

# Complete Genome Sequence of Multi-Drug Resistant *Klebsiella Quasipneumoniae* Isolated for The First Time from A Wastewater Treatment Plant in Slovenia

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## Abstract

### Objectives

This study reports the isolation and genomic characterization of two novel multidrug-resistant isolates of *Klebsiella quasipneumoniae*, isolated from a municipal wastewater treatment plant in Slovenia.

### Methods

Both genomes were sequenced on an Illumina MiSeq platform. De novo genome assembly was performed using Velvet 1.2.10. The genomes were annotated using RAST tool kit (RASTtk). The resistome was identified using tools available from the Center for Genomic Epidemiology.

### Results

High-quality contigs were obtained using and both belong to the MSLT ST 138. KB-61219-C generated 196 contigs and an estimated genome length of 5.958.270 bp. KB-161219-D generated 197 contigs and an estimated genome length of 5.955.847 bp.

### Conclusion

This study reports the isolation and genomic characterization of two novel multidrug-resistant isolates of *Klebsiella quasipneumoniae*, isolated from a municipal wastewater treatment plant in Slovenia.

In recent years, the fate of antimicrobial resistance genes (ARGs) released to wastewaters has received increasing interest [1] and there is a worldwide consensus that raw municipal wastewater, treated effluent and wastewater sludge are reservoirs of ARGs and crucial hotspots for the evolution and spread of antibiotic resistance [2]. *Klebsiella quasipneumoniae* is a common intestinal commensal bacterium that is widely distributed in the environment. It is an important human pathogen causing both nosocomial and community-acquired infections [3]. Infections caused by Carbapenemase-producing Enterobacteriaceae (CPE) are of greatest interest in clinical practice because carbapenems are often the last resort for antimicrobial action [4].

*Klebsiella quasipneumoniae* isolates KB-161219-C and KB-

161219-D were obtained from untreated and treated wastewater, respectively, in Ljubljana, Slovenia, in December 2019. DNA was extracted using the DNAeasy Blood & Tissue Kit (Qiagen, Netherlands) and quantified using the Qubit dsDNA HS assay Kit (Invitrogen, USA) in a Qubit 3.0 fluorometer (Invitrogen, USA), following the manufactures instructions. Sequencing libraries were prepared with the Nextera DNA Flex Library Preparation Kit (Illumina, USA) and barcoded with Nextera DNA CD indexes (Illumina, USA). The genomes were sequenced using the Miseq Reagent Kit V3 500 cycles (Illumina, USA) on a Miseq sequencer (Illumina, USA).

High-quality contigs were obtained using Velvet 1.2.10 and both belong to the MSLT ST 138. KB-61219-C generated 196 contigs and an estimated genome length of 5.958.270 bp. KB-

161219-D generated 197 contigs and an estimated genome length of 5.955.847 bp. The complete genomes were annotated using RAST tool kit (RASTtk). KB-161219-C has 6.006 protein coding sequences (CDS), 76 transfer RNA (tRNA), and 4 ribosomal RNA (rRNA). The annotation included 1.063 hypothetical proteins and 4.943 protein with functional assignments. KB-161219-D contains 6.013 protein coding sequences (CDS), 76 transfer RNA (tRNA), and 4 ribosomal RNA (rRNA). The annotation included 1.067 hypothetical proteins and 4.946 protein with functional assignments. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers SAMN15818748 and SAMN15818747. Assembled contigs were submitted to ResFinder 4.0 in the web service of the Center for Genomic Epidemiology and results are summarized in [Table 1].

This study reports the isolation and genomic characterization of two novel multidrug-resistant isolates of *Klebsiella quasipneumoniae*, isolated from a municipal wastewater treatment plant in Slovenia. The detection and characterization of these isolates show the spread of pathogenic and multidrug-resistant strains into the environment, especially concerning due to fact that CPE harbour highly transferable and broad-spectrum resistance plasmids. This work highlights the need to monitor bacteria carrying beta-lactam resistance genes in the environment, food and clinical settings, in order to better understand their impact and role in the transmission of ARGs [1-5].

**Table 1: Antimicrobial resistance genes of *Klebsiella quasipneumoniae* using the tool ResFinder 4.0 in the web service of the Center for Genomic Epidemiology**

Isolate	Resistance gene	Identity	Alignment Length/Gene Length	Coverage	Predicted Phenotype	Accession
KB161219D	fosA5	93.33	420/420	99,7	Fosfomycin resistance	EU195449
	aac(6')-Iib	99.82	543/543	100,0	Aminoglycoside resistance	L06163
	aac(3)-Ia	98.69	534/534	100,0	Aminoglycoside resistance	X15852
	aadA2b	99.87	780/780	100,0	Aminoglycoside resistance	D43625
	aac(6')-Ib3	100.00	555/555	100,0	Aminoglycoside resistance	X60321
	aadA1	99.62	792/792	100,0	Aminoglycoside resistance	JQ414041
	blaOKP-B-18	99.65	860/861	99,8	Beta-lactam resistance	AM850920
	blaOXA-10	99.88	801/801	100,0	Beta-lactam resistance	J03427
	blaOXA-17	99.88	801/801	100,0	Beta-lactam resistance	DQ902344
	blaGES-5	100.00	864/864	100,0	Beta-lactam resistance	DQ236171
	oqxA	93.62	1176/1176	100,0	Quinolone resistance	EU370913
	oqxB	95.53	3153/3153	100,0	Quinolone resistance	EU370913
	aac(6')-Ib-cr	99.61	519/519	100,0	Fluoroquinolone and aminoglycoside resistance	EF636461
	catB2	100.00	633/633	100,0	Phenicol resistance	AF047479
catA1	99.85	660/660	100,0	Phenicol resistance	V00622	

	cmlA1	99.92	1260/1260	100,0	Phenicol resistance	M64556
	tet(B)	100.00	1206/1206	100,0	Tetracycline resistance	AP000342
	sul1	100.00	840/840	100,0	Sulphonamide resistance	U12338
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	aac(6')-Ib3	100.00	555/555	100,0	Aminoglycoside resistance	X60321
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	sul1	100.00	840/840	100,0	Sulphonamide resistance	U12338
	blaOKP-B-18	99.65	860/861	99,9	Beta-lactam resistance	AM850920
	blaOXA-10	99.88	801/801	100,0	Beta-lactam resistance	J03427
	blaGES-5	100.00	864/864	100,0	Beta-lactam resistance	DQ236171
	blaOXA-17	99.88	801/801	100,0	Beta-lactam resistance	DQ902344

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### Competing interests

None declared.

### Ethical approval

Not required.

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