



Prevalence and genetic profiles of *Escherichia coli* from mangroves and mangrove associated foods off Goa, India



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ABSTRACT

A total of 120 samples comprising of water (45), sediment (45) and mangrove originated food (30) collected from mangrove ecosystems of Goa were screened for *Escherichia coli* employing ISO-16654 method. Seventy-one (59.16%) samples were positive for *E. coli*. The *E. coli* isolates were further characterized by serotyping, virulence gene profiling and pulsed field gel electrophoresis (PFGE). Water and sediment samples were analyzed for physico-chemical parameters. The serotypes reported were O1, O10, O13, O17, O36, O41, O50, O68, O105, O116, O141, O148, O159, O162 and rough types while, 23 strains could not be typed. The *stx1* and *stx2* genes were detected in 33(46.47%) and 16(22.53%) isolates, respectively. The *XbaI* restriction digestion patterns of the *stx* positive strains were diverse. Interestingly, few strains isolated from diarrheal patients and from water, sediment and food from mangrove sources were genetically similar. The study showed that the mangrove ecosystem could be a potential reservoir for pathogenic *E. coli*.

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1. Introduction

Mangroves provide a unique ecological niche to different microbes which play an important role in nutrient recycling as well as various environmental activities. Mangrove forests are a large ecosystem distributed in 112 countries and territories comprising a total area of about 181,000 km² in over a quarter of the total coastline of the world (Sahoo and Dhal, 2009). According to State Forest Report (2013), an extent of 4445 km² mangrove area is present in India of which majority lies in the West or East coast estuaries (TNAU, 2013). These estuarine mangroves have been characterized for salt resistant plants growing in inter tidal areas along sheltered seacoasts and estuaries in the tropical and subtropical regions (GOA-ENVIS, 2013). Mangroves act as a sink for nutrients and provide large quantities of detritus organic matter to nearby coastal waters (Prasad and Ramanathan, 2008), which serve as an important food resource for in-shore marine biota. Therefore, the mangrove ecosystem is a protected nursery habitat for fishes, shrimps and crustaceans. However, mangroves are highly sensitive ecosystems and get easily affected by various anthropogenic factors. Human domestic sewage and industrial

effluents adds organic, inorganic as well as microbial pollutants into mangrove ecosystem (Pawar, 2013).

Recent studies have reported the occurrence of pathogenic microorganisms namely, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Escherichia coli* in mangrove ecosystems (Grisi and Gorchach-Lira, 2010; Rodrigues et al., 2011). *E. coli* is a dominant bacterium in sewage, which can compete with the native microflora (Ramaiah et al., 2007). The presence of fecal indicator bacteria like *E. coli* primarily suggests sewage contamination in mangroves. The prevalence of *E. coli* in water bodies due to anthropogenic activity has been previously reported (Chandran et al., 2013). *E. coli* causes several serious consequences which range from low fever, bloody diarrhea, stomach cramps, nausea, vomiting and low fever in humans, while, some complications may lead to renal failure, anemia, dehydration, spontaneous bleeding, organ failures and death (Jafari et al., 2012).

In the State of Goa (India), the total area covered by the estuaries is approximately 12,000 ha. Out of which the mangrove forests occupy 2000 ha (GOA-ENVIS, 2013). The mangroves of Goa have been explored by locals for biota such as several fishes (*Etroplus suratensis*, *Caranx malabaricus*, *Sparus berda*), crabs (*Scylla serrata*, *Fiddler crab*) and mud Clam (*Polymesoda erosa*) as commercial food (Clemente, 2008; MSI, 2013). Rapid urbanization, population density and industrialization in Goa have drastically affected the mangrove ecosystem and in-turn the indigenous biota. Therefore, biota present at the mangrove also may get affected.

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Indigenous bacterial flora (Desai et al., 2004; De Sousa and Bhosle, 2012; Khandeparker et al., 2011) and pathogenic bacteria (Rodrigues et al., 2011; Ramaiah et al., 2007; Nagvenkar and Ramaiah, 2009) have been isolated from mangrove ecosystems of Goa. The organic/inorganic content has also been determined (Attri et al., 2011; Krishnan and Loka Bharathi, 2009; Paula et al., 2009; Krishnan et al., 2007), however, the potential effects on the local population are not known. The objective of the present study was to isolate and characterize *E. coli* strains from the mangroves and mangrove associated foods.

2. Materials and methods

2.1. Sampling area

Sampling was carried out across Mandovi (15°21'–15°31'N and 73°45'–73°49'E) and Zuari estuaries (15°25'N and 15°25'E) of Goa, India. The samples (water, sediment and mangrove originated biota) were collected from 15 different locations. Sampling sites were located across the areas from where mangrove associated biota are frequently harvested for human consumption (Fig. 1).

2.2. Sampling

A total of 120 samples comprising of sediments ($n = 45$), water ($n = 45$) and mangrove originated biota (fishes, crabs and mud clam) ($n = 30$) were collected in the month of October (post-monsoon). Approximately 10 gm of sediment samples were collected from 10 cm depth by Van Veen grab in sterile polythene bags. For water samples, 100 ml of water was collected in sterile screw cap tubes. All samples were transported to the laboratory in chilled conditions and processed for total viable counts and screened for *E. coli*.

Also, five *E. coli* strains isolated from diarrheal patients having a history of consumption of mangrove originated food were acquired from Goa Medical College, Goa, India.

2.3. Physico-chemical characteristics of water

Physio-chemical parameters: temperature, pH and salinity were determined in the field during sampling using a multi-meter (Cole-Parmer). Total dissolved solids (TDS) and dissolved oxygen

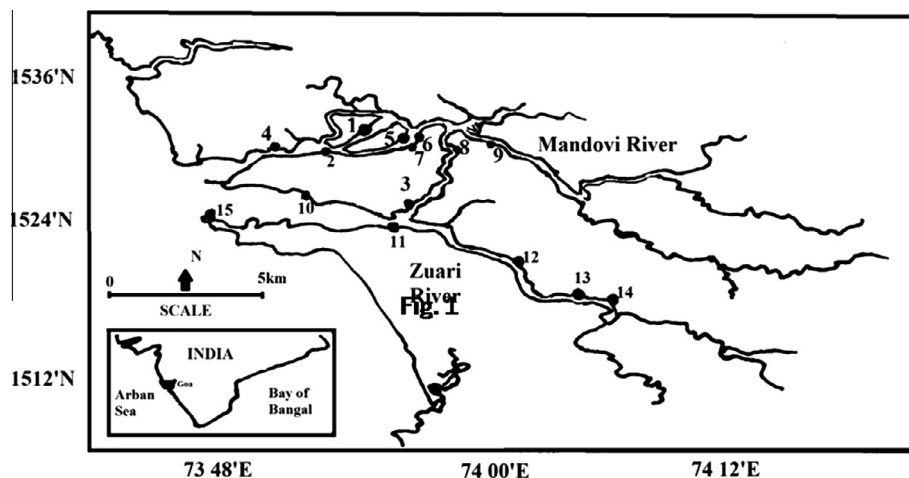
(DO) were determined by standard methods as described by Trivedi and Goel (1986). Bacterial load was determined by total plate count, while, the load of enteric bacteria was determined on McConkey's agar.

2.4. Isolation of *E. coli*

E. coli was isolated as described in ISO-16654 method. Approximately 5 ml of water or 5 gm of sediment sample was inoculated in 45 ml of McConkey's broth and incubated at 18 h at 37 °C for enrichment. A loopful of enriched broth was streaked on Eosin methylene blue agar plates and incubated at 37 °C for 24 h. Dark purple colonies with a metallic sheen were isolated as *E. coli*. These presumptive isolates were stored in nutrient broth. These isolates were further confirmed to be *E. coli* stains by Gram staining, sugar fermentation and IMViC test.

2.5. Detection of the *stx* genes

All the isolates obtained from mangrove associated areas and 5 *E. coli* isolates from diarrheal patients were screened for the presence of Shiga-like toxin 1 (*stx1*) and *stx2*. The PCR was performed as described by Vidal et al. (2004). DNA was extracted by snap chill method. In brief, overnight grown (5 ml) bacterial culture was centrifuged at 13,000g for 10 min. The pellet was then suspended in 100 µl of sterile distilled water and kept in boiling water for 10 min. The suspension was transferred immediately to –20 °C for 10 min. The treated cell suspension was centrifuged at 13,000g for 10 min. And the supernatant was used as a DNA template which was tested for the presence of the *stx1* and *stx2* genes by PCR. Primers used for the *stx1* and *stx2* genes were as described by Vidal et al. (2004). A reaction mixture was prepared for a total volume of 25 µl containing 10x PCR buffer, 1.5 mM of MgCl₂, 2 mM dNTP mixture, 0.5 µM of each primer and 50 ng of DNA template. The reaction conditions were set as initial denaturation at 94 °C for 2 min followed by denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and final extension at 72 °C for 2 min. *E. coli* ATCC 8739 was used as a positive control. The amplified DNA products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide and visualized under Alpha-Imager Gel Doc system. The 348 bp and 584 bp amplicons were obtained for the *stx1* and *stx2* genes, respectively.



North Goa : 1. Ribandar 2. Mercas 3. Madkai 4. Bettim 5. Divar 6. Old Goa
7. Bainginium 8. Kumbharjua 9. Marcel 10. Shridao

South Goa: 11. Cortalim 12. Durbhat Ferry 13. Rachol Ferry 14. Curtorim 15. Vasco

Fig. 1. Map showing sampling locations in the study area of Mandovi-Zuari mangrove ecosystem.

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