EFFICACY OF HYBRID CAPTURE BASED METAGENOMIC SEQUENCING FOR MONITORING ANTIMICROBIAL RESISTANCE IN WASTEWATER

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Background: Wastewater(WW) is a valuable resource for passive public health surveillance. Shotgun metagenomic approaches are rapidly evolving and enable deep insight into overall genetic richness including antimicrobial resistance(AMR), however certain challenges remain, especially around sensitivity. Target enrichment using hybrid capture(xHYB) could allow comprehensive detection of multiple target genes in complex matrices compared to conventional metagenomic sequencing methods.
Methods: Illumina DNA Prep Library Kit(Illumina, SanDiego,CA) and QIAseq xHYB AMR panel(Qiagen, Hilden,Germany) were parallelly tested using WW samples collected between Sep2020-Jun2023 from buildings and local wastewater treatment plant (WWTP). For xHYB, different input DNA concentrations, 100, 50 and 10ng were also tested for sensitivity. Enrichment and library prep were performed following manufacturer's protocol and sequenced on NextSeq2000 using P2 300cycle v3(2x150bp) kit. Resistome analysis was performed using ResPipe.
Results: Sample-set comprised WW from hospital, non-hospital building and local WWTP. Using xHYB an average 41 million reads per sample was recorded with 95% reads having a mean PHRED score >30; this was 40% more total reads than that recorded using conventional Illumina kit. Top 10 normalized AMR read counts for both kits were comparable across the samples tested however, xHYB recorded more unique AMRgenes(ARG) compared to Illumina(552/154). Across the samples, macrolide(msrE, mphE), tetracyline(tetA, tetO, tetQ), sul1 and aminoglycoside genes were predominant. Beta-lactamases; KPC and TEM were relatively more abundant in the hospital WW(HWW) samples. Total resistome load was higher in HWW than non-HWW and WWTP samples. Although the total unique ARGs detected were similar, ~9% of ARGs were not shared across the different input DNA. NDM beta-lactamase was only detected in case of 50 and 10ng input DNA.
Conclusions: HWW was found to be a hotspot of AMR in the sewershed with higher resistome load and higher relative abundance for clinically significant ARGs. xHYB AMR-panel was more sensitive in detecting unique ARGs in WW compared to conventional shotgun metagenomic method, although the resistome profile of the top abundant genes was similar. Differential amplification of certain ARGs suggests that input DNA can influence the resistome diversity, particularly in case of relatively less abundant genes. In the context of WW-AMR monitoring, hybrid-capture approach can be a tool for mining emerging AMR signals.