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Carbapenemase-producing *Klebsiella pneumoniae*: Method development for a reliable AMR monitoring in wastewater

Inhalt

Infectious diseases caused by multidrug-resistant pathogens present a growing global public health threat causing millions of deaths each year. The surveillance of antimicrobial resistance (AMR) in wastewater will become mandatory as regulated by the revised European urban wastewater treatment directive (2024). We therefore aimed at developing reliable detection methods to monitor influent wastewater. Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) were chosen as the primary target due to their Public Health relevance. A filtration-based workflow 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. CP-KP concentrations ranged from 102 to 104 colony-forming units (cfu)/100 ml. All samples were positive for CP-KP, and 93 % of the subcultures were confirmed as *K. pneumoniae* (n= 297), including 79 % verified CP-KP (n= 253). 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 with clinical relevance among CP-KP 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*, monitoring

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