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AMR monitoring in wastewater: detection and characterization of carbapenemase-producing *Klebsiella pneumoniae*

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Infectious diseases caused by multidrug-resistant pathogens represent a growing global public health threat, leading to millions of deaths each year. In 2024, the revised European urban wastewater treatment directive was adopted, mandating the surveillance of antimicrobial resistance (AMR) in wastewater. The AMELAG project aims to develop reliable detection methods to monitor influent wastewater. Due to its clinical relevance, carbapenemase-producing *Klebsiella pneumoniae* (CPKP) were selected as initial screening target for the proof-of-concept study. A filtration-based method was employed, involving colony enumeration on selective media, species verification and susceptibility testing, followed by PCR screening for key resistance genes and whole genome sequencing (WGS) of selected isolates. CPKP counts ranged between 102-104 colony-forming units (cfu)/100 ml. Overall, 326 subcultures were obtained, with 297 *K. pneumoniae* (93 %) including 253 (79 %) CPKP. PCR screening identified blaOXA-48 as the most common carbapenemase gene across the isolate collection, followed by blaNDM-1, blaKPC, and blaVIM. WGS revealed a broad range of sequence types among CPKP isolates, i.e. ST15, ST35, ST37, ST147, ST258, ST273, ST307, and ST485. Overall, the combination of culture-based methods and molecular analysis demonstrates a promising framework for monitoring and characterizing AMR in influent wastewater.

Keywords

Antimicrobial resistance, wastewater surveillance, carbapenemase-producing *Klebsiella pneumoniae*

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Junior Scientist Status

Yes, I am a Junior Scientist.

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