Prevalence and characterization of *Escherichia coli* in the Kelantan River and its adjacent coastal waters

Chui Wei Bong, Siong Kiat Chai, Lay Ching Chai, Ai Jun Wang and Choon Weng Lee

ABSTRACT

The presence of *Escherichia coli* in river and sea water may cause different levels of infections and constitutes a risk to public health. In this study, water samples were collected from 15 sites along the Kelantan River, estuaries and its adjacent coastal waters to investigate the prevalence and diversity of *E. coli*. A membrane filtration technique was used to enumerate *E. coli* and phylogenetic grouping was performed using triplex polymerase chain reaction. *E. coli* abundance ranged from 3.1×10 to 1.6×10^5 colony forming units 100 mL^{-1}, and total suspended solids correlated significantly with *E. coli* abundance ($r^2 = 0.165$, $p < 0.001$) and rainfall ($r^2 = 0.342$, $p < 0.001$). Phylogenetic group B1 and A (59.4%) were the most prevalent, whereas groups B2 and D were least abundant. The higher abundance of phylogenetic group D at upstream sites of the Kelantan River suggested fecal contamination mainly of animal origin. Canonical-correlation analysis showed phylogenetic group B2, and phylogenetic groups A and D were greater in waters with higher inorganic nutrients (e.g. NH_4, NO_2 and NO_3), whereas phylogenetic group B1 appeared to have better salinity tolerance between phylogenetic groups.

Key words | *E. coli*, fecal pollution, Kelantan River, phylogenetic groups

Chui Wei Bong (corresponding author)
Siong Kiat Chai
Choon Weng Lee
Laboratory of Microbial Ecology,
Institute of Biological Sciences,
University of Malaya,
50603 Kuala Lumpur,
Malaysia
E-mail: *cwbong@um.edu.my*

Chui Wei Bong
Siong Kiat Chai
Lay Ching Chai
Choon Weng Lee
Institute of Ocean and Earth Sciences,
University of Malaya,
50603 Kuala Lumpur,
Malaysia

Siong Kiat Chai
Institude of Graduate Studies,
University of Malaya,
50603 Kuala Lumpur,
Malaysia

Ai Jun Wang
Laboratory for Coast and Ocean Geology, Third Institute of Oceanography,
Ministry of Natural Resources of People's Republic of China,
Xiamen 361005,
China

INTRODUCTION

Escherichia coli is a coliform subgroup commonly found in the intestine of both humans and animals. It is used as a marker for fecal pollution in aquatic system (Byamukama *et al.* 2005) and as an indicator to assess food hygiene and food safety. *E. coli* are mostly non-harmful but there are some pathogenic strains. These are responsible for infections of the human digestive system (e.g. enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC), and diffusely adherent *E. coli* (DAEC)) (Hamelin *et al.* 2006), and human extraintestinal infections, such as nosocomial bacteremia, urinary tract infection, and myositis (Galvin *et al.* 2010). Most of these pathotypes are of public health concern because their infectious dose is low and they are easily transmitted via food and water (Matic *et al.* 1997).

E. coli can be categorized into phylogroups A, B1, B2, and D based on the existence or absence of the following genes: (i) *chuA* (required for EHEC heme transport); (ii) *jyaA* (unknown function); and (iii) TSPE4.C2 (unknown DNA segment) (Clermont *et al.* 2000). Most commensal and obligatory pathogens (EHEC, ETEC, and EIEC) are found in phylogroup A and B1. Group A is predominantly found in humans, whereas group B1 is predominantly in animals. Phylogenetic group B2 and D are mostly virulent extraintestinal strains (Luna *et al.* 2010), whereas the virulent

doi: 10.2166/ws.2020.018

groups responsible for chronic and mild diarrhea are spread across the four phylogenetic groups. Phylogroups A and B1 are persistently found in environment and have been recognized as emerging gastrointestinal strains that are closely linked to antibiotic resistance (Escobar-Páramo *et al.* 2004).

In this study we focused on the Kelantan River, which is 248 km long and is formed by the confluence of the Galas and Lebir Rivera that run across the state of Kelantan in Peninsular Malaysia. It has a catchment area of 13,100 km^2 and is dominated by sedentary soils (hills) and alluvial soils (riverine floodplains) (Adnan & Atkinson 2011). The river passes through four main townships of Kota Bharu, Kuala Krai, Pasir Mas, and Tumpat prior to discharge into South China Sea (Pradhan & Youssef 2011). The river flow is affected by monsoon rainfall throughout the year, with maximum annual rainfall capacity of 1,750 mm during the northeast monsoon season (November to January).

For Kelantan local residents, the Kelantan River serves as an important water source for domestic use, transportation, irrigation, sand mining, agriculture, and aquaculture industries. To date, there are about 128 sand mining operations along the Kelantan River, causing the water of the Kelantan River to turn turbid since the early 1990s (Yen & Rohasliney 2013). *E. coli* and total coliform in the Kelantan River are frequently found at concentrations that exceed limits set by the Interim Marine Water Quality Standard (IMWQS) and the Water Quality Index (Basri *et al.* 2015; Bamaiyi *et al.* 2017). This may result in the higher incidence rates of food and water-borne diseases in Kelantan relative to other states in Malaysia. Being in the monsoon belt, the incidence of flooding in Kelantan is also frequent and the high flood waters can overwhelm sanitation systems, stir up rivers, and increase occurrence of both *E. coli* and total coliform between the Kelantan River Delta and its adjacent coastal waters (Ahmad *et al.* 1996; Vignesh *et al.* 2013).

As there is growing concern about the occurrence of *E. coli* in aquatic environment (Ghaderpour *et al.* 2015; Sen *et al.* 2019), it is good to have a better understanding of *E. coli* epidemiology and ecology in the Kelantan River. This study aimed to determine the distribution and genetic diversity of *E. coli* and their association with environmental variables in Kelantan River and its adjacent coastal waters.

MATERIALS AND METHODS

Sampling and physicochemical analysis

Twenty samplings were carried out regularly at 15 sites along the Kelantan River and its adjacent coastal sites from November 2014 until August 2016 (total number of samples = 300) (Figure 1). Replicate water samples were collected in a sterile Schott bottle and kept cold for no more than 6 h until processed in the laboratory. In situ parameters, including surface water temperature and conductivity, were measured using a conductivity, meter (YSI-30, USA) and a pH meter (Thermo Scientific Orion 4-Star, USA) was used to record pH. For dissolved oxygen (DO) measurements, water samples were collected with DO bottles and fixed instantly with manganous chloride and alkaline iodide solutions. The mixtures were then mixed and delivered to the laboratory for analysis. The DO concentration was calculated based on the Winkler titration method (Grasshoff *et al.* 1999).

For total suspended solids (TSS), sample volume was determined and filtered through a glass microfiber filter/F grade (GF/F) filter paper. TSS was measured as the weight increase of filter after drying at 70 °C and particulate organic matters (POM) was calculated based on weight loss after combustion in a microwave furnace (model MF-05, USA) at 500 °C for 3 h. Dissolved inorganic nutrients (nitrate (NO_3), nitrite (NO_2), ammonium (NH_2), phosphate (PO_4) and silicate (SiO_4)) were analyzed according to Parsons *et al.* (1984).

Isolation and enumeration of coliform and *E. coli*

The isolation and enumeration of coliform and *E. coli* in water samples were carried out using a membrane filtration technique. The membrane was placed on CHROMagar ECC for *E. coli* and coliforms (in triplicates) and incubated at 37 °C overnight (modified Method EPA 1604). Presumptive *E. coli* (blue colonies) on CHROMagar ECC were selected and purified on CHROMagar ECC before storing in glycerol solution. Enumeration of *E. coli* was carried out by counting the number of blue colonies, and colonies with mauve color were counted as total coliform. Abundance of total coliform and presumptive *E. coli* were expressed as colony forming units per 100 milliliters (CFU 100 mL^{-1}).

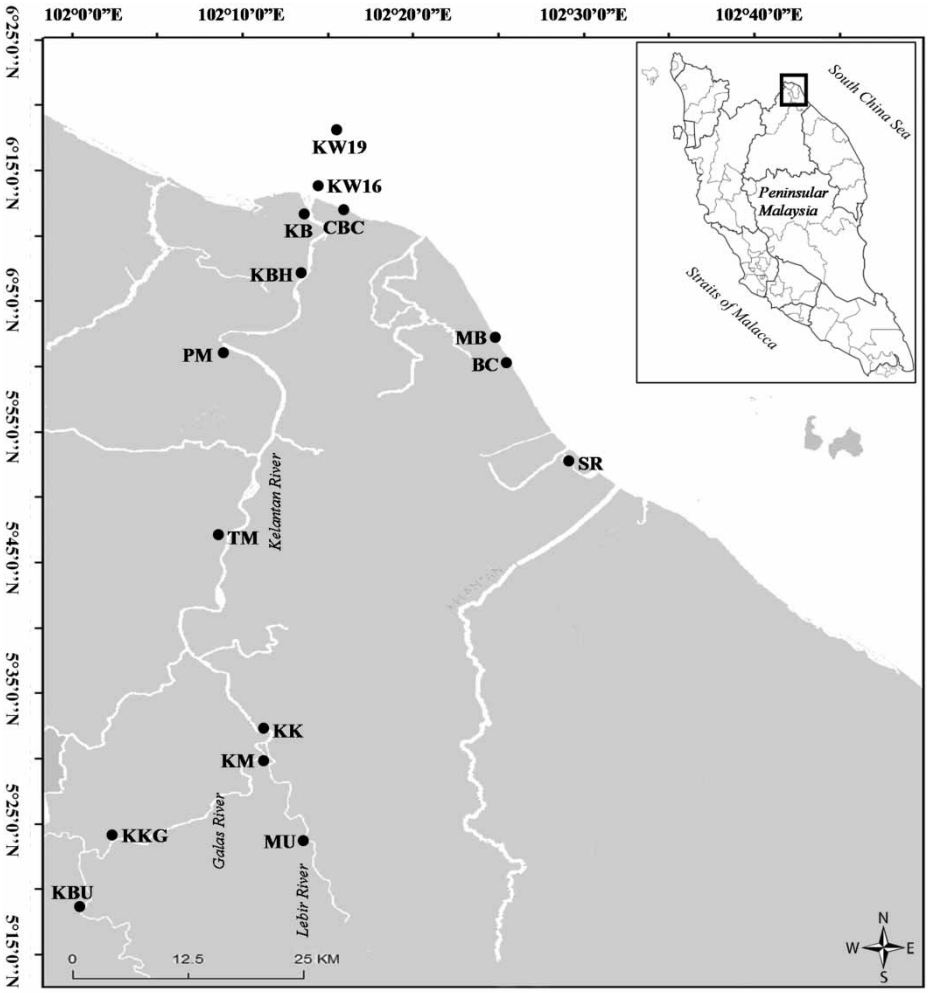


Figure 1 | Map showing sampling sites along the Kelantan River and its adjacent waters. KBU: Kampung Batu Udang; KKG: Kampung Kuala Gris; MU: Manik Urai; KM: Kampung Merbau; KK: Kampung Kuala Krai; TM: Tanah Merah; PM: Pasir Mas; KBH: Kota Bahru; KB: Kuala Besar; KW16: Offshore 1, KW19: Offshore 2; CBC: Cahaya Bulan Beach; MB: Melawi Beach; BC: Bachok Beach; SR: Semarak River.

Identification of *E. coli*

Polymerase chain reactions (PCR) for the detection of *phoA* gene (housekeeping gene) was carried out using primers *PhoA-F* (5′-GTCACAAAAGCCCGGACACCATAAATGCCT-3′) and *PhoA-R* (5′-TACACTGTCATTACGTTGCG-GATTTGGCGT-3′). This PCR amplifies a 903 bp fragment, which was used to confirm the presumptive *E. coli* (Yu & Thong 2009). Twenty-five microliters of reaction mixture consisting of with 1X Green GoTaq reaction buffer (pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, USA), 1 mM of $MgCl_2$, 140 μM of each deoxynucleotide triphosphate (dNTP), 0.1 μM of both primers and 5.0 μl of DNA. The PCR was carried out in a 2,720 thermal cycler (Applied Biosystems, Singapore) with the following conditions: initial denaturation at 94.0 °C for 2 min, 40 cycles at 92 °C for 0.5 min, 59 °C for 0.5 min, 72 °C for 0.5 min; and a final extension for 5 min at 72 °C. *E. coli* ATCC 25922 was used as the positive control and sterile water was included as the negative control.

Phylogenetic grouping of *E. coli*

Triplex PCR was employed to assess *E. coli* phylogroups (A, B1, B2, and D) (Table 1) (Clermont *et al.* 2000). Primer pairs used were ChuA.F (5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.R (5′-TGCCGCCAGTACCAAAGACA-3′); YjaA.F

Table 1 | Characterization of *E. coli* phylogroups based on presence or absence of *chuA, yjaA,* and TSPE4-C2

	Primer		
Phylogenetic group	***ChuA***	***YjaA***	**TspE4.C2**
A	−	+	−
	−	−	−
B1	−	−	+
	−	+	+
B2	+	+	+
	+	+	−
D	+	−	+
	+	−	−

(5′-TGAAGTGTCAGGAGACGCTG-3′) and YjaA.R (5′-ATGGAGAATGCGTTCCTCAAC-3′); and TspE4C2.F (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.R (5′-CGCGCCAACAAAGTATTACG-3′), which generated 279, 211, and 152 bp fragments, respectively. Characterization of *E. coli* was based on the existence and absence of *ChuA*, *YjaA*, and *TspE4.C2* (Table 1). Twenty microliters of reaction consisting of 1X Green GoTaq buffer (pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, USA), 120 μM dNTP, 1.5 mM of $MgCl_2$, 5 μL of DNA, 0.4 μM of *jyaA* primers and 0.24 μM of each *ChuA* and TSPE4-C2 primers was used for identification of phylogenetic grouping. The conditions for the PCR were initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min, and 72 °C for 0.5 min; final extension at 72 °C for 7 min.

Detection of *E. coli* virulence genes

All the validated *E. coli* were subjected to two multiplex PCR assays (M1 and M2) to detect for virulence genes linked to *E. coli* strains that cause intestinal disease (Chapman *et al.* 2006). Twenty-five microliters of reaction mixture consisting of 1× Green GoTaq buffer (pH 8.5), 0.5 U of *Taq* DNA polymerase (Promega, USA), 1.65 mM of $MgCl_2$, 220 μM dNTP, 0.24 μM of each primer, and 5 μl DNA was used for both PCR assays. Primer sequence and PCR amplification conditions were adopted from Gómez-Duarte *et al.* (2009). The primer sequences used for identification of virulence genes in this study are shown in Table 2 and PCR was carried out under the following conditions: initial denaturation at 94 °C for 2 min, 40 cycles at 92 °C for 0.5 mi, 59 °C for 0.5 min, and 72 °C for 0.5 min; and final extension at 72 °C for 5 min. Six positive controls (*E. coli* 2060-004, E2348/69, JM221, E9034A, C1845, and EC-12) were used and sterile water was used as the negative control.

Table 2 | Primer sequence for virulence gene detection in *E. coli* in PCR assay M1 and M2

	Primers	**Sequence**	**Expected band**	**Reference**
M1	VT.1	5′-GAGCGAAATAATTTATATGTG-3′	518 bp	Gómez-Duarte *et al.* (2009)
	VT.2	5′-TGATGATGGCAATTCAGTAT-3′		
	eae.1	5′-CTGAACGGCGATTACGCGAA-3′	917 bp	
	eae.2	5′-CGAGACGATACGATCCAG-3′		
	bfpA.1	5′-AATGGTGCTTGCGCTTGCTGC-3′	326 bp	
	bfpA.2	5′-GCCGCTTTATCCAACCTGGTA-3′		
	aggR.1	5′-GTATACACAAAAGAAGGAAGC-3′	254 bp	
	aggR.2	5′-ACAGAATCGTCAGCATCAGC-3′		
M2	LT.1	5′-GCACACGGAGCTCCTCAGTC-3′	218 bp	
	LT.2	5′-TCCTTCATCCTTTCAATGGCTTT-3′		
	ST.1	5′-GCTAAACCAGTAGAG(C) TCTTCAAAA-3′	147 bp	
	ST.2	5′-CCCGGTACAG(A) GCAGGATTACAACA-3′		
	daaE.1	5′-GAACGTTGGTTAATGTGGGGTAA-3′	542 bp	
	daaE.2	5′-TATTCACCGGTCGGTTATCAGT-3′		
	virF.1	5′-AGCTCAGGCAATGAAACTTTGAC-3′	618 bp	
	virF.2	5′-TGGGCTTGATATTCCGATAAGTC-3′		
	ipaH.1	5′-CTCGGCACGTTTTAATAGTCTGG-3′	933 bp	
	ipaH.2	5′-GTGGAGAGCTGAAGTTTCTCTGC-3′		

Statistical analysis

PAST software was employed to carry out statistical analysis (Hammer *et al.* 2001), and $p < 0.05$ or at 95% confidence interval were accepted as significant. Values were compared using variance and Tukey's test analysis, and relationships between variables measured were tested using correlation and linear regression analyses. Principal component analysis (PCA) was used to determine physicochemical variables, which affect the water quality of Kelantan River, estuaries, and its adjacent waters. Canonical-correlation analysis (CCA) was used to compare the categories of variables measured and phylogenetic groups, and the relationship between the categories is represented in a two-dimensional graph.

RESULTS

Water quality parameters

Surface water temperature was relatively invariant among the sites, ranging from 28.8 ± 2.0 °C to 31.0 ± 2.2 °C (coefficient of variation, $CV = 3\%$). Salinity was low (<1) in the Kelantan River and its tributaries, but increased in the estuaries (4.4 ± 4.9–11.9 ± 11.3) and its adjacent coastal sites (22.3 ± 8.9–30.5 ± 2.1). The pH ranged from 7.1 ± 0.5 to 8.2 ± 0.3 ($CV = 5\%$), and DO concentration was at healthy levels (>270 μM, $CV = 8\%$). TSS and POM concentrations fluctuated between 55 ± 28–300 ± 241 mg/L and 17 ± 18–52 ± 36 mg/L, respectively, and were higher in the river (>150 mg/L TSS and >30 mg/L POM) compared to the estuaries and coastal sites [significance was measured using Student's *t*-test with 104 degrees of freedom: $t(104) = 6.61$, $p < 0.001$; $t(104) = 5.71$, $p < 0.001$] (Table 3). Of the three nitrogen species measured, NO_3 was the dominant species and its average concentrations varied over a wide range from 0.03 to 0.61 mg/L ($CV = 37\%$), with higher concentrations detected in the river compared to the estuaries and coastal sites. A high NO_3 level was observed in Krai (0.61 mg/L). NH_4 concentrations were low among sites, and varied from 0.01 to 0.09 mg/L with the exception of Kota Bahru (0.18 mg/L); whereas NO_2 concentrations were generally low (<0.02 mg/L). For the other inorganic nutrients measured, SiO_4 fluctuated over a wider range between 1.09 and 24.24 mg/L. High SiO_4 levels were observed upstream with the highest concentration observed at Merbau. For PO_4, concentrations were low and varied from 0.01 to 0.03 mg/L (Figure 2).

Abundance of coliform and *E. coli*

Coliform and *E. coli* were found at all sampling sites (Figure 3(a)). The average coliform concentrations ranged from 1.0×10^3 to 3.3×10^4 CFU 100 mL^{-1}. However, the beaches of Melawi and Bachok, and the estuary at Semarak exhibited coliform concentrations that were one order of magnitude higher (2.01×10^5, 3.22×10^5 and 3.15×10^5, CFU 100 mL^{-1}, respectively). For *E. coli*, the average abundance ranged from 3.1×10^1 to 1.6×10^5 CFU 100 mL^{-1}. The highest abundance of *E. coli* was detected in river water at Kota Bahru, whereas for estuaries and coastal sites, the highest *E. coli* abundance was observed in the Semarak River.

Distribution of phylogenetic groups and pathotypes of *E. coli*

In this study, a total of 2,341 *E. coli* were isolated. In the Kelantan River and its adjacent coastal waters, all four phylogenetic groups were observed (Figure 3(b)). Phylogenetic group B1 ($n = 695$, 29.7%) was the most dominant, followed by groups A ($n = 688$, 29.4%), D ($n = 597$, 25.5%), and B2 ($n = 361$, 15.4%). The phylogenetic groups were not distributed homogeneously among the sites. Phylogenetic group A was more frequently isolated from rivers and estuaries compared to its adjacent coastal sites. Higher *E. coli* phylogenetic group A counts were observed upstream at Kampung Batu Udang ($n = 86$) followed by Manik Urai ($n = 62$). The counts decreased until Tanah Merah ($n = 28$), before increasing from the downstream site of Pasir Mas ($n = 70$) to the estuaries where the highest count observed was at Kuala Besar ($n = 129$). In contrast, phylogroup B1 was most abundant in Merbau ($n = 91$) and the counts decreased downstream before increasing at Kota Bahru to the estuary site KW16. In comparison, group B1 dominated in the coastal sites. For phylogenetic group B2 and D, which were the least abundant, the highest counts were

Table 3 | Water quality parameter measured in this study

River station	Location	Temperature (°C)	pH	Salinity (ppt)	DO (μM)	TSS (mg L^{-1})	POM (mg L^{-1})
Kampung Batu Udang (KBU)	5°17′48.4″N, 102°01′10.4″E	29.0 ± 1.7	7.2 ± 0.4	0.4 ± 0.8	320 ± 36	300 ± 241	52 ± 36
Kampung Kuala Gris (KKG)	5°23′28.0″N, 102°04′5.8″E	28.8 ± 2.0	7.2 ± 0.4	0.3 ± 0.6	300 ± 39	259 ± 234	41 ± 31
Manik Urai (MU)	5°23′16.6″N, 102°14′12″E	29.0 ± 1.7	7.2 ± 0.4	0.6 ± 1.1	308 ± 36	233 ± 214	37 ± 22
Kampung Merbau (KM)	5°29′29.6″N, 102°11′33″E	29.3 ± 2.2	7.2 ± 0.4	0.4 ± 0.8	291 ± 17	208 ± 325	32 ± 30
Kuala Krai (KK)	5°31′53″N, 102°11′47.7″E	28.8 ± 2.0	7.1 ± 0.5	0.3 ± 0.4	288 ± 32	286 ± 380	40 ± 36
Tanah Merah (TM)	5°46′38.46″N, 102°09′2.34″E	30.0 ± 2.5	7.3 ± 0.4	0.2 ± 0.4	318 ± 51	236 ± 178	39 ± 21
Pasir Mas (PM)	6°01′22.3″N, 102°09′14.1″E	31.0 ± 2.2	7.3 ± 0.5	0.5 ± 0.8	310 ± 33	249 ± 226	41 ± 28
Kota Bharu (KBH)	6°07′40.1″N, 102°14′2.2″E	30.1 ± 2.0	7.2 ± 0.3	0.7 ± 0.9	309 ± 30	157 ± 102	32 ± 15
Estuaries and Coastal							
Kuala Besar (KB)	6°12′20.7″N, 102°14′3.9″E	30.2 ± 1.7	7.5 ± 0.2	4.4 ± 4.9	309 ± 38	70 ± 37	19 ± 6
KW16	6°13′52.83″N, 102°14′19.27″E	29.7 ± 1.5	7.8 ± 0.6	11.9 ± 11.3	298 ± 34	92 ± 70	21 ± 10
KW19	6°18′45.42″N, 102°15′43.44″E	29.6 ± 1.4	8.2 ± 0.3	29.8 ± 3.5	278 ± 24	55 ± 28	17 ± 18
Cahaya Bulan Beach (CBC)	6°11′53.58″N, 102°16′14.80″E	31.0 ± 1.7	7.8 ± 0.2	28.5 ± 4.2	277 ± 47	101 ± 77	23 ± 14
Melawi Beach (MB)	6°01′17.2″N, 102°25′12.0″E	30.5 ± 2.1	7.9 ± 0.4	30.5 ± 2.1	271 ± 49	135 ± 99	29 ± 22
Bachok Beach (BB)	6°0′32.97″N, 102°25′35.75″E	30.3 ± 1.9	8.1 ± 0.2	30.3 ± 1.9	266 ± 46	121 ± 67	61 ± 143
Semarak (SR)	5°53′24.3″N, 102°28′49.5″E	30.8 ± 2.0	7.7 ± 0.4	22.3 ± 8.9	290 ± 65	88 ± 39	22 ± 9

observed at Kampung Batu Udang ($n = 70$ and $n = 103$, respectively) and the counts were lower at coastal waters compared to river waters. In this study, pathogenic strains (EHEC, EPEC, EAEC, ETEC, DAEC, or EIEC) were not detected. However, one *E. coli* isolate from Manik Urai carried the *eae* gene whilst another from Kampung Batu Udang possessed the *LT* gene.

DISCUSSION

A relatively stable temperature pattern typical of tropical water was observed for all sampling sites. Salinity varied greatly between river, estuaries and coastal water but were in the range previously reported for other riverine systems in Malaysia (You *et al.* 2016; Lim *et al.* 2018). High TSS is the pervasive water quality problem in Malaysia (Dow 1995). The high TSS concentration observed at the Kelantan River contained silt and clay, which can be attributed to the upstream deforestation activities, sand mining activities, agriculture, livestock husbandry, and dredging operations (Yen & Rohasliney 2013). This resulted in a turbid and brownish river water. The floating fine silt and detritus from the catchment area carried by rainwater during monsoon season (Prasanna & Ranjan 2010) also increased the TSS and affected the water quality in the Kelantan River Delta (Table 3). In this study we observed that TSS did not correlate with DO ($r^2 = 0.001$, $p > 0.10$), suggesting that the TSS in the Kelantan River had generally low organic content (<20%).

NO_3 concentration in this study was higher than NO_2 [$t(216) = 15.07$, $p < 0.001$] and NH_4 [$t(216) = 8.72$, $p < 0.001$], similar to that reported by Yen & Rohasliney (2013). The detectable concentrations were within the criterion (7 mg L^{-1}) set by the Malaysian Interim National Water Quality Standard (INWQS, Department of Environment 2019). Agriculture is the second largest sector and made up 24.6% or USD1.2 billion of the gross domestic product of Kelantan in 2016 (Department of Statistics Malaysia 2017). Over the last few decades, intensive land use together with technological development have led to large-scale planting of commercial crops (rubber, oil palm, tobacco) and other agriculture farming, which directly increased the use of fertilizers, manure, and soil in Kelantan (Samsurijan *et al.* 2018). Furthermore, anthropogenic activities associated with domestic sewage and waste effluents containing nitrogenous compounds, may also contribute to elevated

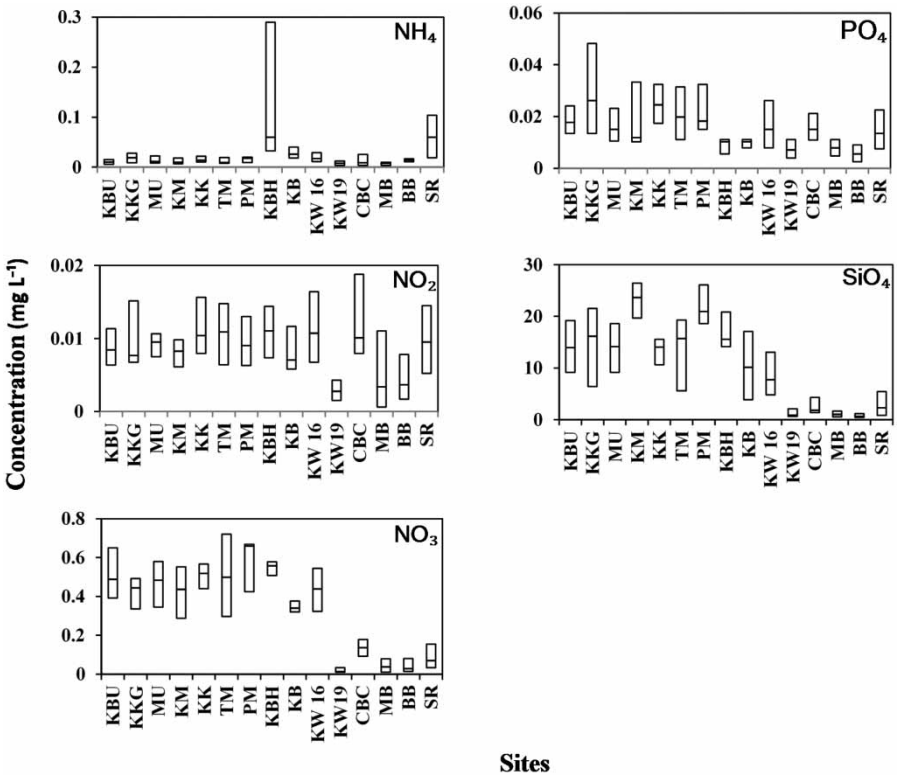
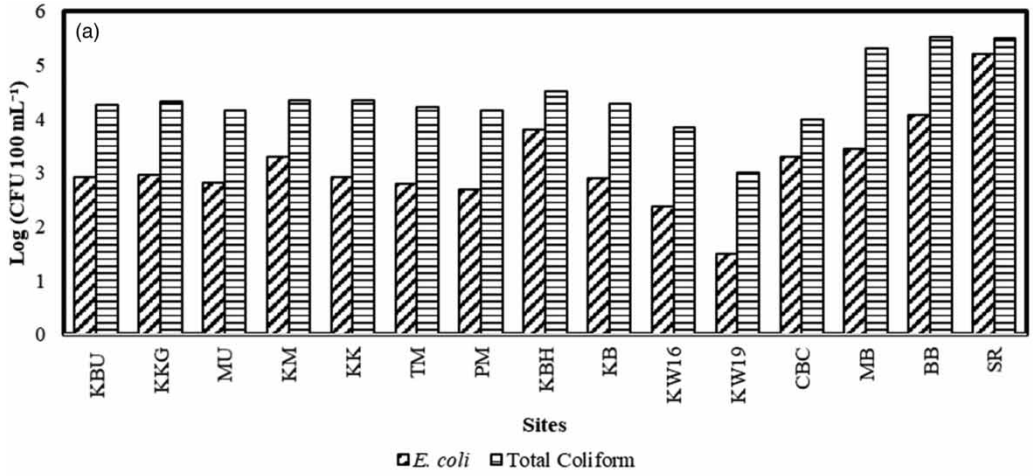


Figure 2 | Boxplot for dissolved inorganic nutrients for the Kelantan River and its adjacent waters.

levels of NO_3 in the river and its adjacent waters (Yen & Rohasliney 2013; Shamsuddin *et al.* 2016). In this study, the NH_4 concentration was lower than the NO_3 concentration indicating less impact of industrial effluents along the Kelantan River, estuaries and its adjacent coasts. For SiO_4, high concentrations were detected in the river (>13 mg L^{-1}) and the concentrations decreased in the estuaries–coastal waters sites. The main source of SiO_4 in the Kelantan River is the mining activities that begin after the convergence of the two tributaries (Galas River and Lebir River) and continues until the estuary delta of the Kelantan River, which severely impacts the transport and displacement of river sediments (Yen & Rohasliney 2013). Besides natural and chemical weathering of sedimentary soils and rocks, human activities are also sources of SiO_4 in the river (Shaari *et al.* 2015; Wang *et al.* 2017). The decrease in SiO_4 concentration may be attributed to dilution, lack of silica enrichment, utilization of silica by aquatic organisms (e.g. diatoms), and plants that grow along the river. In this study, the overall inorganic nutrients detected were in the range reported in tropical and sub-tropical waters (Lee & Bong 2006; Sakai *et al.* 2016).

Coliform and *E. coli* were highly prevalent in the Kelantan River surface waters, estuaries, and its adjacent coasts. The average abundance of *E. coli* detected at all sampling sites exceeded both the recommended *E. coli* allowable limit by NWQS of class II for rivers in Malaysia (100 CFU/100 ml) and Malaysia IMWQS (Department of Environment 2019) except KW19. This is indicative of fecal pollution, which is consistent with previous studies on the Kelantan River (Bamaiyi *et al.* 2015; Basri *et al.* 2015). Fecal pollution here is mainly due to the direct untreated sewage discharge from the houses and floating toilets built along the riverbanks. Furthermore, there is inadequate sewage treatment facilities in Kelantan where the use of individual septic tanks that connect to multi-points of sewage treatment plants (SPAN 2016) only partially treat the sewage before being discharged into the river (Sakai *et al.* 2016). This could subsequently cause river water quality deterioration. In this study, we found



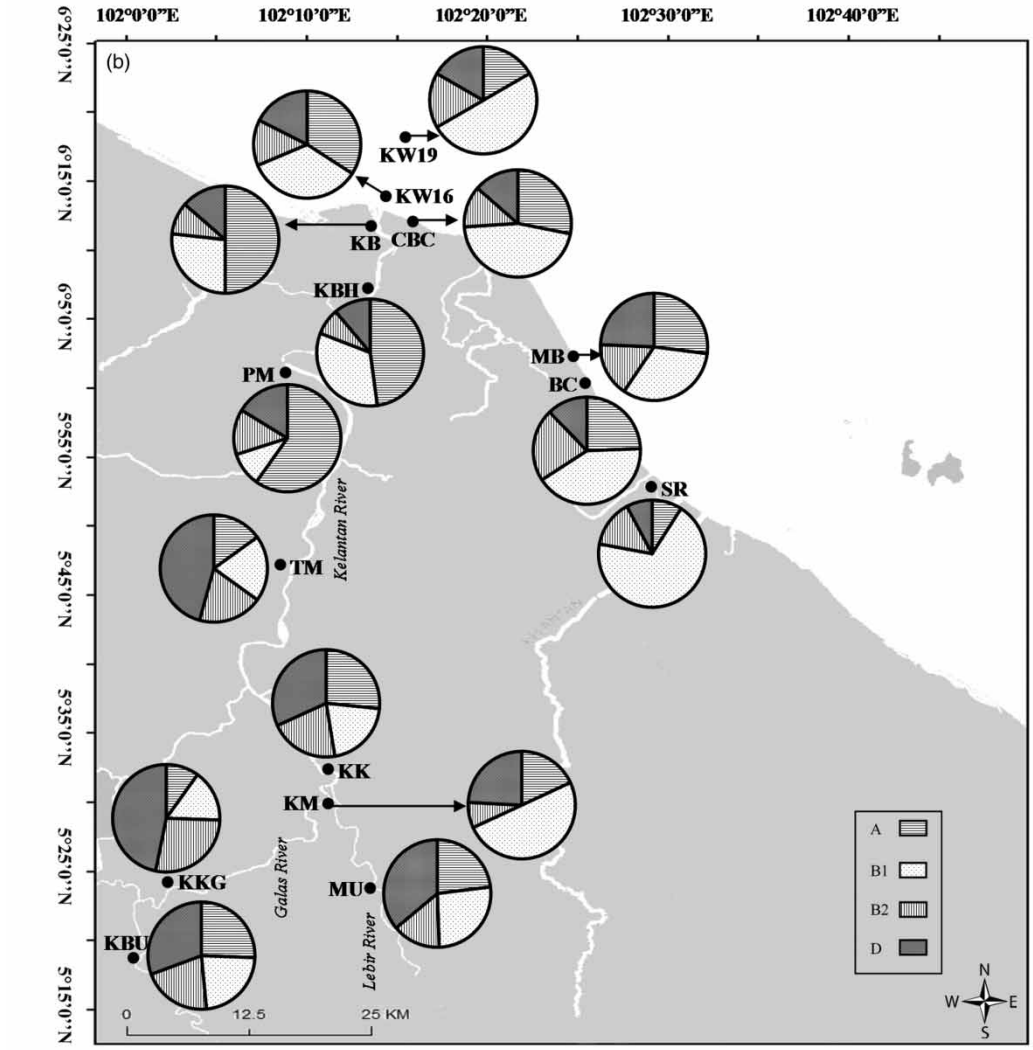


Figure 3 | (a) Average total coliform and *E. coli* [log (CFU 100 mL^{-1})] in the Kelantan River and its adjacent coastal waters and (b) the distribution of *E. coli* phylogenetic groups by sampling sites. A: phylogenetic group A; B1: phylogenetic group B1; B2: phylogenetic group B2; D: phylogenetic group D.

that the average abundance of *E. coli* in estuarine and coastal waters was one order of magnitude higher compared to the river waters. This may be caused by the untreated sewage released directly from the houses and toilets built near the beach and the coastal upwelling process, which provides a nutrient source and support for *E. coli*. Furthermore, *E. coli* can rapidly adapt to, and tolerate, different abiotic (availability of nutrients, pH, moisture, temperature, salinity) and biotic (grazing) stress factors (Van Elsas *et al.* 2011; Alves *et al.* 2014), and could further enhance their fitness in aquatic environments. Studies have shown the capability of *E. coli* to grow and proliferate in marine environments through alkaline pH adaptation (Hughes 2008) and or change in their genetic structure (Van Elsas *et al.* 2011). The prevalence of *E. coli* in coastal, estuaries, and river waters of Kelantan may pose health risks for the local residents who have direct or indirect contact with water through recreational activities or seafood consumption.

Our present study showed that *E. coli* group B1 was the most prevalent followed by A, D, and B2, of which groups B1 and A comprised 59% of the total *E. coli* in this study. Our findings are similar to previous studies that reported environmentally persistent *E. coli* are of groups B1 and A rather than virulent types B2 and D (Figueira *et al.* 2011; Ghaderpour *et al.* 2015). At upstream sites of the Kelantan River, higher abundances of D phylogroup strains were observed suggesting that fecal contamination was mainly from animal origin. Studies have shown that the *E. coli* population structure differs significantly between humans and animals (Carlos *et al.* 2010). It has been reported that livestock and poultry are the main reservoir for D phylogroup. Based on statistics from the Department of Veterinary Services Malaysia (2016), poultry farming, with a total of 1.84 million population, is the major livestock activity operating in Kelantan. Poultry could therefore be a major animal fecal pollution source in the river. The difference between phylogenetic groups among the sites in this study may be attributed to hydrological conditions, different sources of pollution, selective pressures in the waters, and land use (Lyautey *et al.* 2010; Van Elsas *et al.* 2011). Distinct survival rates, together with all these parameters, will structure the *E. coli* community distribution and diversity in aquatic environments (Berthe *et al.* 2013).

In this study, PCA implied that TSS and SiO_4 were the elements that influence the Kelantan River estuaries and its adjacent coastal water quality. PCA 1 and PCA 2 explained 72.10% and 19.75% of the total variance of the explanatory physical variables measured (Figure 4(a)). Our results showed that TSS correlated with *E. coli* abundance ($r^2 = 0.165$, $p < 0.001$). The positive correlation between *E. coli* and TSS suggested that *E. coli* was transported in the river bound to particulate matter. Research indicates that the attachment of *E. coli* to sediment organic matter with clay content can increase their survival in aquatic environments (Pachepsky & Shelton 2011; Liang *et al.* 2017). Suspended solids not only provide organic and inorganic nutrients but also provide protection against adverse factors (ultraviolet radiation, metal toxicity, grazing, attack by bacteriophage) (Medema *et al.* 2003). On the other hand, secretion of extracellular polymeric substances by microorganisms at the outer cell surface (Liao *et al.* 2015) for cell aggregation, adhesion, and protection is one of the survival strategies for cells to survive and adapt in hostile environments (Vu *et al.* 2009; Bruckner *et al.* 2011). TSS also correlated with rainfall ($r^2 = 0.342$, $p < 0.001$). This finding concurs with other studies that showed rainfall is the primary process affecting river volume and flow, which can directly increase the level of TSS through runoff (Shen & Julien 1993; Vaze & Chiew 2003). Our study therefore suggested that rainfall indirectly affects the distribution and abundance of *E. coli* in the Kelantan River, estuaries and its adjacent coasts.

In order to illuminate the factors influencing the *E. coli* phylogroups occurrence and distribution, a CCA was conducted (Figure 4(b)). Our CCA showed distinct differences in survival among strains belonging to different phylogenetic groups. Phylogenetic group A was greater in deteriorated water containing NH_4 and NO_2, whereas phylogenetic group D was greatest with NO_3. In contrast, *E. coli* phylogenetic group B2 seemed to thrive in waters with higher DO. Abundance of phylogenetic group B1 appeared to have better salinity tolerance compared to other phylogenetic groups. This explained why phylogenetic group B1 dominated at coastal sites, whereas phylogenetic group D dominated upstream of Kelantan River with higher concentration of NO_3. However, more research is needed to validate these findings.

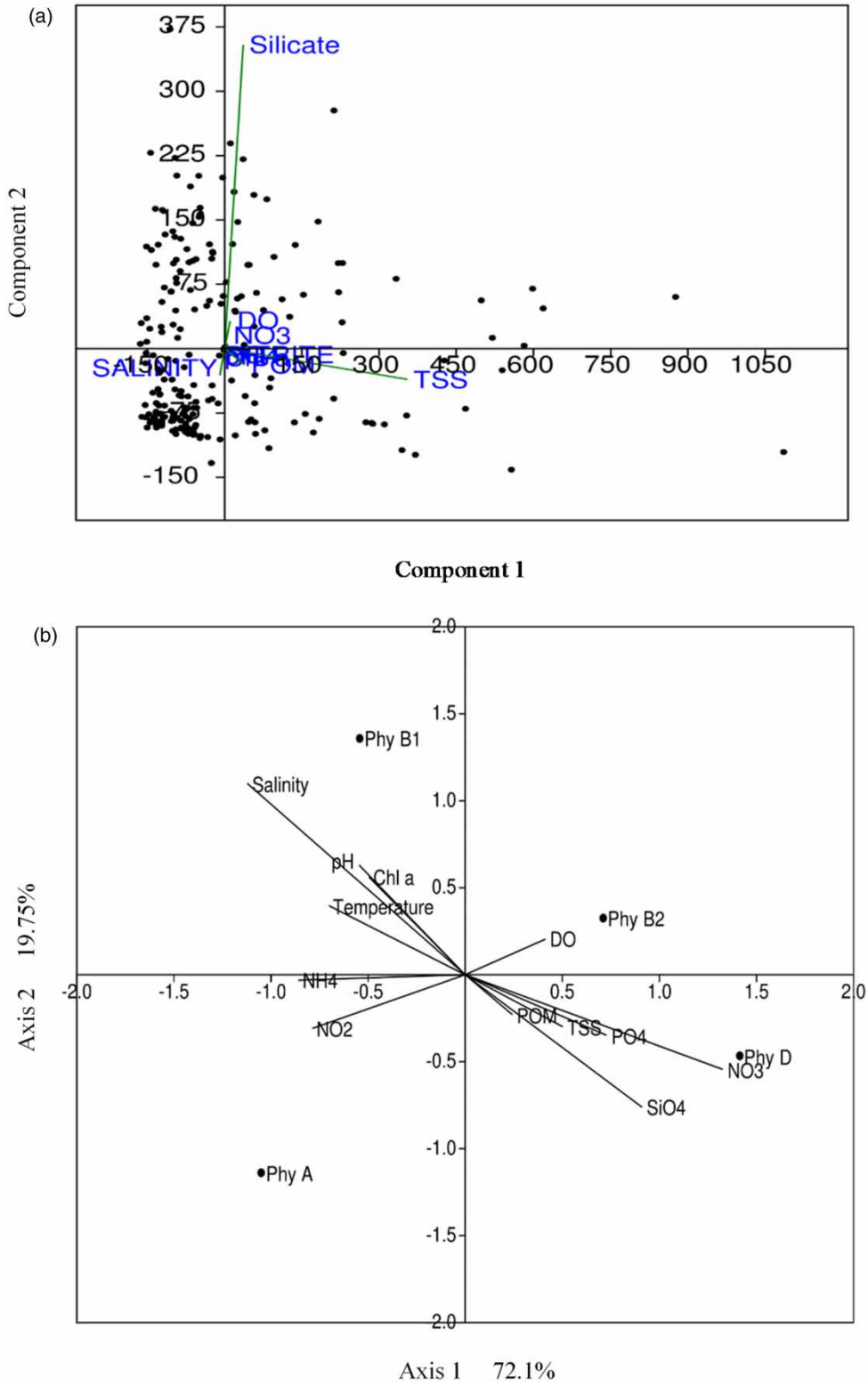


Figure 4 | (a) PCA ordination biplot showing the physicochemical variables affecting the water quality of the Kelantan River, estuaries and its adjacent waters and (b) CCA between *E. coli* phylogroups and physicochemical variables.

CONCLUSION

Our study of the Kelantan River, estuaries, and its adjacent coastal waters showed that NO_3 was the dominant nitrogen species and PCA analysis demonstrated that TSS and SiO_4 were the physicochemical factors that influence the water quality. The coliform and *E. coli* counts detected in this study exceeded INWQS and Malaysian Marine Water Quality Standards, suggesting the prevalence of fecal pollution. TSS was significantly correlated with *E. coli* abundance and rainfall. All four *E. coli* phylogroups were detected, but most were commensal groups B1 and A. Phylogenetic group D and B2 were the least abundant. CCA analysis demonstrated that phylogenetic group B2 seemed to thrive in water with higher DO. However, phylogenetic groups A and D were greater in deteriorated water containing NH_4, NO_2 and NO_3, whereas phylogenetic group B1 appeared to have better salinity tolerance among the phylogenetic groups.

ACKNOWLEDGEMENT

This work was supported by the Institution Centre of Excellence Phase II Fund, Ministry of Higher Education (grant number: IOES-2014D), University of Malaya Centre of Excellence (grant number: RU009D-2015 and TU001F-2018), and Bilateral Cooperation of Maritime Affairs of China (contract no: HC140502).

REFERENCES

Adnan, N. A. & Atkinson, P. M. 2011 Exploring the impact of climate and land use changes on streamflow trends in a monsoon catchment. *International Journal of Climatology* **31** (6), 815–831.

Ahmad, A. R., Zulkefli, M., Ahmed, M., Aminuddin, B. Y., Sharma, M. L. & Mohd Zain, M. 1996 Environmental impact of agricultural inorganic pollution on groundwater resources of the Kelantan Plain, Malaysia. In: *ACIAR Proceedings*. Australian Centre for International Agricultural Research, pp. 8–21.

Alves, M. S., Pereira, A., Araújo, S. M., Castro, B. B., Correia, A. & Henriques, I. 2014 Seawater is a reservoir of multi-resistant *Escherichia coli*, including strains hosting plasmid-mediated quinolones resistance and extended-spectrum beta-lactamases genes. *Frontiers in Microbiology* **5**, 426.

Bamaiyi, P. H., Sani, N. I. M., Mohamad, K., Hisham, N. E. B., Rostman, S. B. C., Kamir, N. F. M., Nor, M. N. M. & Khan, M. A. K. G. 2015 The 16S rRNA bacterial biodiversity of the flood water during massive flood session in Kota Bharu, December 2014. In: 1st Kelantan International Health Conference, 28–30 July 2015, Renaissance Hotel Kota Bahru.

Bamaiyi, P. H., Izreen, M. S. N., Mohamad, K., Eizzati, B. H. N., Siti-Bainum, C. R., Farhana, M. K. N., Nor, M. N. M., Kadkhodaei, S. & Khan, G. K. M. A. 2017 Isolation and polymerase chain reaction identification of bacteria from the 2014–2015 flood of Kota Bharu, Kelantan, Malaysia. *Asian Biomedicine* **10** (6), 549–565.

Basri, Z. D. M., Othman, Z. & Wahid, M. A. 2015 Quantification of pathogenic bacteria in floodwater in Malaysia. Fifth International Conference on Geotechnique, Construction Materials and Environment. 16–18 November, Osaka, Japan.

Berthe, T., Ratajczak, M., Clermont, O., Denamur, E. & Petit, F. 2013 Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and their survival in estuary water. *Applied and Environmental Microbiology* **79** (15), 4684–4693.

Bruckner, A. W., Faisal, M. & Alnazry, H. H. 2011 A paradigm shift for fisheries management to enhance recovery, resilience, and sustainability of coral reef ecosystems in the Red Sea. In: *Sustainable Fisheries: Multi-Level Approaches to a Global Problem*. (W. W. Taylor, A. J. Lynch & M. G. Schechter, eds). American Fisheries Society, Bethesda, MD, pp. 85–111.

Byamukama, D., Mach, R. L., Kansiime, F., Manafi, M. & Farnleitner, A. H. 2005 Discrimination efficacy of fecal pollution detection in different aquatic habitats of a high-altitude tropical country, using presumptive coliforms, *Escherichia coli*, and *Clostridium perfringens* spores. *Applied and Environmental Microbiology* **71** (1), 65–71.

Carlos, C., Pires, M. M., Stoppe, N. C., Hachich, E. M., Sato, M. I., Gomes, T. A., Amaral, L. A. & Ottoboni, L. M. 2010 *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC Microbiology* **10** (1), 161.

Chapman, T. A., Wu, X. Y., Barchia, I., Bettelheim, K. A., Driesen, S., Trott, D., Wilson, M. & Chin, J. J. C. 2006 Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. *Applied and Environmental Microbiology* **72** (7), 4782–4795.

Clermont, O., Bonacorsi, S. & Bingen, E. 2000 Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology* **66** (10), 4555–4558.

Department of Environment 2019 *Malaysia Marine Water Quality Standards and Index*. https://www.doe.gov.my/portalv1/en/standard-dan-indeks-kualiti-jabatan-alam-sekitar (accessed 22 August 2019).

Department of Statistic 2017 *GDP by State, 2010–2016*. https://www.dosm.gov.my/v1/index.php?r=column/cthemeByCat&cat=102&bul_id=VS9Gckp1UUpKQUFWS1JHUnJZS2xzdz09&menu_id=TE5CRUZCblh4ZTZMODZIbmk2aWRRQT09 (accessed 22 August 2019).

Department of Veterinary Services Malaysia 2016 *Bilangan Ternakan 2016* (Number of livestocks 2016). http://www.dvs.gov.my/dvs/resources/user_1/DVS%20pdf/perancangan/2018/Perangkaan%202016%202017/3.Muka_Surat_1-15_.pdf (accessed 23 August 2019).

Dow, K. M. 1995 *An Overview of Pollution Issues in the Strait of Malacca (No. 5-95)*. Maritime Institute of Malaysia.

Escobar-Páramo, P., Grenet, K., Le Menac'h, A., Rode, L., Salgado, E., Amorin, C., Gouriou, S., Picard, B., Rahimy, M. C., Andremont, A. & Denamur, E. 2004 Large-scale population structure of human commensal *Escherichia coli* isolates. *Applied and Environmental Microbiology* **70** (9), 5698–5700.

Figueira, V., Serra, E. & Manaia, C. M. 2011 Differential patterns of antimicrobial resistance in population subsets of *Escherichia coli* isolated from waste- and surface waters. *Science of the Total Environment* **409** (6), 1017–1023.

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D. & Cormican, M. 2010 Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Applied and Environmental Microbiology* **76** (14), 4772–4779.

Ghaderpour, A., Ho, W. S., Chew, L. L., Bong, C. W., Chong, V. C., Thong, K. L. & Chai, L. C. 2015 Diverse and abundant multi-drug resistant *E. coli* in Matang mangrove estuaries, Malaysia. *Frontiers in Microbiology* **6**, 977.

Gómez-Duarte, O. G., Bai, J. & Newell, E. 2009 Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacter* spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. *Diagnostic Microbiology and Infectious Disease* **63** (1), 1–9.

Grasshoff, K., Kremling, K. & Ehrhardt, M. 1999 *Methods of Seawater Analysis*. Wiley, New York, 600 pp.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T. A., Bekal, S., Fairbrother, J. M., Harel, J., Maynard, C., Masson, L. & Brousseau, R. 2006 A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in *Escherichia coli* isolates from Great Lakes recreational waters. *Applied and Environmental Microbiology* **72** (6), 4200–4206.

Hammer, Ø., Harper, D. A. & Ryan, P. D. 2001 PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4** (1), 9.

Hughes, B. S. 2008 Evolutionary patterns of *Escherichia coli* growth in seawater determined with a host to coast environmental laboratory analog. *Aquatic Microbial Ecology* **53** (3), 243–255.

Lee, C. W. & Bong, C. W. 2006 Carbon flux through bacteria in a eutrophic tropical environment: Port Klang waters. In: *The Environment in Asia Pacific Harbours* (E. Wolanski, ed.). Springer, Dordrecht, pp. 329–345.

Liang, X., Liao, C., Soupir, M. L., Jarboe, L. R., Thompson, M. L. & Dixon, P. M. 2017 *Escherichia coli* attachment to model particulates: the effects of bacterial cell characteristics and particulate properties. *PLoS ONE* **12** (9), e0184664.

Liao, C., Liang, X., Soupir, M. L. & Jarboe, L. R. 2015 Cellular, particle and environmental parameters influencing attachment in surface waters: a review. *Journal of Applied Microbiology* **119** (2), 315–330.

Lim, J. H., Lee, C. W., Bong, C. W., Affendi, Y. A., Hii, Y. S. & Kudo, I. 2018 Distributions of particulate and dissolved phosphorus in aquatic habitats of Peninsular Malaysia. *Marine Pollution Bulletin* **128**, 415–427.

Luna, G. M., Vignaroli, C., Rinaldi, C., Pusceddu, A., Nicoletti, L., Gabellini, M., Danovaro, M. & Biavasco, F. 2010 Extraintestinal *Escherichia coli* carrying virulence genes in coastal marine sediments. *Applied and Environmental Microbiology* **76** (17), 5659–5668.

Lyautey, E., Lu, Z., Lapen, D. R., Wilkes, G., Scott, A., Berkers, T., Edge, T. A. & Topp, E. 2010 Distribution and diversity of *Escherichia coli* populations in the South Nation River drainage basin, eastern Ontario, Canada. *Applied and Environmental Microbiology* **76** (5), 1486–1496.

Matic, I., Radman, M., Taddei, F., Picard, B., Doit, C., Bingen, E., Denamur, E. & Elion, J. 1997 Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* **277** (5333), 1833–1834.

Medema, G. J., Shaw, S., Waite, M., Snozzi, M., Morreau, A. & Grabow, W. 2003 Catchment characterisation and source water quality. In: *Assessing Microbial Safety of Drinking Water: Improving Approaches and Methods* (A. Dufour, M. Snozzi, W. Koster, J. Bartram, E. Ronchi & L. Fewtrell, eds). WHO OECD, London, pp. 111–158.

Method EPA 1604 Isolation and enumeration of coliform and *E. coli*. *Total Coliforms and Escherichia coli in Water by Membrane Filtration Using A Simultaneous Detection Technique (MI Medium)*, EPA821-R-02-024. Off. Water, US Environmental Protection Agency, Washington, DC.

Pachepsky, Y. A. & Shelton, D. R. 2011 *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. *Critical Reviews in Environmental Science and Technology* **41** (12), 1067–1110.

Parsons, T. R., Maita, Y. & Lalli, C. M. 1984 *A Manual of Biological and Chemical Methods for Seawater Analysis*. Pergamon Press, Oxford.

Pradhan, B. & Youssef, A. M. 2011 A 100-year maximum flood susceptibility mapping using integrated hydrological and hydrodynamic models: Kelantan River Corridor, Malaysia. *Journal of Flood Risk Management* **4** (3), 189–202.

Prasanna, M. B. & Ranjan, P. C. 2010 Physicochemical properties of water collected from Dhamra estuary. *International Journal of Environmental Sciences* **1** (3), 334–342.

Sakai, N., Sakai, M., Haron, D. E. M., Yoneda, M. & Mohd, M. A. 2016 Beta-agonist residues in cattle, chicken and swine livers at the wet market and the environmental impacts of wastewater from livestock farms in Selangor State, Malaysia. *Chemosphere* **165**, 183–190.

Samsurijan, M. S., Rahman, N. N. A., Ishak, M. I. S., Masron, T. A. & Kadir, O. 2018 Land use change in Kelantan: review of the environmental impact assessment (EIA) reports. *Geografia-Malaysian Journal of Society and Space* **14** (4), 322–331.

Sen, K., Berglund, T., Soares, M. A., Taheri, B., Ma, Y., Khalil, L., Lu, J. & Turner, R. J. 2019 Antibiotic resistance and pathogenicity of *E. coli* isolated from a constructed wetland dominated by a crow roost, with emphasis on ESBL and AmpC containing *E. coli*. *Frontiers in Microbiology* **10**, 1034.

Shaari, H., Mohd, M. S., Abdullah, N. A. & Bidai, J. 2015 Heavy metals monitoring in *Psammotaea elongata* from Semerak Lagoon, Kelantan, Malaysia. *Oriental Journal of Chemistry* **31** (2), 993–999.

Shamsuddin, A. S., Ismail, S. N. S., Abidin, E. Z., Bin, H. Y. & Juahir, H. 2016 Contamination of nitrate in groundwater and evaluation of health risk in Bachok, Kelantan: a cross-sectional study. *American Journal of Applied Sciences* **13** (1), 80–90.

Shen, H. W. & Julien, P. 1993 Chapter 12: erosion and sediment transport. In: *Handbook of Hydrology* (D. R. Maidment, ed.). McGraw-Hill, New York, NY.

SPAN 2016 Water and sewerage. In: *National Environmental Health Action Plan (NEHAP) Conference*. http://nehapmalaysia.moh.gov.my/wp-content/uploads/2016/03/Paper-1-Water-Sewerage.pdf (accessed 22 August 2019).

Van Elsas, J. D., Semenov, A. V., Costa, R. & Trevors, J. T. 2011 Survival of *Escherichia coli* in the environment: fundamental and public health aspects. *The ISME Journal* **5** (2), 173.

Vaze, J. & Chiew, F. H. 2003 Comparative evaluation of urban storm water quality models. *Water Resources Research* **39** (10), SWC5-10.

Vignesh, K. S., Figueroa, J. A. L., Porollo, A., Caruso, J. A. & Deepe Jr, G. S. 2013 Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity* **39** (4), 697–710.

Vu, B., Chen, M., Crawford, R. J. & Ivanova, E. P. 2009 Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules* **14** (7), 2535–2554.

Wang, A. J., Bong, C. W., Xu, Y. H., Hassan, M. H. A., Ye, X., Bakar, A. F. A., Li, Y. H., Lai, Z. K., Xu, J. & Loh, K. H. 2017 Assessment of heavy metal pollution in surficial sediments from a tropical river-estuary-shelf system: a case study of Kelantan River, Malaysia. *Marine Pollution Bulletin* **125** (1–2), 492–500.

Yen, T. P. & Rohasliney, H. 2013 Status of water quality subject to sand mining in the Kelantan River, Kelantan. *Tropical Life Sciences Research* **24** (1), 19.

You, K. G., Bong, C. W. & Lee, C. W. 2016 Antibiotic resistance and plasmid profiling of *Vibrio* spp. in tropical waters of Peninsular Malaysia. *Environmental Monitoring and Assessment* **188** (3), 171.

Yu, K. X. & Thong, K. L. 2009 Multiplex PCR for simultaneous detection of virulence genes in *Escherichia coli*. *Malaysian Journal of Science* **28** (1), 1–14.

First received 12 November 2019; accepted in revised form 21 January 2020. Available online 10 February 2020