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Detection of multiple potentially pathogenic bacteria in Matang mangrove estuaries, Malaysia

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ABSTRACT

The deltaic estuarine system of the Matang Mangrove Forest Reserve of Malaysia is a site where several human settlements and brackish water aquaculture have been established. Here, we evaluated the level of fecal indicator bacteria (FIB) and the presence of potentially pathogenic bacteria in the surface water and sediments. Higher levels of FIB were detected at downstream sampling sites from the fishing village, indicating it as a possible source of anthropogenic pollution to the estuary. Enterococci levels in the estuarine sediments were higher than in the surface water, while total coliforms and *E. coli* in the estuarine sediments were not detected in all samples. Also, various types of potentially pathogenic bacteria, including *Klebsiella pneumoniae*, *Serratia marcescens* and *Enterobacter cloacae* were isolated. The results indicate that the Matang estuarine system is contaminated with various types of potential human bacterial pathogens which might pose a health risk to the public.

1. Introduction

Few estuaries in Malaysia or the region have the reputation of being a world-class demonstration site of exemplary mangrove forestry management as the estuaries of the Matang Mangrove Forest Reserve (MMFR), Perak state, Malaysia. MMFR is a silviculture production forest managed on a sustainable basis without a single hectare loss of forest since its reservation in 1902, mainly for the production of charcoal and piling poles. While timber production is the main objective for the reservation of MMFR, the protection of fisheries resources for food security is perhaps a greater socio-economic and ecological role played by MMFR. Perak produces the highest or 28.7% of the total fish landings in Malaysia. The MMFR with its large expanse of sheltered waters is site to 7666 floating fish cages, and cockle culture covers an extensive area of 4726 ha within and outside the estuaries (Ariffin and Nik Mustafa, 2013). Brackish water shrimp ponds thrive outside the perimeters of the MMFR but draw and discharge sea water into it. There are presently 385 shrimp ponds covering a total area of about 223 ha.

Although the MMFR demonstrates how a sustainably managed forest could provide significant ecological services and economic benefits, it also illustrates a coastal zone of increasing multiple-use conflicts and the dilemma of land and water management practiced on a sectoral basis (Chong et al., 2010). Increasingly, anthropogenic influences on water quality in the MMFR may take a toll of its resources in spite of the excellent (land) management of the mangrove. Of the total of 34 permanent settlements in the MMFR, 28 are fishing villages sited by the mangrove estuaries. A survey by Lim and Mohamad Parit (2001) indicated 5300 households with a total population of 31,800 people mostly (79%) associated with sea fishing in some way. Kuala Sepetang is now a striking fishing settlement of both old and modern homes. The quaint human abodes that line the watercourse are built on tall stilts made from the nibong palm (*Oncosperma tigillarium*), and alarmingly, among them the stark presence of 'open-discharge' toilets.

In this study, we aimed to investigate the general public health risk related to human bacterial pathogens, which could be contributed by fecal contamination due to human settlement along the estuaries. In most countries, microbiological quality and safety monitoring of coastal and estuarine waters is an essential component of the national risk management program (Touron et al., 2007), due to the growing number of human bacterial infections associated with recreational and commercial uses of marine resources (Tamplin, 2001). In general anthropogenic activities,

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including human settlements, industrialization, agricultural and aquacultural practices, contribute greatly to the degradation of water quality and safety (May et al., 2006). An increasing number of potential human bacterial pathogens, including pathogenic *Escherichia coli* (*E. coli*), and species of *Vibrio*, *Klebsiella*, and *Salmonella* have been reported from estuarine and marine environments globally (Peng et al., 2005; Islam and Tanaka, 2004; Lee et al., 2011).

The level of pollution in the coastal environment requires particular attention due to potential microbiological risks that are linked to the consumption of contaminated aquaculture products, with eventual socio-economic consequences (Touron et al., 2007; Retnam et al., 2013). In 2008, a ban was placed on Malaysian seafood products by the European Union (EU) due to health risks posed by seafood from Malaysia which caused export losses amounting to almost 200 million USD dollars (Retnam et al., 2013). Therefore, it is important to monitor the presence of waterborne pathogens in the coastal and estuarine environments, not just to protect the people from infectious diseases, but also to protect Malaysia's developing aquaculture industry.

In this study, the Matang mangrove estuary was investigated for its water quality (enumeration of total coliforms, *E. coli* and Enterococci) and safety (Enterobacteriaceae) using a chromogenic medium that allows for simultaneous isolation and identification of various species of Enterobacteriaceae. CHROMagar Orientation™ (CHROMagar, Paris, France) was used in this work as the single detection and isolation medium for isolation of Enterobacteriaceae and Gram-positive Enterococci (Merlino et al., 1996).

2. Materials and methods

2.1. Study area

The Matang mangrove estuaries form a complex system of interconnected waterways that criss-cross the MMFR (Fig. 1). The MMFR situated in the state of Perak is the single largest tract of mangrove forest (41,000 ha) remaining in Peninsular Malaysia (Fig. 1). Sangga Besar River is the main waterway traversed by fishing boats between Kuala Sepetang and the fishing ground in coastal waters. The upper reach of Sangga Besar is joined by the Sepetang River which brings down the main riverine discharge from upstream. The Selinsing River is the longest distributary meandering 18 km downstream and discharging into the Malacca Strait. Few boats ply the long waterway which has a few fish cages located near the river mouth. Extensive coastal mudflats line the coast just outside the outer mangrove fringe.

Sampling stations were set up from the upper estuary to the coastal mudflat area downstream (Fig. 1). All stations were located and marked by GPS coordinates for future reference. Station A was located upstream of Sepetang River, while station B was situated near to the Kuala Sepetang village. Station D was situated at a cockle culture site. Station E was situated just off the small island village of Bagan Kuala Sangga where many floating cage nets were concentrated. Two further sampling stations were selected on the relatively unpolluted Selinsing River, stations G and H, located on the upstream and downstream, respectively.

2.2. Water and sediment sampling

Four samples of water and sediment were collected at each station during the wet period in October 2011 and dry period in May 2012. The water samples were collected using a 4.2 l Van Dorn sampler and separately stored in acid-washed bottles. River bed sediment was sampled by an Ekman grab (Wildco, USA) and a portion of the top 5 cm of sediment, was scooped into a 50 ml sterile bottle, after removing debris and shells. Filled sediment bottles were capped before they were placed together in a zip-lock polyethelene bag according to station. All water and sediment samples were always kept in an ice filled chest, before their transfer to the laboratory. In the laboratory, all water and sediment samples were stored in a 4 °C freezer until microbiological analyses were carried

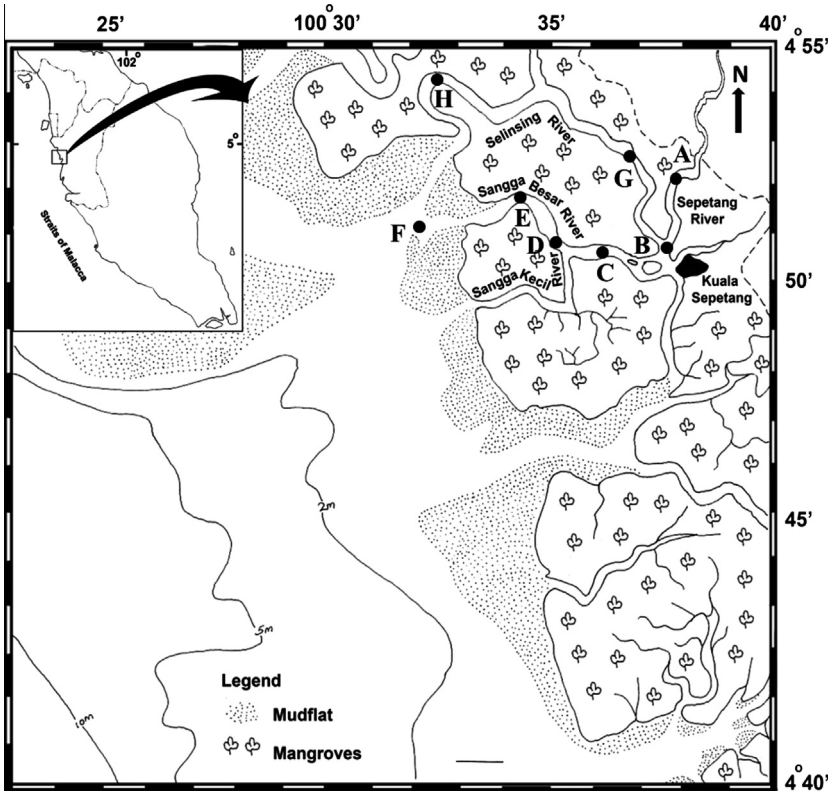

Fig. 1. Locations of eight sampling stations (A–H) along Sepetang, Sangga Besar and Selinsing Rivers in Matang mangrove festuaries, Perak, Malaysia.

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