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Enterococcus species diversity and molecular characterization of biomarker genes in *Enterococcus faecalis* in Port Blair Bay, Andaman and Nicobar Islands, India

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ABSTRACT

This study was performed to evaluate the abundance and diversity of *Enterococcus* sp. and the distribution of biomarker genes in *Enterococcus faecalis* in Port Blair Bay, Andaman and Nicobar Islands. The *Enterococcus* sp. densities at the seven sampling stations were highly influenced by tidal fluctuations and season. The distributions and diversities of species varied in the inner and outer regions of Port Blair Bay. Among the 1816 total isolates, the occurrence of fecal *Enterococcus* was high (1.78×10^4 CFU/100 mL) in Phoenix Bay. Moreover, 67.76% of the isolates were identified as *Enterococcus*, and the most frequently identified species were *E. hirae*, *E. avium* and *E. faecalis*. Assessments of antibiotic resistance and biomarker genes revealed the maximum occurrence in the Aberdeen Bay isolates. The most prevalent biomarker genes observed in the *E. faecalis* isolates were *gelE* and *asa1*, whereas *cyl* was not found among the isolates. *In silico* sequence analysis of biomarker genes of *E. faecalis* also revealed that they are evolutionarily well conserved with those of earlier reports. Further, multivariate analysis distinguished the JB, PB and OS stations from the other stations according to distinctive microbial densities and compositions. In addition, the Shannon-Wiener diversity indices and box-whisker plots further facilitated and supported the multivariate results.

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1. Introduction

Coastal waters generally contain both pathogenic and non-pathogenic microbes derived from runoff, sewage, industrial effluent, agricultural activities, wild life and indigenous microorganisms. These pathogens can be hazardous to the health of bathers and consumers when an infective dose colonizes a suitable growth site of the body and leads to disease (Elmanama et al., 2005; WHO,

1998). *Enterococcus* bacteria are part of the normal intestinal flora of animals and humans, and they are released into the environment directly or via sewage outlets (Farrel et al., 2003). Studies conducted by the Environmental Protection Agency (EPA) to determine the correlation between bacterial indicators and digestive system illness have reported that the most prominent indicators for health risk from recreational water contact are *E. coli* and *Enterococcus* sp. in fresh and salt waters, respectively (USEPA, 2003).

In the last few decades, *Enterococcus* sp. has emerged as the most important causative organism of nosocomial infection and as a specific threat to public health (Heymann, 2006). The distribution of infectious *Enterococcus* sp. in the environment via water may cause an increase in the prevalence of these strains in humans (Irani et al., 2011). *Enterococcus faecalis* and *Enterococcus faecium* are important opportunistic pathogens (Harwood et al., 2004). Enterococci are able to grow at a temperature of 45 °C and pH 9.6 in 6.5% NaCl broth and to survive at 60 °C for 30 min (Sherman, 1937). Among the enterococci, *E. faecalis* can cope with

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unfavorable conditions by maintaining cell viability under starvation for extended periods and become resistant to UV radiation, heat, and differing/high concentrations of sodium hypochlorite, hydrogen peroxide, ethanol, and acid (Hartke et al., 1998). Major factors for the pathogenesis of enterococci include their resistance to a wide range of antibiotics and virulence factors. Genes encoding virulence factors include *asa1*, *esp*, *hyl*, *gelE*, *ef0591*, *aadE*, *pbp4* and *cylA* (Kuzucu et al., 2005).

The coastal waters of the Andaman and Nicobar (A & N) Islands are pristine compared with those of the highly populated mainland India (Sahu et al., 2013). The diversity and distribution of *Enterococcus* sp. in the environment and coastal waters are not reflective of the pollution rate; however, the population density and distribution of virulence factors and the pathogenicity of this indicator organism can define the microbial pollution rate and the source of possible contamination in a particular zone. The objectives of this study were as follows: (i) to determine the seasonal impact on population density and the distribution of *Enterococcus* sp. in Port Blair Bay; (ii) to evaluate the species diversity and dominance of *Enterococcus* sp. among selected stations; and (iii) to examine the antibiotic susceptibility and distribution of biomarker genes in *E. faecalis* during various seasons. This study is the first to attempt to correlate the microbial pollution trend

with the diversity of biomarker genes in *E. faecalis* from Port Blair Bay, A & N Islands.

2. Methods

2.1. Study area

Port Blair Bay is located in South Andaman Island and extends from east to west and southwest, opening to the Andaman Sea on the eastern side (Fig. 1). This bay area is influenced by the anthropogenic activities of Port Blair city, where the majority of the population of the A & N Islands is congregated. In the present study, seven stations were selected within Port Blair Bay, from the inner part of the bay to the Open Sea station, including Flat Bay (FB), Minnie Bay (MB), Junglighat Bay (JB), Haddo Harbor (HH), Phoenix Bay (PB), Aberdeen Bay (AB) and Open Sea (OS) (Fig. 1).

2.2. Sample collection and analysis of microbial and physicochemical characteristics

Sampling was performed quarterly according to seasonal preference (February, May, August and November) from seven

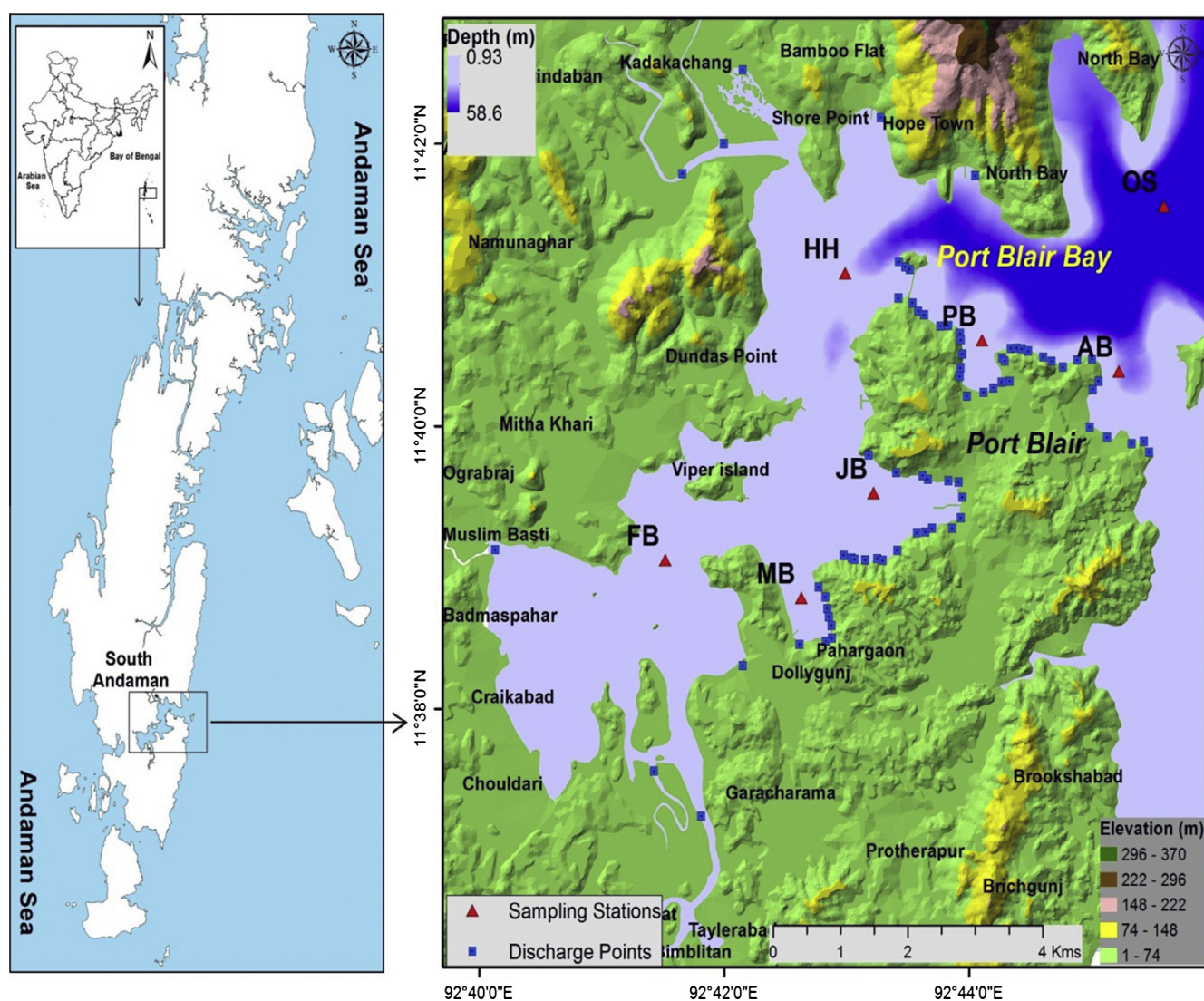


Fig. 1. Sampling stations with drainage points along Port Blair Bay, Andaman and Nicobar Islands.

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