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Whole-genome sequencing-based profiling of carbapenamase- (OXA-48) and ESBL- (CTX-M-15) producing *Klebsiella pneumoniae* ST392 clone isolated in a Croatian Intensive Care Unit

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Background:

- Carbapenem-resistant ***Enterobacteriaceae*** (CRE) represent a significant global public health concern due to increasing resistance rates and high mortality [1].
- *Carbapenemase-producing* ***Klebsiella pneumoniae*** represents a major cause of lower respiratory tract infections (LRTIs) associated with intensive care units (ICUs)
- Rapid and accurate detection of carbapenem-resistant isolates is essential for infection control and effective antibiotic therapy [2].
- **This study aimed** to characterise the phenotypic and genotypic antimicrobial resistance in OXA-48-producing *Klebsiella pneumoniae* causing LRTIs in the ICU of the University Hospital of Split, Croatia.

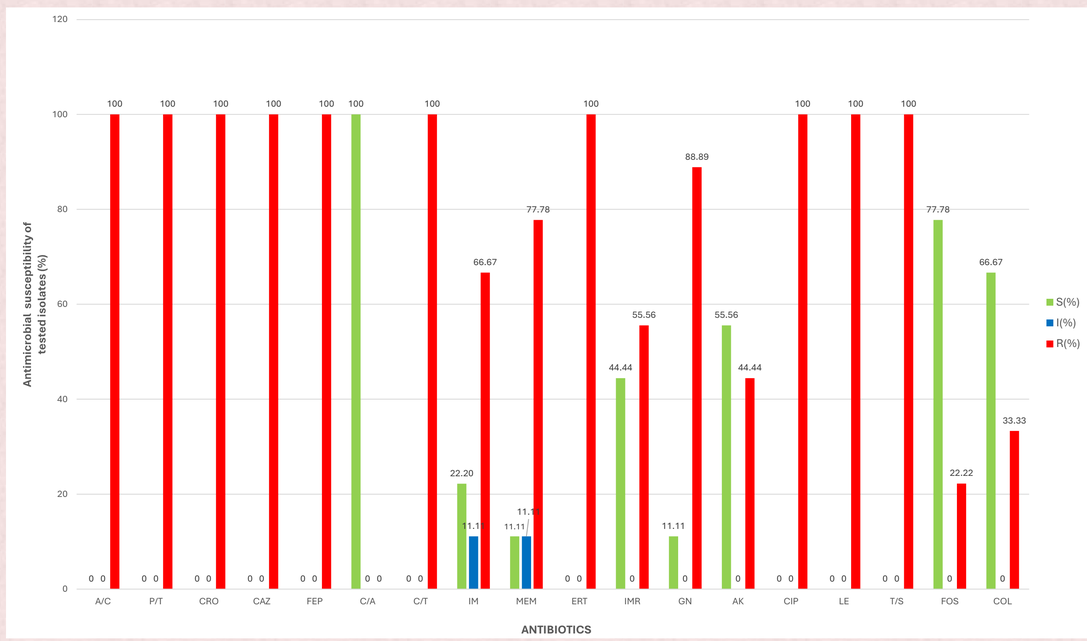
Material/methods:

- The inclusion criteria were clinically, laboratory and radiologically diagnosed LRTIs in ICU patients and OXA-48-producing *Klebsiella pneumoniae* isolated from LRT specimens (January 2023 to January 2024).
- Isolates were identified using MALDI-TOF-MS (Bruker, Germany), tested for antimicrobial susceptibility (AST) using disc diffusion method and interpreted according to EUCAST (v.13.0). Carbapenem-resistant strains were screened for carbapenemase production (O.K.N.V.I. RESIST-5, CORIS, Belgium).
- A selected number of isolates with phenotypically detected OXA-48 production were analysed by whole-genome sequencing (WGS).
- Reads generated by Illumina sequencing were assembled *de novo* into contiguous sequences using Shovill v0.9.0, with subsampling read depth down to 150x and assembling using SPAdes v.4.0. ABRicate was used to identify antimicrobial resistance genes using NCBI AMRFinderPlus database.
- Multilocus sequence typing (MLST) was determined using genomic sequences as input data against the standard MLST schemes.

Results:

- AST results of nine non-duplicate isolates are shown in **Figure 1**. All isolates were **multidrug-resistant** with full resistance to amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftriaxone, ceftazidime, cefepime, ceftolozane/tazobactam, ertapenem, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole.
- High-level resistance was observed to imipenem/cilastatin (67%), meropenem (78%), imipenem/cilastatin/relebactam (56%), gentamicin (89%) and amikacin (45%).
- All isolates were susceptible to ceftazidime/avibactam, while moderate resistance was observed to fosfomycin (22%) and colistin (33%).
- Three isolates were selected for WGS and results are shown in **Table 1**.
- All isolates possessed antimicrobial-resistance genes for β-lactamases (***bla*$_{CTX-M-15}$, *bla*$_{SHV-11}$, *bla*$_{TEM-1}$, *bla*$_{OXA-48}$, *bla*$_{OXA-1}$**), ***fosA6*** gene for fosfomycin, ***aac3-IIa, aph(6)-Id*** and ***aac(6')-Ib*** for aminoglycoside resistance, ***qnrB1*** gene for quinolone resistance and ***sul2*** and ***dfrA14*** for trimethoprim/sulfamethoxazole resistance.
- Additionally, all isolates possess **IncL, InFIB, IncFII, IncR** and **ColRNAI** plasmids and belonged to the **ST392 clone**.

Figure 1 **Antimicrobial susceptibility pattern of OXA-48-producing *Klebsiella pneumoniae* isolates**



Note: amoxicillin/clavulanic acid (A/C); piperacillin/tazobactam (P/T); ceftriaxone (CRO); ceftazidime (CAZ); cefepime (FEP); ceftazidime/avibactam (C/A); ceftolozane/tazobactam (C/T); imipenem/cilastatin (IM); meropenem (MEM); ertapenem (ERT); imipenem/cilastatin/relebactam (IMR); gentamicin (GN); amikacin (AK); ciprofloxacin (CIP); levofloxacin (LE); trimethoprim/sulfamethoxazole (T/S); fosfomycin (FOS); colistin (COL)

Table 1 **Resistant isolates and corresponding resistance genes**

Strain	Antibiotics and resistance genes							Plasmid Incompatibility Group	MLST
	β-lactams Class A β-lactamases	β-lactams Class D β-lactamases	FOS	AMINO	CIP	T/S	efflux		
KP-3	*bla*$_{CTX-M-15}$ *bla*$_{SHV-11}$ *bla*$_{TEM-1}$	*bla*$_{OXA-48}$ *bla*$_{OXA-1}$	*fosA6*	*aac3-IIa* *aph(6)-Id* *aac(6')-Ib* *aac(3)-IIe*	*qnrB1*	*sul2* *dfrA14*	*oqxA* *oqxB*	IncL InFIB IncFII IncR ColRNAI	**ST392**
KP-8	*bla*$_{CTX-M-15}$ *bla*$_{SHV-11}$ *bla*$_{TEM-1}$	*bla*$_{OXA-48}$ *bla*$_{OXA-1}$	*fosA6*	*aac3-IIa* *aph(6)-Id* *aac(6')-Ib*	*qnrB1*	*sul2* *dfrA14*	*oqxA* *oqxB*	IncL InFIB IncFII IncR ColRNAI	**ST392**
KP-9	*bla*$_{CTX-M-15}$ *bla*$_{SHV-11}$ *bla*$_{TEM-1}$	*bla*$_{OXA-48}$ *bla*$_{OXA-1}$ *bla*$_{OXA-23}$	*fosA6*	*aac3-IIe* *aph(6)-Id* *aac(6')-Ib*	*qnrB1*	*sul2* *dfrA14*	*oqxA* *oqxB*	IncL InFIB IncFII IncR ColRNAI	**ST392**

Note: CIP: ciprofloxacin resistance protein; FOS: fosfomycin; AMINO: aminoglycosides; T/S: trimethoprim/sulfamethoxazole; MLST: Multilocus sequence typing

Conclusion:

A high-risk OXA-48- and CTX-M-15-producing ***Klebsiella pneumoniae* ST392 clone** has emerged in a Croatian ICU. This multidrug-resistant clone poses significant challenges for clinical management by limiting the effectiveness of standard antibiotic therapies and underscores the urgent need for novel therapeutic approaches.

References:

1. Shao C, Wang W, Liu S, Zhang Z, Jiang M, Zhang F. Molecular epidemiology and drug resistant mechanism of carbapenem-resistant Klebsiella pneumoniae in elderly patients with lower respiratory tract infection. *Front Public Health*. 2021 May 20;9:669173. doi:10.3389/fpubh.2021.669173.
2. Budia-Silva M, Kostyanev T, Ayala-Montaño S, Bravo-Ferrer Acosta J, Garcia-Castillo M, Cantón R, et al. *International and regional spread of carbapenem-resistant Klebsiella pneumoniae in Europe*. Nat Commun. 2024;15:5092. doi:10.1038/s41467-024-49349-z.

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