

TITLE: CHARACTERIZATION AND FAST ANALYSIS OF PATHOGENIC BACTERIA IN BALLAST WATER BY FLOW CYTOMETRY

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ABSTRACT:

Commercial vessels have been using ballast water for stability since the 90s, discharging it into connecting ports in oceanic regions and spreading several microorganisms around the world. However, little is known about the the potential risk of dissemination of these microorganisms. Currently, the detection in ballast water is a failed process due to the lack of knowledge about particularities of microorganism populations inside of the tank. This work aims to analyze water samples from ballast tanks and to verify, in a fast and economically way, the presence of pathogenic bacteria. Ballast water samples were obtained at the Port of Itaguaí on the Strategic Vision bulk carrier from Singapore. In order to perform a 'Representative Sampling' according to the IMO/ONU guidelines, samples were collected from five different tanks. Presence or absence of target bacterial populations inside the tanks were checked through MacConkey Agar II selective medium. From each tank, two samples were striated in Petri dish and the growth was monitored in BOD for 48h at 30°C. Both color and shape of bacterial population that grown up in the selective medium were compared to the standard for *Salmonella*, *Escherichia*, and *Pseudomonas* genera. For bacterial characterization in flow cytometry, samples from tanks and from agar plates were filtered in 5µm nylon mesh and preserved in 0.025ml/L of glutaraldehyde. All samples were stained with SYBR Green and analyzed in flow cytometry (BD Influx Cell Sorter) with a blue laser (488nm). As results, 30% of samples revealed at least one pathogenic bacteria growing inside 40% of the ballast tanks. It were found colonies with yellowish, reddish, and single beige tones respectively characteristic of *Pseudomonas*, *Escherichia*, and *Salmonella* genera. Flow cytometry analyzes revealed only two distinct populations inside the tanks and suggest the needed for additional detectors in better characterization of pathogenic bacteria from ballast tank. Considering these conflicting results, the presence of viable populations that were detected only after laboratory procedures, as growing in selective medium, non-traceable and undesired dissemination is a considerable risk, and may become a public health problem. Considering the need to create methods to detect these agents, before the disposal of the ballast occurs, the Brazilian Navy is still working on the development of a new and fast technique to support naval inspectors and port agents.

Keywords: ballast water, flow cytometry, pathogenic bacteria.

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