DE NOVO PLASMID ASSEMBLY FROM CARBAPENEM RESISTANT ENTEROBACTERIACEAE IN CENTRAL ADRIATIC SEA

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Introduction

Carbapenem-resistant Enterobacteriaceae (CRE), are classified as a critical priority by the World Health Organization (WHO) due to their ability to transfer resistance genes. Factors such as inadequate infection control and inefficient environmental surveillance contribute to the global burden of CRE, resulting in increased morbidity, mortality and costs.

Aim

To investigate the presence and diversity of CRE in the marine environment of the central Adriatic Sea.
In 38 isolates, isolated from Trstenik public beach and Jadro estuary, we detected various carbapenemase genes, such as *bla*$_{KPC-2}$, *bla*$_{OXA-48}$, and *bla*$_{VIM-1}$ Therefore in this study we performed *de novo* assembly of eight plasmids.

Methods and workflow

- DNA was extracted from PFGE gels using the QIAEX II Gel Extraction Kit. On-bead tagmentation of plasmid DNA was done using Illumina DNA Prep. The libraries were pooled and quality checked before sequencing on the Illumina iSeq 100 platform
- SPAdes was used for plasmid-specific assembly, and contigs were functionally characterized using genome databases.
- Antimicrobial resistance genes were identified via CARD and CGE-tools (ResFinder), while virulence genes were detected using VFDB.
- Plasmid typing was performed with MobSuite and PlasmidFinder.

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Results

- Plasmids were found to carry up to five *bla* genes, including *bla*$_{TEM-1}$, *bla*$_{OXA-10}$, *bla*$_{OKP-B}$, and *bla*$_{GES-1}$ in addition to the carbapenemase genes.
- The *bla*$_{VIM-1}$-carrying plasmid identified the first report of the *bla*$_{VIM-1}$ gene in *Enterobacter asburiae* in a marine environment. The *E. asburiae* isolate also harbored *bla*$_{KPC-2}$ on separate conjugative plasmid, which contained multiple antibiotic resistance genes (ARGs) and virulence factors, including those for aminoglycoside, tetracycline and quinolone resistance.
- Sequencing of conjugative *bla*$_{KPC-2}$-bearing plasmid in multidrug-resistant (MDR) *E. bugandensis* and extensively drug resistant (XDR) *E. coli*, identified plasmid replicons, such as IncP6 and IncR, as key vehicles for ARG dissemination.
- Plasmid analysis of XDR *K. pneumoniae* isolate highlighted the role of IncFIB plasmids in the environmental spread of *bla*$_{KPC-2}$.

Conclusions

- Enterobacteriaceae isolates carried blaKPC-2 on diverse conjugative plasmids that harboured multiple resistance genes, insertion sequences and plasmid replicons, favouring their horizontal spread.
- The presence of virulence determinants along with their potential of dissemination through horizontal gene transfer, underscores the complexity and adaptability for persistence of these 'critical-priority' pathogens in aquatic environments.

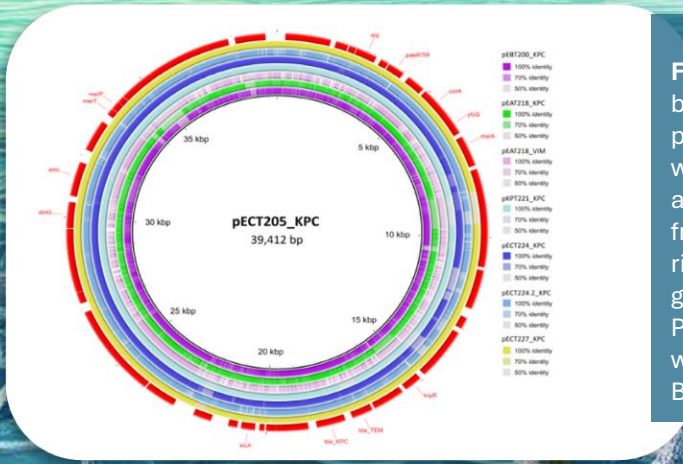

Figure 1. Mapping of blaKPC-2-harbouring plasmid pECT205_KPC with other de novo assembled plasmids from this study. The red ring depicts the coding genes identified by Prokka. The plasmids were visualized using BRIG software

Table 1. Molecular characteristics of eight *de novo* assembled resistance plasmids.

Plasmid	Incompatibility group	Resistance genes	Virulence genes
pECT218_KPC	Col440I, FII(pECLA)	*aac(6')-Ib3, qnrB6, sul1, tet(A)*	*astA*
pEAT218_VIM	Col440I, N L/M(pOXA-48)	*aac(6')-Ib3, bla*$_{OXA-10}$ *bla*$_{VIM-1}$, *sul1, tet(A)*	*astA*
pKPT221_KPC	A/C2, FIB(K), FIB(pKPHS1), FII, P6	*aadA1, bla*$_{KPC-2}$, *bla*$_{TEM-1}$, *bla*$_{OKP-B-16}$, *bla*$_{OKP-B-5}$, *bla*$_{OKP-B-3}$, *fosA5, OqxA, sul1, tet(A), dfrB1*	*clpK2, traT, yagZ/ecpA, yagX/ecpC*
pECT224_KPC	Col440I, ColRNAI, A/C, FII, P6, R	*aac(6')-Ib-cr bla*$_{KPC-2}$, *sul1, dfrA27*	
pECT224.2_KPC	ColRNAI	*bla*$_{KPC-2}$	
pECT227_KPC	Col(IRGK),P6, Q1	*aac(6')-Ib3, bla*$_{KPC-2}$, *bla*$_{TEM-1}$	
pEBT200_KPC	Col(IRGK) ColRNAI FIA(HI1), R	*aac(6')-Ib3, armA, bla*$_{GES-1}$, *bla*$_{OXA-10}$, *bla*$_{KPC-2}$, *bla*$_{TEM-1}$, *msr(E), mph(E)*	*traT*
pECT205_KPC	Col440I, P6	*aac(6')-Ib-cr, bla*$_{KPC-2}$, *bla*$_{TEM-1}$, *sul1, dfrA27*	