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Baseline

Occurrence and distribution of bacteria indicators, chemical tracers and pathogenic vibrios in Singapore coastal waters

Shin Giek Goh [a], Stéphane Bayen [b,c], David Burger [b], Barry C. Kelly [b], Ping Han [b], Vladan Babovic [b], Karina Yew-Hoong Gin [a,d,*]

[a] National University of Singapore, Department of Civil and Environmental Engineering, 1 Engineering Drive 2, Blk E1A #07-03, 117576 Singapore, Singapore
[b] Singapore-Delft Water Alliance, National University of Singapore, 1Engineering Drive 2, Singapore 117576, Singapore
[c] Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore, Ste-Anne-de-Bellevue, Quebec H9X 3V9, Canada
[d] NUS Environmental Research Institute (NERI), T-Lab Building #02-01, Engineering Drive 1, 117411 Singapore, Singapore

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ABSTRACT

Water quality in Singapore's coastal area was evaluated with microbial indicators, pathogenic vibrios, chemical tracers and physico-chemical parameters. Sampling sites were grouped into two clusters (coastal sites at (i) northern and (ii) southern part of Singapore). The coastal sites located at northern part of Singapore along the Johor Straits exhibited greater pollution. Principal component analysis revealed that sampling sites at Johor Straits have greater loading on carbamazepine, while turbidity poses greater influence on sampling sites at Singapore Straits. Detection of pathogenic vibrios was also more prominent at Johor Straits than the Singapore Straits. This study examined the spatial variations in Singapore's coastal water quality and provided the baseline information for health risk assessment and future pollution management.

Singapore is a heavily urbanized island located in Southeast Asia between the Indian Ocean and the South China Sea. The country is bordered by the Johor Straits in the north; the Singapore Straits in the southeast; and the Straits of Malacca in the southwest. The land area of Singapore has increased from 581.5 km^2 from 1960 to 718.3 km^2 in year 2014 (DOS, 2014) and is expected to reach 766 km^2 by 2030 (MND, 2013). Continuous land reclamation activities have caused increases in turbidity and changes in natural habitat (Slijkerman et al., 2011). A rapidly expanding petrochemical industry in the past few decades has also contributed to petroleum and heavy metal contamination of the coastal seas. In addition, urban runoff, agricultural runoff and wastewater discharges could be possible sources of organics, nutrients and pathogens to the coastal waters of Singapore (Slijkerman et al., 2011). One of the consequences of excessive nutrient inputs are higher incidences of harmful algal blooms and low levels of DO, resulting in massive fish kills as observed in 2009, 2014 and 2015 (Lee, 2014; Leong et al., 2012; Ooi et al., 2010; Wong, 2015). In addition, high nutrient level and warm temperature may encourage the growth of pathogenic bacteria, such as vibrios. The coastal waters that contaminated by human fecal wastes could pose higher risk to human health (Arnone and Walling, 2007; Soller et al., 2010). Fecal indicator bacteria which commonly used for water quality monitoring may subject to false positive signals in tropical areas (Ekklesia et al., 2015). As such, wastewater-related chemical tracers which are more specific to human fecal wastes, are recommended as second tier in assessing the water quality (Ekklesia et al., 2015).

In this study, the water quality of Singapore's coastal waters was evaluated based on physical, chemical and microbiological properties. Specifically, the objectives of this study were (i) to examine the spatial variations in coastal water quality; (ii) to compare the relationships between microbial indicators and chemical tracers; and (iii) to investigate the occurrence of vibrios in tropical coastal waters.

Water samples were collected six times from the eight locations from April 2013 to January 2014. Water samples were drawn from three depths – surface (0.5 m below surface), mid and bottom (0.5 m above sediment). The sampling sites are shown in the location map (Fig. 1). Sampling site B, A, H and G which located along the Johor Straits are surrounded with agriculture and aquaculture farms, industry and residential areas. Sampling site C is situated near to the major industrial area in Singapore. The land use near to site D and E at east coast of Singapore Strait is mainly for residential and reserved area, respectively.

Measurements of physico-chemical parameters were conducted in situ (temperature, pH, salinity, conductivity, turbidity, DO and chlorophyll-*a*) using a YSI 6600 Sonde Yellow Springs, OH, US while grab samples were brought back to the lab for analyses of other chemical parameters (TSS, organic carbon, nutrients), bacteria (total coliforms, *Escherichia coli* (*E. coli*) and *Enterococcus*) and chemical contaminants

* Corresponding author.
E-mail address: ceeginyh@nus.edu.sg (K.Y.-H. Gin).

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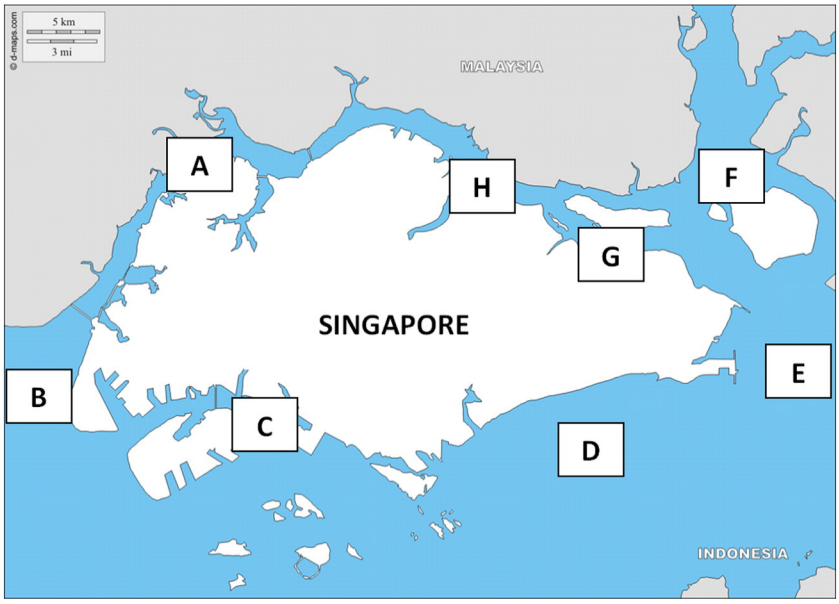

Fig. 1. Location of the 8 sampling locations where water quality was monitored (Base map extracted from d-maps.com).

of emerging concern (chloramphenicol, lincomycin, atrazine, caffeine, carbamazepine, sulfamethoxazole, sulfadiazine, triclosan, sulfamethazine). A list of physico-chemical parameters that were monitored is given in Table 1.

Colilert™ and Enterolert™ (IDEXX Laboratories, Inc., Westbrook, Maine) were used to quantify the cell numbers of *E. coli* and *Enterococcus*, respectively. 100-mL of raw water sample and 10 times dilution of raw water sample were tested for each sample according to manufacturer's instruction.

Vibrios were enumerated according to the method of Dickinson et al. (2013) with some modifications. Briefly, the water samples (0.1 mL, 1 mL, 10 mL and 100 mL) collected from 8 coastal areas were used to determine the most probable number (MPN) by enrichment in alkaline peptone water (Sigma-Aldrich, St Louis, MO). The samples were incubated overnight at 37 °C. 1-mL of enriched sample was centrifuged at 10,000*g* for 10 min to pellet the bacteria. The pellet was resuspended in 200 μL of nuclease-free water and subsequently heated to boiling for 10 min to release the nucleic acids for the polymerase chain reaction (PCR) analysis. The primers used for the detection of vibrios are listed in Table 2. PCR was carried out in a thermal cycler (Eppendorf, Germany) and performed in 25 μL volumes consisting of 12.5 μL of 2× GoTaq® master mix (Promega, Madison, WI), 1.25 μL of each primer (10 μM), 7.5 μL of nuclease-free water and 2.5 μL of extracted DNA. PCR annealing temperatures for different target vibrios are shown in Table 2. In general, the PCR amplification cycle consisted of an initial denaturing cycle at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing temperature (refer Table 2) for 1 min and extension at 72 °C for 1 min. A final extension step at 72 °C for 5 min was also included at the end of 30 cycles. The amplified products were separated by electrophoresis on a 1.5% agarose gel stained with GelRed™ (Biotium, Inc., Hayward, CA). The separated fragments on the agarose gel were visualized under UV light with *E*-Gel® Imager System (Life Technologies, Carlsbad, CA).

Table 1
List of physical and chemical parameters of water quality investigated.

Test parameters	Units	Method
Temperature	°C	
pH		APHA 4500-H + B: Electrometric method
Salinity	ppt	APHA 2520 B: Electrical conductivity method
Conductivity	mS/cm	APHA 2510 B: Electrical conductivity method
Turbidity	NTU	APHA 2130 B: Nephelometric method
Dissolved oxygen (DO)	mg/L	APHA 4500 G: Membrane electrode method
Chlorophyll-*a*	μg/L	APHA 10200H: Chlorophyll
Total suspended solids (TSS)	mg/L	APHA 2540 D: Total suspended solids dried at 103–105 °C
Dissolved organic carbon (DOC)	mg/L	APHA 5310 B: High temperature combustion method
Total organic carbon (TOC)	mg/L	APHA 5310 B: High temperature combustion method
Dissolved nitrate (NO_3)	mg/L	APHA 4500-NO3 I: Cadmium Reduction & Flow Injection Analysis
Dissolved phosphate (PO_4)	mg/L	APHA 4500-P G: Flow injection analysis for orthophosphate
Total phosphate (TP)	mg/L	APHA 4500-P H: Manual digestion & flow injection analysis for total phosphorus
Dissolved ammonia (NH_3)	mg/L	APHA 4500-NH3 H: Flow injection analysis for ammonia

The contaminants of emerging concern were analysed in seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry (Bayen et al., 2014). In short, 1 mL of seawater was filtered (PTFE, 0.22 μm), of which 10 μL was analysed using an Agilent 1290 Infinity LC coupled with a 6490 Triple Quad MS/MS. Chromatographic separation was achieved on a Poroshell 120 SB-C18 column (2.1 mm; 150 mm; 2.7 μm; Agilent Technologies), equipped with a pre-filter (porosity 2 μm, 2.1 mm). Multiple Reaction Monitoring (MRM) transitions reported in various references were selected from earlier work (Bayen et al., 2013). This method, previously validated in our laboratories for seven antibiotics was further validated for three pharmaceutically active compounds (carbamazepine, caffeine and triclosan) and one herbicide (atrazine) (see Table S1 of the Supplementary information).

Preliminary analysis to examine the distribution of data was carried out before the selection of statistical analysis method. The non-parametric statistical method was selected for spatial distribution comparison between the groups and to explore the relationships between groups. The Kruskal-Wallis test was used to examine if there was a significant difference in environmental parameters, nutrient levels, microbial indicators and chemical tracers for different sites. Cluster analysis (CA) was used to classify the sampling sites based on the microbial data and environmental parameters. Hierarchical agglomerative clustering was applied to examine the similarity relationships between the data set where it starts with every case as a separate cluster and

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