

Plasmids show potential to turn non-O1/non-O139 *Vibrio cholerae* into virulent strains in wastewater in Tshwane District, South Africa

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BACKGROUND: Non-O1/non-O139 *Vibrio cholerae* (NOVC) species are typically non-toxic and regarded as etiological agents of infrequent but mild-severe human gastroenteritis. There are limited studies reporting plasmids and their potential role in driving virulence in NOVC isolates, particularly in wastewater. This study aimed to investigate the occurrence and characteristics of plasmids in NOVC isolates circulating in wastewater in the Tshwane district between May 2023 and May 2024.

METHODS: Study isolates were recovered from 341 wastewater samples and identified using phenotypic and molecular methods. Genetic relatedness was assessed by REP-PCR. Representative isolates were subjected to whole genome sequencing (WGS) using Oxford Nanopore Technologies MinION Mk1C platform. Plasmid profiling was performed through BLAST analysis against the NCBI database and annotated with pLannotate in Proksee.

RESULTS: In total, 341 samples were processed from which 145 isolates were classified as NOVC. Rep-PCR clustered isolates into 12 groups and the representative isolates confirmed as NOVC by WGS. Plasmid analysis revealed that 7 of the 12 isolates harboured plasmids. The sizes of these plasmids ranged from 3,200 bp to 28,683 bp. Among these, three distinct plasmid profiles (*pA*, *PVN84* and *p21L*) were identified in the isolates, including some unnamed plasmids which carried multiple resistance determinants, genes encoding toxin-antitoxin systems, as well as replicase and relaxases. Notably, plasmids carrying resistance genes such as *dfrA31*, *dfrA6*, *dfrA1*, *QNR_VIBPA*, *dhfrI*, *qnrVC*, and *qnrVC5* were identified, conferring resistance to commonly used antibiotics, including trimethoprim and quinolones.

CONCLUSION: This study revealed the presence of plasmids with the potential to increase the virulence of NOVC strains in wastewater in Tshwane. This underscores the urgent need for continuous surveillance to prevent the emergence of these strains as a significant public health threat.