



Nanopore-based long-read metagenomics uncover the resistome intrusion by antibiotic resistant bacteria from treated wastewater in receiving water body

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ABSTRACT

Wastewater treatment plant (WWTP) effluent discharge could induce the resistome enrichment in the receiving water environments. However, because of the general lack of a robust antibiotic-resistant bacteria (ARB) identification method, the driving mechanism for resistome accumulation in receiving environment is unclear. Here, we took advantage of the enhanced ARBs recognition by nanopore long reads to distinguish the indigenous ARBs and the accumulation of WWTP-borne ARBs in the receiving water body of a domestic WWTP. A bioinformatic framework (named ARGpore2: <https://github.com/sustc-xylab/ARGpore2>) was constructed and evaluate to facilitate antibiotic resistance genes (ARGs) and ARBs identification in nanopore reads. ARGs identification by ARGpore2 showed comparable precision and recall to that of the commonly adopt BLASTP-based method, whereas the spectrum of ARBs doubled that of the assembled Illumina dataset. Totally, we identified 33 ARBs genera carrying 65 ARG subtypes in the receiving seawater, whose concentration was in general 10 times higher than clean seawater's. Notably we report a primary resistome intrusion caused by the revival of residual microbes survived from disinfection treatment. These WWTP-borne ARBs, including several animal/human enteric pathogens, contributed up to 85% of the receiving water resistome. Plasmids and class 1 integrons were reckoned as major vehicles facilitating the persistence and dissemination of ARGs. Moreover, our work demonstrated the importance of extensive carrier identification in determining the driving force of multifactor coupled resistome booming in complicated environmental conditions, thereby paving the way for establishing priority for effective ARGs mitigation strategies.

1. Introduction

Antibiotic resistance has been hailed as top global health concerned in 21st century by the WHO (“WHO | Antimicrobial resistance,” n.d.). The increasing level of antibiotics in environment could form persistent selection pressure for antibiotic resistant bacteria (ARB) to develop resistance via obtaining antibiotic resistance genes (ARGs), contributing

to proliferate antimicrobial resistance (AMR) in microbial communities (Tyers and Wright, 2019). Importantly, ARGs are frequently encoded on mobile genetic elements (MGEs), which facilitates its transfer and transformation between bacteria among phylogenetically distant lineages (Zhang et al., 2018). For instance, human bacterial pathogens might recruit ARGs from microbes colonized in soil and other environments (Forsberg et al., 2012) through horizontal gene transfer (HGT).

Abbreviations: WWTP, wastewater treatment plant; ARB, antibiotic-resistant bacteria; ARGs, antibiotic resistance genes; AMR, antimicrobial resistance; HGT, horizontal gene transfer; MGEs, mobile genetic elements; epicPCR, Emulsion, Paired Isolation and Concatenation PCR.

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