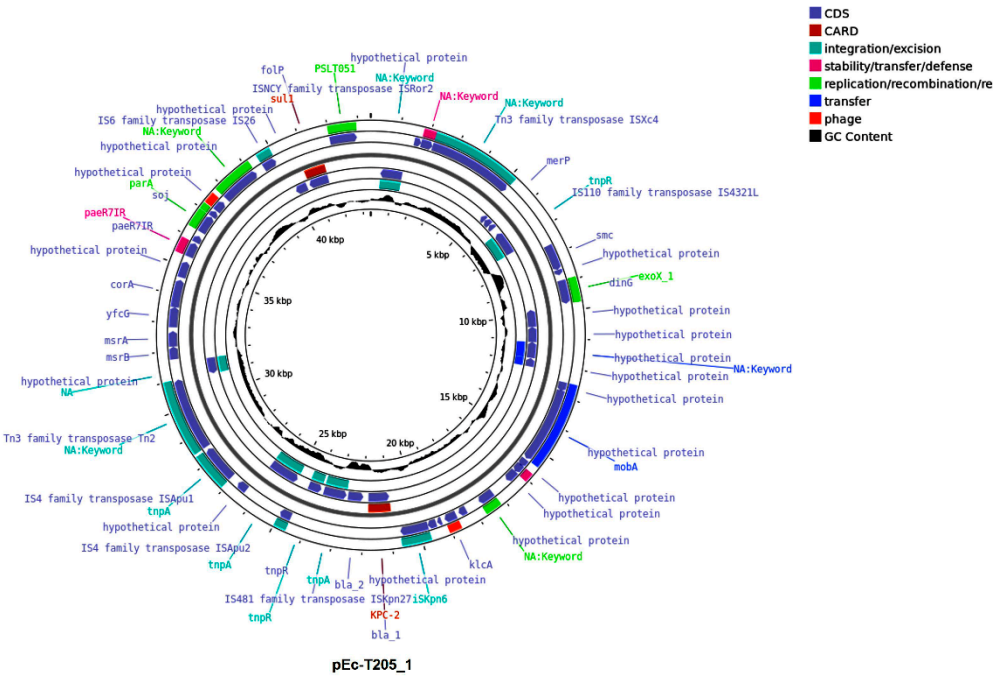
Supplementary Figure S5_Annotated Map of the Putative Plasmid-Derived Contig Assembly_pEc-T224_2



Supplementary Figure S6_Annotated Map of the Putative Plasmid-Derived Contig Assembly_pEc-T227_1



Supplementary Figure S7_Annotated Map of the Putative Plasmid-Derived Contig Assembly_pEb-T200_1



Supplementary Figure S8_Annotated Map of the Putative Plasmid-Derived Contig Assembly_pEc-T205_1

References

Grant, J.R., Enns, E., Marinier, E., Mandal, A., Herman, E.K., Chen, C.Y., Graham, M., Van Domselaar, G. and Stothard, P. (2023) 'Proksee: In-depth characterization and visualization of bacterial genomes', *Nucleic Acids Research*, 51(W1), pp. W484–W492. Available at: https://doi.org/10.1093/nar/gkad326.

Supplementary Table S1. Comparative overview of ARGs, inferred plasmid features, and anthropogenic pressures across Adriatic coastal studies. Includes data from this study and six published datasets, highlighting gene content, mobility markers, and environmental contexts. Plasmid features are inferred from sequence data and do not confirm complete plasmid structures.

Study Focus	ARGs Detected	Plasmid Replicons	MOB Types	IS Elements	Transferability Evidence	Host Species	Sample Sites	Anthropogenic Pressures	References
Plasmid-Associated ARGs in Marine *Enterobacterales* from the Central Adriatic	*bla*$_{KPC}$, *bla*$_{TEM}$, *bla*$_{GES}$, *bla*$_{OXA}$, *aac*$_{A4}$, *ant1*, *folP*, *dfrB1*, *tetA*	IncA/C2, IncP6	MOBP, MOBQ	IS110, IS4, IS6, IS1182, IS630	MOB typing; *tra* genes not confirmed	*E. coli*, *K. pneumoniae*, *E. bugandensis*, *E. asburiae*	Radoševac (Split)	Hospital discharge, sewage	This Study
Carbapenemase-Producing *Enterobacterales* from a Submarine Outfall near Split	*bla*$_{KPC-2}$, *bla*$_{OXA-48}$, *bla*$_{TEM}$, *sul1*, *qnrS*, *aadA*, *aac(6')-Ib*	IncA/C2, IncL, IncFII	MOBP	IS26, ISKpn19	Conjugation confirmed	*E. coli*, *K. pneumoniae*, *C. freundii*	Submarine outfall near Split	Hospital discharge	Kvesić et al., 2022
Antibiotic Resistance Gradients in Coastal Waters of the Adriatic	*bla*$_{KPC}$, *qnr*S, *tetA*, *sul1*, *ermB*	IncFII	MOBP	ISKpn6, ISKpn7	Conjugation inferred	*E. coli*, *Klebsiella* spp.	Multiple marine and coastal points	Tourism, sewage	Kvesić et al., 2021
Colistin-Resistant Bacteria and *mcr*-1 Detection in Coastal Waters	*tetA*, *sul1*, *qnrS*, *ermB*, *bla*$_{CTX-M}$	IncP1, IncQ (implied)	MOBQ, MOBF (implied)	IS26, ISEcp1 (reported by gene context)	Plasmid markers present	*E. coli*, *Klebsiella*, *Serratia* spp.	Tourist beaches, aquaculture	Aquaculture, tourism	Šamanić et al., 2021)

Vancomycin-Resistant *Enterococcus* in Coastal Waters	*vanA*, *vanB*, *aac(6′)-Ie*, *aph(2″)-Ia*, *aph(2″)-Ib/Id*	Not applicable	Not applicable	Not reported	Not applicable	*E. faecium*	Wastewater, beach, clinical	Hospital, urban wastewater	Dželalija et al., 2023
Biogeographical Shifts in ARGs along an Adriatic Trophic Gradient	*sul1*, *qnrS*, *bla*$_{OXA}$, *intI1*, *tetA*, *bla*$_{VIM}$, *bla*$_{TEM}$, *ermB*, *mphA*	Not specified	Not specified	ISs not detailed	Inferred via ARG-IS associations	Mixed marine bacteria	Central Adriatic gradient	Sewage, urban inputs	Dželalija et al., 2024
Marine Resistome Dynamics across an Adriatic Trophic Gradient	*sul1*, *qnrS*, *bla*$_{OXA}$, *bla*$_{TEM}$, *intI1*, macrolide, *van*, imipenem	Not specified	Not specified	ISs not detailed	Inferred via ARG-IS associations	Mixed marine bacteria	Broad Adriatic gradient	Sewage, ports, trophic gradient	Dželalija et al., 2023

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