Title: STANDATIZATION OF DNA-EXTRATION METHOD TO DETECT CTX-M-15 BETA-LACTAMASES GENES (*bla*$_{CTX-M15}$) IN WASTEWATER SAMPLES

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The dairy release of antibiotics due medical, veterinary and livestock approach in environment are directly associated with resistance bacterial dissemination. In water, bacteria from different origins (human, animal and environmental) are able to mix, encouraging the exchange of antibiotic-resistance-bacteria and genetic platforms. In consequence, water constitutes not only a route for the dissemination of resistant bacteria among human a transforming nonpathogenic bacteria into reservoirs of resistance genes and platforms. The presence of ESBL-producing bacteria in rivers and, and sludge has been reported, taking into account the different anthropogenic activities that come together and are considered to be sources of contamination (hospital, municipal, industrial, agricultural and animal production effluents). In this work, we aimed to standardize a DNA-extraction method for detection of CTX-M-15 beta-lactamases alleles (*bla*$_{CT-X-M15}$ gene). For this purpose, an Uropathogenic *Escherichia coli* (UPEC) strain, previously genotyped for CTX-M-15 alleles were submitted to DNA extraction described as following. After 18 hours of cultivation in LB at 37°C, the bacterial suspension were diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} e 10^{-10}. Each diluted suspension was homogenized with 1L of wastewater and filter by using a 22um pore membrane (Millipore). After the filtration, each membrane was transferred to an individual 15mL tube containing 1mL of TE (Tris-EDTA) buffer. The tubes were submitted to sonication by ultrasound during 5 minutes. TE suspension of each tube were transferred to 1,5mL microtube and centrifuged. The supernatant were discarded and the pellets were submitted to DNA chloroform -isoamilic alcohol DNA extraction. After ethanol washing and sterilized deionized water suspension, each DNA solution was submitted to PCR detection of CTX-M-15 genes. The genomic DNA of UPEC strain extracted by Wizard kit (Promega) was used as positive control to amplification reaction. After agarose electrophoresis, CTX-M-15 alleles were detected up to 10^{-2} bacterial diluition. These results showed that the DNA extraction methodology purposed is suitable for ESBL genes detection in wastewater.

Key words: CTX-M-15 beta-lactamases, antibiotic water

Finalcial support: CNPq, FAP/DF