

**DANI  
DOKTORATA  
BIOTEHNIČKOG  
PODRUČJA**

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**2024 | DANI DOKTORATA  
BIOTEHNIČKOG PODRUČJA**



**hrzz**  
Hrvatska zaklada  
za znanost



AUSTRIAN INSTITUTE  
OF TECHNOLOGY

KLINIKA  
ZA INFektivNE  
BOLESTI



UNIVERSITY  
HOSPITAL FOR  
INFECTIOUS DISEASES

"DR. FRAN MIHALJEVIĆ"

1893



# ***Klebsiella* spp. resistant to last-resort antibiotics from treated wastewater**

Ana Puljko<sup>1</sup>, Ivan Barišić<sup>2</sup>, Marko Jelić<sup>3</sup>, Nikolina Udiković Kolić<sup>1</sup>

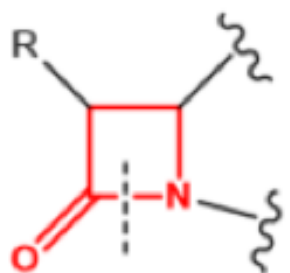
<sup>1</sup>Ruđer Bošković Institute, Zagreb, Croatia, <sup>2</sup>Austrian Institute of Technology, Vienna, Austria,

<sup>3</sup>University Hospital for Infectious Diseases, Zagreb, Croatia

➤ **Antimicrobial resistance – the greatest challenge to global health**

➤ **Last resort antibiotics – carbapenems**

β-lactam antibiotic  
(penicillin,  
cephalosporin,  
carbapenem)

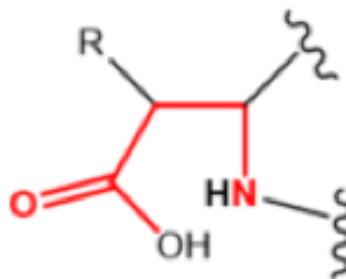


β-lactam ring

β-lactamase  
(e.g. carbapenemase)

hydrolysis

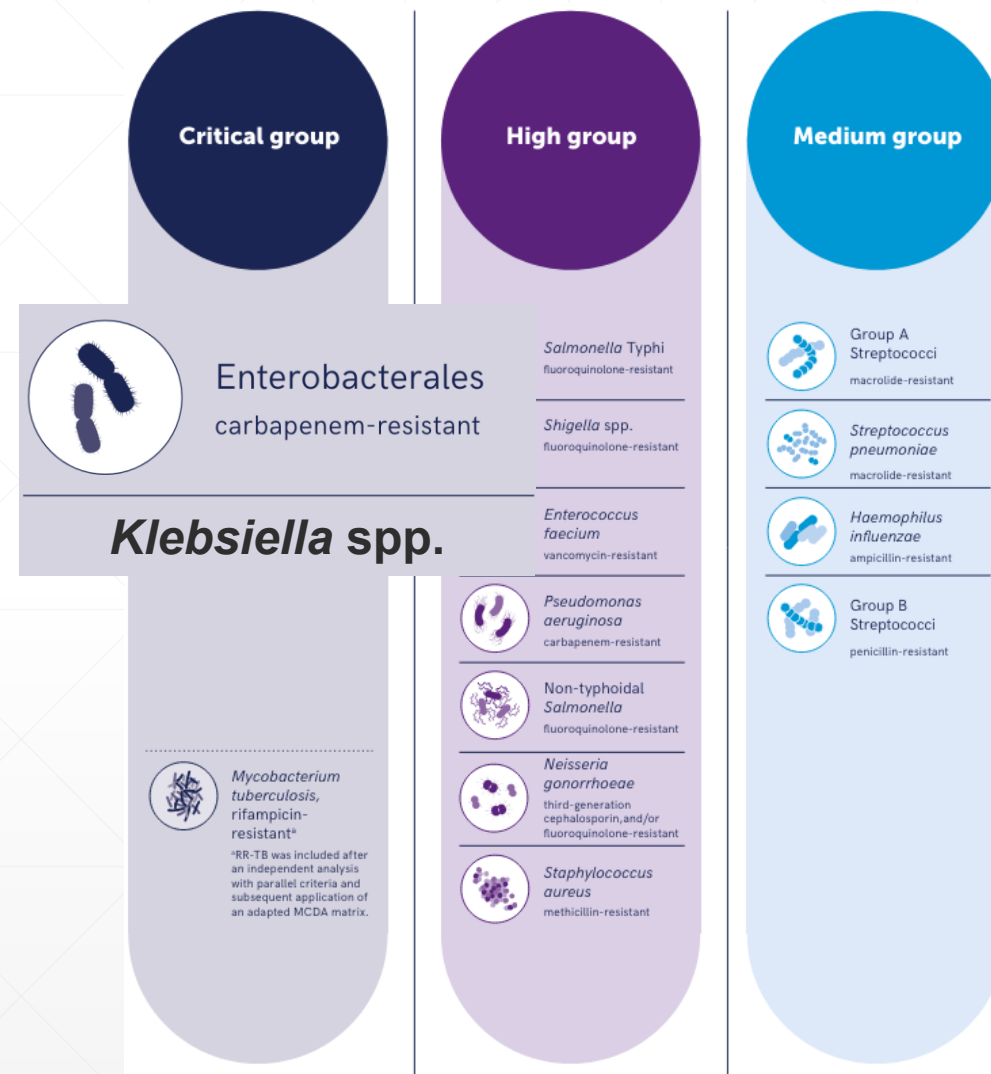
Inactivated



**Colistin (Polymixin)**

– another last-resort antibiotic

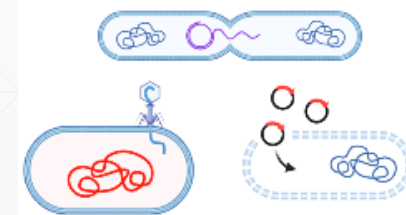
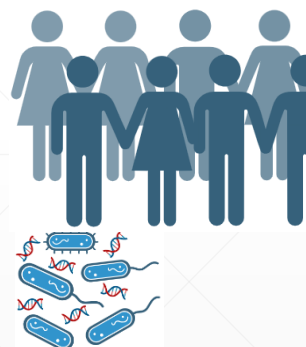
WHO priority pathogens list for R&D of new antibiotics





## AIM

Detect and analyze carbapenemase-producing *Klebsiella* spp. from treated wastewater from Zagreb WWTP



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al gene  
(HGT)

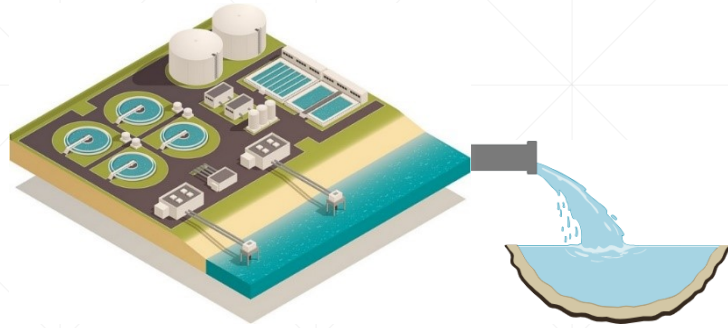
# INTRODUCTION

# MATERIALS AND METHODS

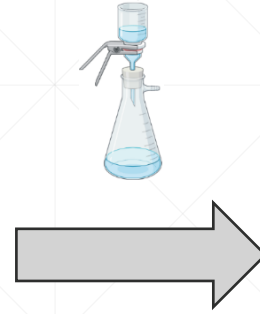
# RESULTS

# CONCLUSIONS

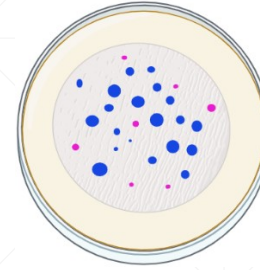
Zagreb WWTP



Treated wastewater



Cultivation



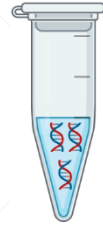
ChromAgar mSupercarba

Identification



MALDI-TOF MS

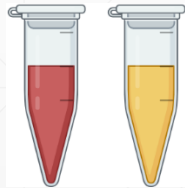
*Klebsiella* spp.



Genomic DNA isolation

Phenotypic testing

Carbapenemase production

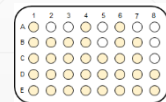


Carba NP

Antibiotic susceptibility testing



Kirby-Bauer method

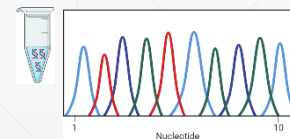


Microdilution

Detection of ARGs



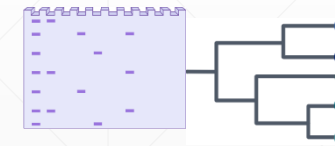
PCR



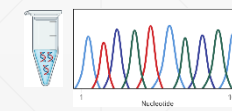
Sanger sequencing

Genotypic testing

Molecular epidemiology



Pulse Field Gel Electrophoresis (PFGE)



Multilocus Sequence typing (MLST)

Whole genome sequencing (WGS)



Ion Torrent PGM



Data analysis

Genetic context of ARGs  
Plasmid replicons



# INTRODUCTION

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Treated wastewater

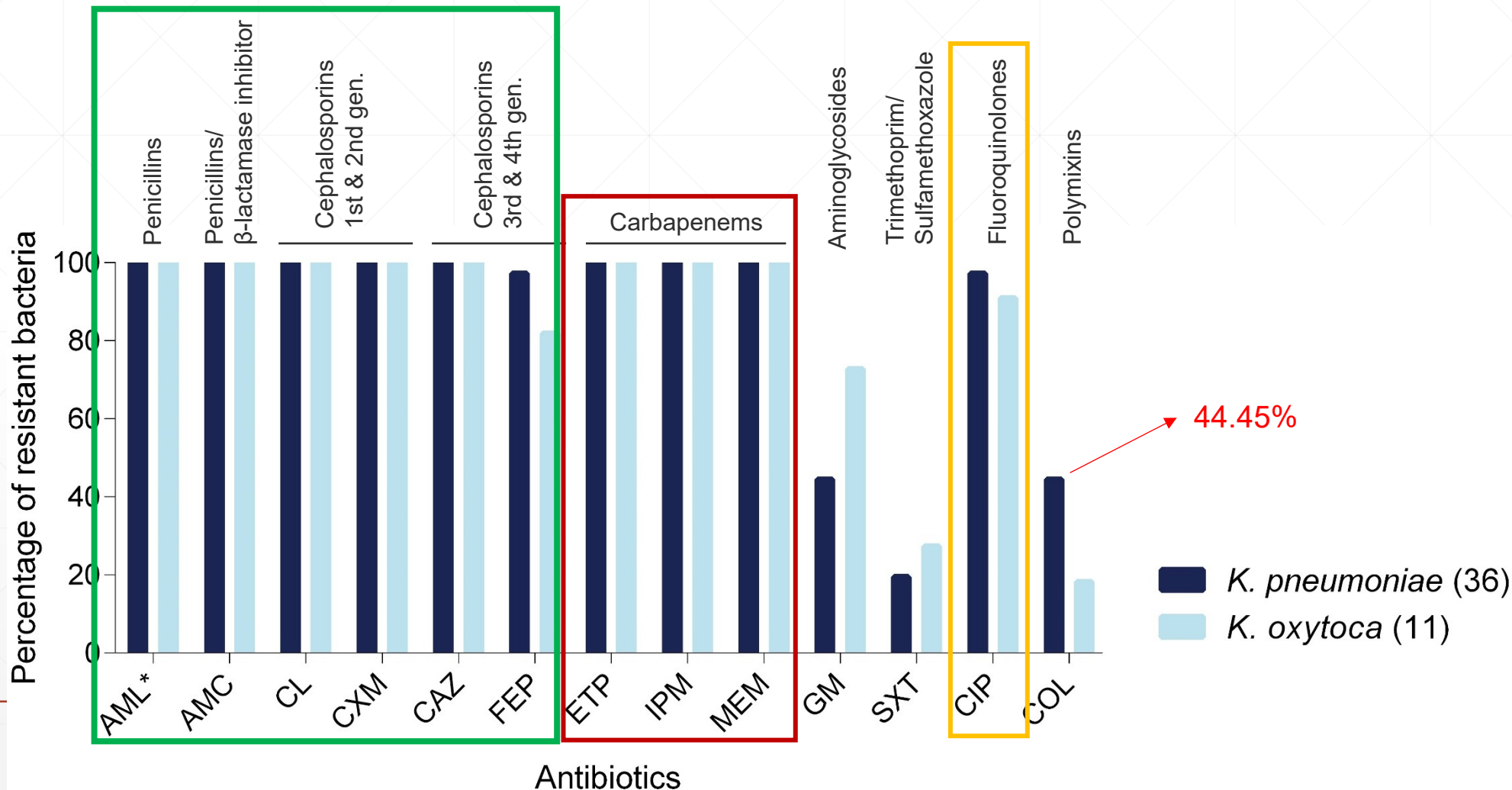
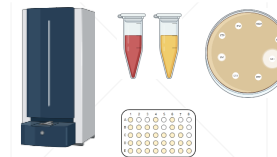


47 *Klebsiella* spp.

36 *Klebsiella pneumoniae*

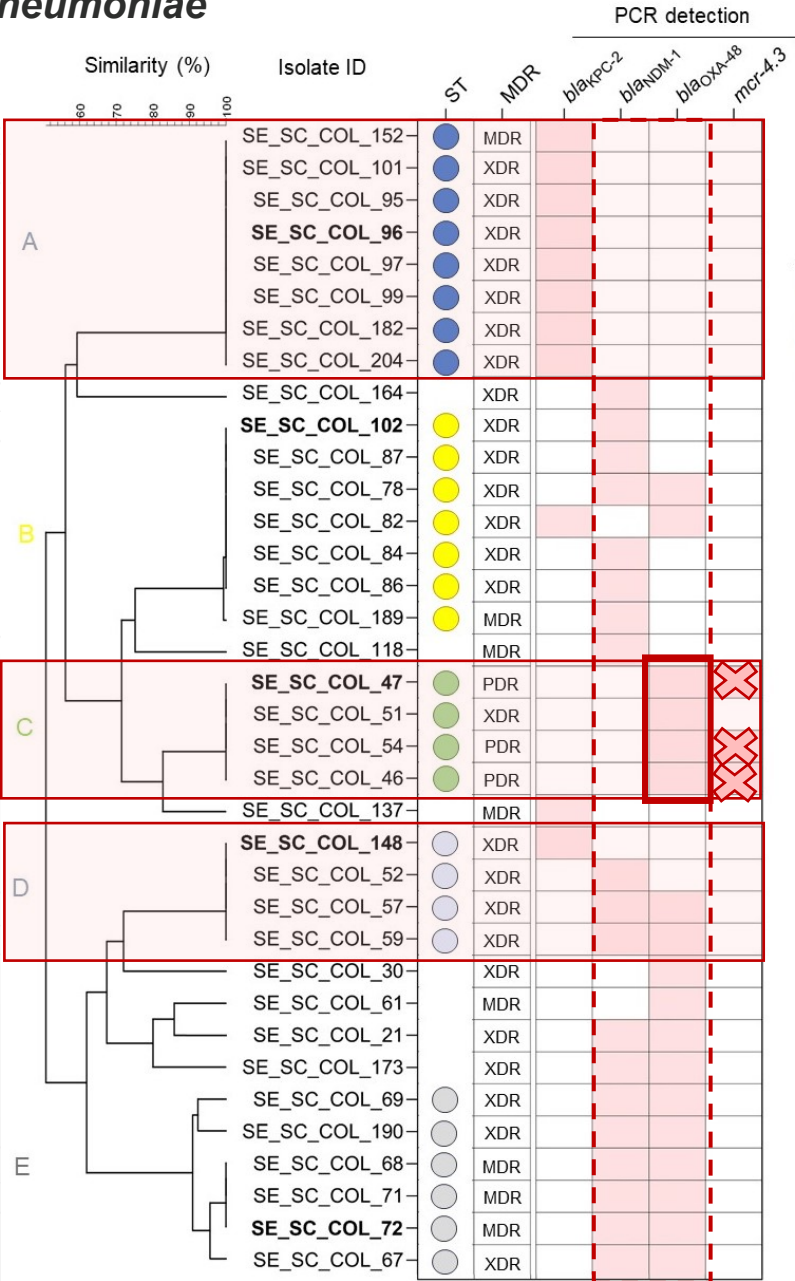
11 *Klebsiella oxytoca*

Carbapenemase activity

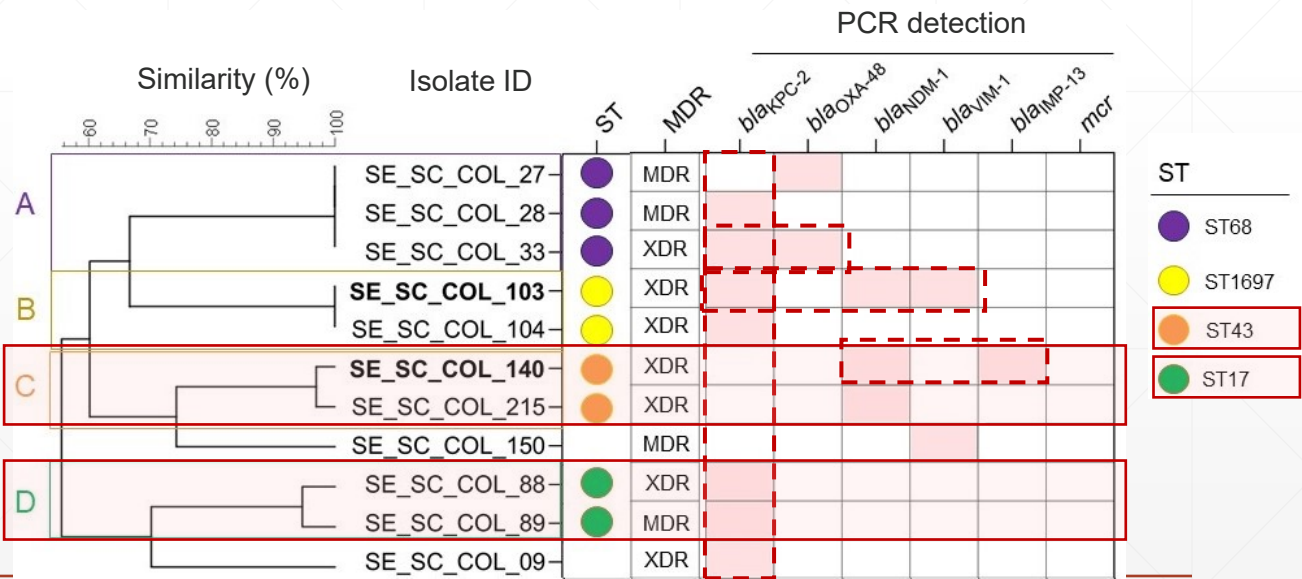




*K. pneumoniae*



*K. oxytoca*



INTRODUCTION

MATERIALS AND METHODS

RESULTS

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Isolate ID	Species	ST	β-lactamase genes															Antibiotic resistant genes for other antibiotic classes																							
			<i>bla</i> KPC-2	<i>bla</i> OXA-48	<i>bla</i> NDM-1	<i>bla</i> IMP-13	<i>bla</i> TEM-1	<i>bla</i> OXA-1	<i>bla</i> OXA-9	<i>bla</i> CTX-M-3	<i>bla</i> CTX-M-15	<i>bla</i> SHV-1	<i>bla</i> SHV-12	<i>bla</i> SHV-98	<i>bla</i> CARB-2	<i>bla</i> OXY-1-7	<i>bla</i> OKP-B	<i>qnr</i> B	<i>qnr</i> S	<i>aac</i> (6')-Ib-cr	<i>dfr</i> A14	<i>sul</i> 1	<i>sul</i> 2	<i>aac</i> (6')-Ib-cr	<i>aac</i> (3)-I	<i>aph</i> (3')-I	<i>aad</i> A1	<i>mcr</i> -4.3	Mutations	<i>fos</i> A	<i>cat</i> B3	<i>oqx</i> AB	<i>qac</i> E	<i>aar</i> -3	<i>mph</i> A	<i>mph</i> E	<i>msr</i> E	<i>tet</i> A			
SE_SC_COL_47	<i>K. pneumoniae</i>	629																																							
SE_SC_COL_72	<i>K. pneumoniae</i>	ND																																							
SE_SC_COL_96	<i>K. quasipneumoniae</i> subps. <i>similipneumoniae</i>	3590																																							
SE_SC_COL_102	<i>K. quasipneumoniae</i> subps. <i>similipneumoniae</i>	1697																																							
SE_SC_COL_103	<i>K. quasipneumoniae</i> subps. <i>similipneumoniae</i>	1697																																							
SE_SC_COL_148	<i>K. quasipneumoniae</i> subps. <i>similipneumoniae</i>	1803																																							
SE_SC_COL_140	<i>K. michiganensis</i>	43																																							

Antibiotic classes:

✓

β-lactams

✓

Fluoroquinolones

✓

Trimethoprim/Sulfonamides

✓

Aminoglycosides

✓

Polymyxin

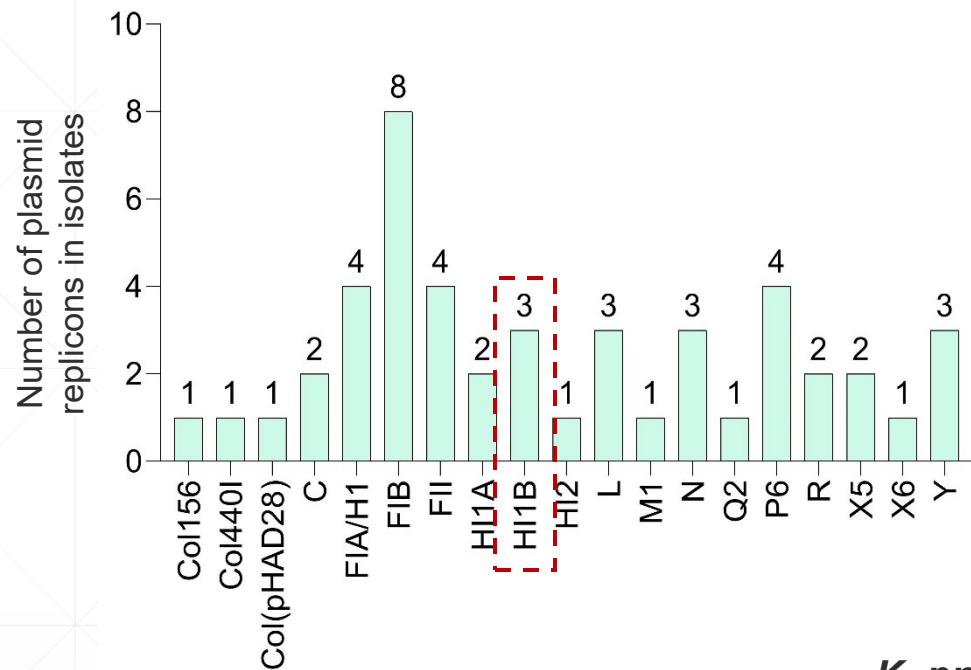
Additional resistance (not phenotypically assessed)

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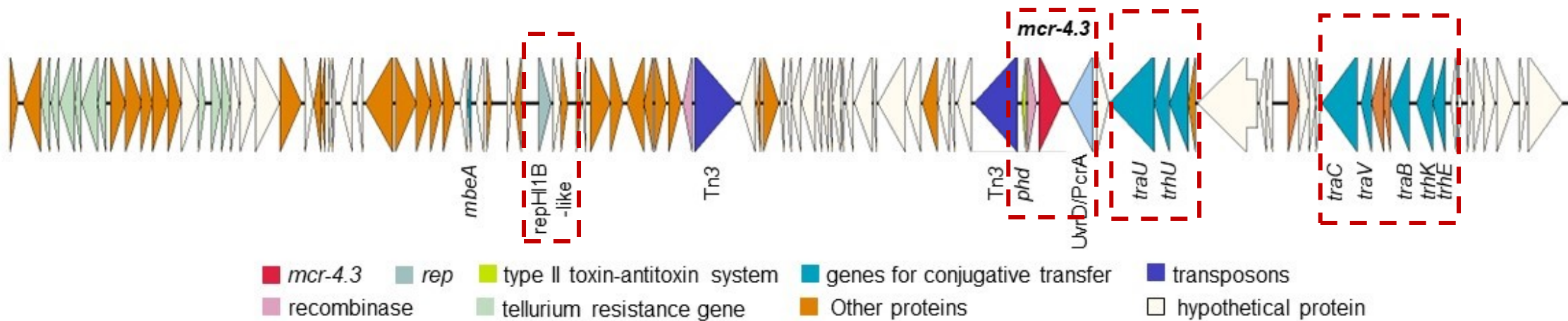


transfer

*bla*<sub>OXA-48</sub>, *bla*<sub>KPC-2</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>VIM-1</sub>, *bla*<sub>IMP-13</sub>

*bla*<sub>OXA-48</sub> – IncL plasmid

*K. pneumoniae* ST629 SE\_SC\_COL\_47  
(110,506 bp)





## CONCLUSIONS

- Human-associated *Klebsiella* strains were found in treated wastewater from Zagreb WWTP
- All isolates had carbapenemase genes and produced carbapenemases
- Clinically important *K. pneumoniae* ST629 harbored two ARGs for resistance to two last-resort antibiotics associated with plasmid transfer
- Treated wastewater could potentially serve as secondary reservoir and source for carbapenemase-producing *Klebsiella* and their further spread into the environment
- More effective wastewater treatment should be implemented to reduce the spread of these highly resistant pathogens into the environment



# Acknowledgement

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**Austrian Institute of Technology, Vienna, Austria**  
**Ivan Barišić, PhD**



**Department of Clinical Microbiology**  
**University Hospital for Infectious Diseases „Fran Mihaljević”,**  
**Zagreb, Croatia**  
**Marko Jelić, PhD**



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