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DETECTION OF CARBAPENEMASES AND EVALUATION OF THEIR REMOVAL CAPACITY BY SEWAGE TREATMENT PLANTS IN THE METROPOLITAN REGION OF BELÉM

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ABSTRACT

Antimicrobial resistance (AMR) presents a significant threat to public health, particularly with the increasing resistance seen in gram-negative bacteria, leading to higher mortality rates and healthcare expenses. Wastewater treatment plants serve as major sources for spreading AMR in the environment. β-lactam antimicrobials, commonly used in treating infections, are hindered by the rapid dissemination of β-lactamase enzymes, making drugs like carbapenems, previously relied upon as last-resort treatments, ineffective. Quantitative real-time Polymerase Chain Reaction (RT-qPCR) has emerged as a quick and effective method for identifying antimicrobial resistance genes (ARGs) in sewage samples, including genes for carbapenemase.

Keywords: Carbapenemase. Antimicrobial resistance. Gram-negative. Public health.

1 INTRODUCTION

Antimicrobial resistance (AMR) has become one of the leading emerging threats to public health, causing serious challenges to the success of prevention and treatment of persistent diseases.[1] Antibiotic resistance has sharply increased in gram-negative bacteria over the past two decades and, in many cases, has been associated with increased mortality and healthcare costs.[2] In this scenario, wastewater treatment plants are already considered reservoirs and main sources for the dissemination of AMR in the environment.[3]

β-lactam antimicrobials are the most widely used worldwide for the treatment of infections in humans and animals. A significant threat to the use of these agents is the rapid spread of β-lactamase enzymes - enzymes that catalyze the hydrolysis of the beta-lactam ring, inactivating the antimicrobial and thus preventing it from exerting activity against enzymes responsible for cell wall synthesis, mainly among Gram-negative bacteria. This event has rendered these drugs obsolete in a short period of time, including carbapenems which are considered the most reliable last-resort treatment for bacterial infections caused by extended-spectrum β-lactamase-producing bacteria.[4]

The expansion in whole genome sequencing and the subsequent increase in antibiotic resistance targets have paved the way for high-throughput qPCR (HT-qPCR), to analyze hundreds of antimicrobial resistance genes (ARGs) in a single run.[5] These tests have rapid response times and require little practical time to operate, making them a fast method for robust water quality assessments. Therefore, the present study aims to evaluate the antibiotic resistance profile and detection of bla and int genes in gram-negative bacilli from sewage treatment systems (STS) in the Metropolitan Region of Belém, operated by the Pará Sanitation Company (COSANPA). Through the development of a qPCR assay capable of simultaneously detecting the presence of genes related to microbial resistance and distinguishing them, with the objective of monitoring what is present in different stages of wastewater treatment in the greater RMB.

2 MATERIAL & METHODS

Collections will be made at three Wastewater Treatment Plants (WWTP-1, WWTP-2, and WWTP-3) in neighborhoods of the metropolitan region of Belém, according to demand from the Pará Sanitation Company (COSANPA). Samples will be collected in sterile 1L polypropylene bottles. These bottles will be kept refrigerated in a cooler until arrival at the laboratory for processing on the same day as collection. This work will involve monthly analysis for a period of 3 months. Laboratory manipulation preparation will involve obtaining cells using the membrane filtration technique. Samples will be filtered through 0.22 µm membranes, placed in 50 mL Falcon tubes with STE buffer, and stored at -20°C. The samples will have their total DNA extracted using SPINeasy DNA/RNA Kit for Soil (MPBIO) commercial kits, following the manufacturer's protocol. The quantification of the extracted product will be conducted using a Qubit™ fluorometer. DNA integrity will be visualized through 1% agarose gel electrophoresis.

The products from the previous steps will be qualitatively analyzed for beta-lactam resistance genes bla_{IMP} and bla_{KPC}. The reaction will be conducted on the QuantStudio 12K Flex system (Thermo Fisher Scientific), using 96-well plates. The final volume of each sample will be 9 µL, containing 5 µL of TaqMan Fast Advanced Master Mix for qPCR (Thermo Scientific), 0.5 µL of TaqMan Antibiotic Resistance assays (Thermo Scientific), and up to 3.5 µL of extracted DNA sample. TaqMan Antibiotic Resistance assays consist of a pair of unlabelled PCR primers and a TaqMan probe with a fluorescent dye (FAM) at the 5' end,

and a minor groove binder (MGB) and a non-fluorescent quencher (NFQ) at the 3' end. For positive and negative controls, respectively, positive DNA samples for the genes of interest and ultrapure water will be used. The temperature configuration will proceed as follows: 1 cycle of incubation at 25 °C for 2 minutes, followed by 1 cycle for reverse transcription at 53 °C for 10 minutes, polymerase activation in 1 cycle at 95 °C for 2 minutes, and finally amplification in stages at 95 °C (3 seconds) and 60 °C (30 seconds), for 40 cycles. Amplified samples (CT from 1 to 40) will be considered positive.

3 RESULTS & DISCUSSION

The following graphs show the presence of Carbapenemase in the sewage treatment plants of the metropolitan region of Belém. The resistance genes were detected on the inlet and outlet of the WWTP.

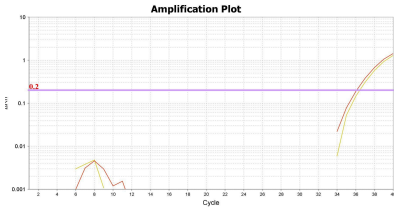

Figure 1 WWTP-1blaIMP
Inlet (Yellow) and outlet (Red) samples

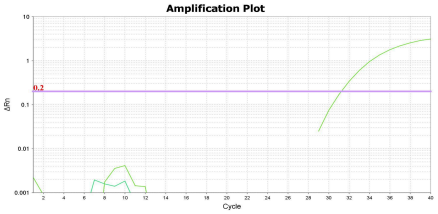

Figure 2 WWTP-2 blaIMP
Inlet (Green) and outlet (Turquoise) samples

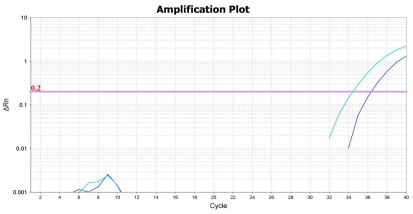

Figure 3 WWTP-3blaIMP
Inlet (Blue) and outlet (Dark Blue) samples

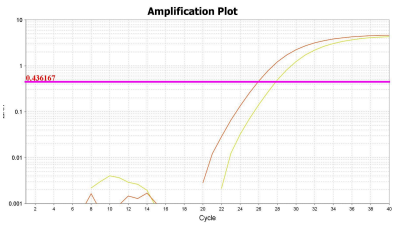

Figure 4 WWTP-1 blaKPC
Inlet (Yellow) and outlet (Red) samples

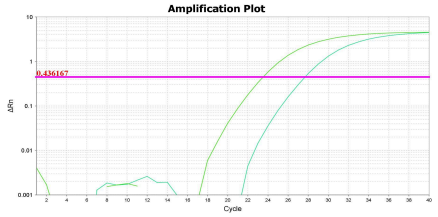

Figure 5 WWTP-2 blaKPC
Inlet (Green) and outlet (Turquoise) samples

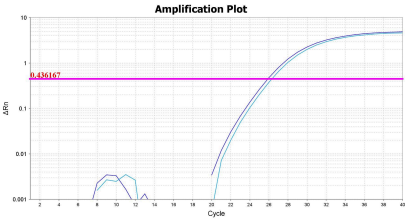

Figure 6 WWTP-3 blaKPC
Inlet (Blue) and outlet (Dark Blue) samples

The test outcome is determined by the presence or absence of amplification, where a positive diagnosis signifies the crossing of the amplification line at the threshold, while a negative diagnosis indicates the absence of amplification. This fundamental principle highlights the reliability and sensitivity of the assay in detecting the target analyte. Additionally, it underscores the importance of meticulous interpretation of results, as they carry critical implications for subsequent clinical or investigative interventions. The dichotomous nature of the diagnostic outcome emphasizes the significance of precision and accuracy in experimental procedures, ensuring robust and dependable conclusions.

4 CONCLUSION

The conclusion of this study reveals a concerning reality regarding the effectiveness of wastewater treatment. The detected presence of carbapenemase genes both at the inlet and outlet of the treatment stations in Anita Gerosa, Bengui and Porto indicates significant failures in the removal process of pathogenic agents. This finding underscores the urgency of implementing more effective measures to mitigate the spread of antimicrobial resistance through the wastewater treatment system. Furthermore, it emphasizes the importance of continuous and enhanced surveillance to ensure environmental and public health safety.

REFERENCES.

1 .DADGOSTAR, Porooshat. Antimicrobial resistance: implications and costs. Infection and drug resistance, p. 3903-3910, 2019.

2 EICHENBERGER, Emily M.; THADEN, Joshua T. Epidemiologia e mecanismos de resistência de bactérias Gram-negativas extensivamente resistentes a medicamentos. Antibióticos , v. 8, n. 2, pág. 37, 2019.

3 OLIVEIRA, Micaela et al. Bactérias ambientais e patogênicas resistentes a carbapenêmicos isoladas de uma estação de tratamento de águas residuais abrigam mecanismos distintos de resistência a antibióticos. Antibióticos , v. 10, n. 9, pág. 1118, 2021.

4 ZHANG, Luhua et al. Prevalência e caracterização de bactérias produtoras de β-lactamases e carbapenemases de espectro estendido em esgotos hospitalares, efluentes tratados e rios receptores. Revista internacional de pesquisa ambiental e saúde pública , v. 17, n. 4, pág. 1183, 2020.

5 WASEEM, Hassan et al. Contribuições e desafios do qPCR de alto rendimento para determinação da resistência antimicrobiana no meio ambiente: uma revisão crítica. **Moléculas** , v. 24, n. 1, pág. 163, 2019.

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