

TITLE: ASSOCIATION OF GRAM-NEGATIVE BACILLI HARBORING EXTENDED-SPECTRUM BETA-LACTAMASES WITH FECAL CONTAMINATION IN WATERS FROM A BRAZILIAN URBAN ESTUARY

AUTHORS: COSTA, W.F.¹; PARANHOS, R.²; PICÃO, R.C.¹; LAPORT, M.S.¹

INSTITUTIONS: ¹DEPARTAMENTO DE MICROBIOLOGIA MÉDICA, INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, CEP 21941-902, RIO DE JANEIRO – RJ, BRAZIL); ²DEPARTAMENTO DE BIOLOGIA MARINHA, INSTITUTO DE BIOLOGIA, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ (AVENIDA CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, CEP 21941-902, RIO DE JANEIRO – RJ, BRAZIL)

ABSTRACT:

Gram-negative bacilli producing extended-spectrum β -lactamases (ESBL) is a global health problem that can have water matrices as hotspots for its dissemination, leading to human, animal, and environmental health risks. We aimed to investigate the occurrence and distribution of these bacteria in Guanabara Bay (GB) waters, a heavily polluted and tourist tropical estuary in Rio de Janeiro, Brazil, over 12 consecutive months, verifying its association with fecal indicators. Subsurface (1 m depth) and bottom (6-20 m depth) water samples were collected monthly from Sep/2018 to Aug/2019 at three points with different pollution gradients inside GB. Potential ESBL-producers were recovered from 72 water samples on agar marine and chromogenic medium (CHROMagar) supplemented with ceftriaxone ($8 \mu\text{g.mL}^{-1}$) and amphotericin B ($1 \mu\text{g.mL}^{-1}$). Strains were identified by MALDI-TOF MS. Gram-negative bacilli were characterized for ESBL phenotype and genotype by disc-approximation and PCR, respectively. Fecal indicator counts (MPN.100mL⁻¹), thermotolerant coliforms (TC) and *Escherichia coli* (EC), were recorded from sampling points. Over 12 months, 1,558 colony-forming units were isolated and 706 (45.3%) were identified as: *Enterobacterales* (93), non-*Enterobacterales* gram-negative bacilli (264), and other microorganisms (349). Among the gram-negative bacilli, of 141 strains tested for ESBL production, 76 (53.9%) were positive. ESBL-producers belonged to *Escherichia* (38), *Aeromonas* (16), *Acinetobacter* (12), *Citrobacter* (7), *Enterobacter* (2), and *Pseudomonas* (1), which were isolated specially from GB34 subsurface (39/76). Search for ESBL-coding genes detected *bla*_{CTX-M-14} (15), *bla*_{CTX-M-12} (11), *bla*_{CTX-M-8} (11), *bla*_{TEM} (10), and *bla*_{SHV} (7). The 12 months average of fecal indicators in GB's waters varied of the highest in GB34 subsurface (1.4×10^5 and 1.3×10^5 of TC and EC, respectively) to minor in GB1 bottom (2.7×10^1 and 2.2×10^1). Significant correlations were observed among ESBL-producers and EC ($r=0.9167$, $p=0.0101$) and TC ($r=0.9209$, $p=0.0091$). Thus, the occurrence and distribution of ESBL-producing gram-negative bacilli in GB waters is likely related to sewage contamination.

Keywords: ESBL, fecal coliforms, anthropogenic pollution, Guanabara Bay, antibiotic resistance

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