

Correlation of coliform bacteria's resistome phenotype and genotype in municipal sewage is made possible by minion nano pore sequencing

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ABSTRACT

The risk assessment of the acquisition of ambient Antibiotic Resistance Genes (ARGs) by pathogenic populations during treatment is attracting more and more attention in Wastewater Treatment Facilities (WWTPs), which serve as the interface between human society and the natural environment. However, due to a lack of reliable Resistome profiling techniques, genotype and resistance phenotype are still insufficiently associated in human pathogens found in sewage samples. The resistance genes of Multiple Antibiotic Resistant (MAR) coliform bacteria, a frequent indicator of human enteric

pathogens in sewage samples, were quantified here using Minion sequencing. Within 30 hours of collecting the samples, our pipeline could produce the results, and the Resistome quantification was accurate and comparable to that based on the Illumina platform. Long Nano pore reads also facilitated the genome reconstruction of a representative MAR strain, from which we identified an instance of chromosomal integration of an environmental resistance gene acquired through plasmid exchange with a porcine pathogen. This allowed for the simultaneous identification of the carrier populations of ARGs detected. This study showed how to use minion sequencing to quickly monitor and simultaneously track evolutionary changes in environmental ARGs to address any potential health risks.gap.

Key Words: Minion sequencing; Pathogenic;Chromosomal integration; Multiple antibiotic resistance

INTRODUCTION

This article aims to emphasize that the lifestyle medicine movement is a sign of the inadequate nutrition education currently provided for healthcare. The World Health Organization has classified Antibiotic Resistance Genes (ARGs) as emerging environmental pollutants due to the development of antibiotic-resistant bacteria and the resulting rise in human illness and mortality. Recent investigations using metagenomics methods have revealed that putative ARGs are prevalent in almost all environments. The potential acquisition of resistance by clinical pathogens in environmental settings is a matter of constant concern given the pervasiveness of environmental ARGs. Domestic wastewater systems are perfect hubs for the horizontal spread of ARGs across microorganisms due to direct contact between pathogenic bacteria and environmental ARG carriers and the constant selection pressure imposed by antibiotic residues in the sewage. Wastewater Treatment Plants (WWTPs) are receiving more social and scientific attention on risk assessment of the acquisition of environmental ARGs by clinical pathogens during the treatment processes, despite some researchers finding the core Resistome of wastewater treatment systems is different from that of human pathogens.

Coliform bacteria are the most often utilised indicator for the hygienic quality of water before and after treatment within the microbial communities of WWTPs, and faecal coliforms are regularly surveyed as an indicator of the probable presence of human enteric pathogens. Faecal coliforms from human, animal, and/or environmental sources may be detected in water samples as Multiple Antibiotic-Resistant (MAR) coliforms. In order to make it easier to assess the risk of environmental ARGs in wastewater treatment procedures, research on the Resistome (composition of ARGs) of MAR coliforms would contribute significant genetic information. By overcoming the inherent limitation of ARG primers, sequencing-based ARG detection provides a more accurate quantification as compared to qPCR approaches. Thus, it has become more and more common to research the distribution of environmental ARGs in many ecosystems, including wastewater treatment systems, using ARG identification based on high-throughput shotgun sequencing. The majority of sequencers, however, were unable to perform

the real-time Resistome profiling needed to direct the resistance control procedures. Furthermore, using these vast sequencing platforms to detect the ARG carrier populations is difficult because of the short read length and fragmented assembly.

Oxford Nano pore Technologies Ltd. has developed a different technique for producing long-read sequences quickly through DNA sequencing. Because they are bordered by or interspersed with insertion sequences, genes acquired through horizontal gene transfer, such as resistance genes, typically have an intrinsic repetitive nature. This results in many gaps in the short-read assemblies. The lengthy reads generated by minion sequencing could clarify the relationship between repetitive sections and make it easier to assemble the host of the resistance genes' entire genome.

Additionally, minion sequencing may offer a portable and time-saving framework that might shorten the time between sample collection and results delivery, allowing Resistome monitoring practises to adopt a real-time quick approach. Although the minion sequencing error rate raises the question of whether it is practical for this use, the fundamental computational problems for mistake correction and de novo assembly of Nano pore long reads could be resolved by a variety of specifically built bioinformatics techniques. Van der Helm et al. measured the Resistome of functionally chosen metagenomics libraries using minion sequencing.

The resistance genes carried by the MAR coliform bacteria cultivated from the influent of a nearby Wastewater Treatment Plant (WWTP) were examined in this work using minion sequencing. The study's process is in summary form. We set out to create techniques for examining taxonomic data and predicting the resistance profile of these faecal coliforms, particularly ARGs on mobile genetic components. Additionally, the resistance profile predicted by the minion platform and the short-read Illumina technology were compared. Additionally, we revealed resistant plasmids shared between coliforms in WWTP and human and porcine pathogens by reporting a hybrid assembly of combined minion and Illumina data to identify a pathogenic MAR coliform strain's chromosomal antibiotic resistance island's composition and location of insertion.

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