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Comparative Analysis of Metagenomic and Isolate Sequencing: A Case Study targeting carbapenemase-producing *Klebsiella pneumoniae* in influent wastewater in Germany

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Metagenomic sequencing captures abundant DNA of a samples, enabling cultivation-free analysis of microbial communities. Wastewater (WW) metagenomics offers a powerful tool for monitoring pathogen spread and antimicrobial resistance genes (ARG) that may threaten public health. Here, we compared metagenomic data with cultured isolate sequences of *Klebsiella pneumoniae* (KP), a major healthcare-associated pathogen according to the WHO.

Twelve metagenomes from two WW treatment plants in Germany were sequenced with Illumina and 56 cultured KP isolates from the same samples with Illumina and ONT. Hybrid assemblies enabled high-quality genome reconstruction from isolates which were compared to metagenomes by applying modern tools for ARG screening.

Genomic analysis of the isolates revealed multiple sequence types and resistance patterns. Even though ONT data allowed for more contiguous assemblies they missed several small plasmids and ARGs, indicating that a combined approach might be beneficial for full genomic analysis. Metagenomes yielded few KP reads, insufficient for genome recovery. Around 50% of metagenomic reads were unclassifiable, reflecting dataset complexity. Cultivation-based sequencing was essential for detailed genomic profiling, while metagenomics provided broader community and resistome insights, including resident environmental species.

This study highlights the complementary strengths and limitations of each approach for pathogen and ARG monitoring in WW.

Keywords

metagenomics, wastewater, *Klebsiella pneumoniae*, ARG, AMR

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Postdoc

Junior Scientist Status

No, I am not a Junior Scientist.

Author: Dr DESIRÒ, Daniel (Robert Koch-Institut)

Co-authors: Dr BAJIC, Vladimir (Robert Koch-Institut); Dr WERNER, Katharina (Umweltbundesamt); HELAL, Mustafa (Universitätsklinikum Schleswig-Holstein); Dr DRILLER, Maximilian (Robert Koch-Institut); Dr WOLF,

Silver A. (Robert Koch-Institut); Dr BLUMENSCHKEIT, Christian (Universität Leipzig); Dr WALTHER, Birgit (Umweltbundesamt); Dr HÖLZER, Martin (Robert Koch-Institut)

Presenter: Dr DESIRÒ, Daniel (Robert Koch-Institut)

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