Microbiological Quality of Selected Aquatic Environments in Langkawi Island

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ABSTRACT   Field samplings were conducted during the dry period in April 2004 on nine rivers in the Northeast part of Langkawi Island, Kedah, Malaysia. The microbiological quality of river water was determined by enumerating the indicator organisms such as total faecal coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) and pathogenic enteric bacteria such as *Salmonella* spp., *Vibrios* spp and *Shigella* spp. TC, FC and FS were detected in only 22%, 11% and 22% of the marine samples respectively. The mean concentrations of TC, FC and FS were 8.9×10^2, 3×10^1 and 4.2×10^3 cfu/100ml respectively. On the other hand, all the freshwater river samples had 100 % TC, FC and FS, with a mean concentration of 7.9×10^3, 4.9×10^3 and 4.4×10^3 cfu/100ml, respectively. *Salmonella* spp. were detected in most of the freshwater rivers while *Vibrios* spp. were predominantly found in estuarine marine rivers. Overall, the microbiological quality of the natural, marine waters of NE Langkawi is marginal as the bacterial counts for the total coliforms, faecal coliforms were below the maximum acceptable level but the fecal streptococci count was slightly higher than the permissible limit. This is especially so for the freshwater rivers. No correlation between the indicator organisms and pathogenic bacteria was determined.

ABSTRAK   Persampelan kerja lapangan telah dijalankan untuk 9 batang sungai di bahagian Timur Laut Pulau Langkawi, Kedah, Malaysia semasa musim kemarau pada 9 April 2004. Kualiti air sungai telah ditentukan melalui enumerasi organisma penunjuk seperti jumlah koliform tahi (total faecal coliforms, TC) koliform tahi (faecal coliforms, FC) dan streptococci, FS) telah dikesan hanya pada 22%, 11%, dan 22% daripada jumlah sample marin yang dikumpulkan. Kepekatan min untuk TC, FC, dan FS adalah masing – masing 8.9×10^2, 3×10^1 dan 4.2×10^3 cfu/100ml. Sebaliknya, semua sample air tawar mempunyai 100%, TC, FC, dan FS, dengan kepekatan min masing – masing 7.9×10^3, 4.9×10^3 and 4.4×10^3 cfu/100ml. *Salmonella* sp. telah dikesan dalam kebanyakkan sungai air tawar manakala, *Vibrio* sp. pula ditemui dalam sungai marin estuarin. Secara keseluruhan, kualiti mikrobiologi air marin semulajadi di NE Langkawi didapati sederhana oleh sebab TC, FC dan FS adalah pada di bawah paras nilai maksimum yang dibenarkan terutamanya untuk sungai air tawar. Tiada korelasi dapat ditentukan di antara organisma penunjuk dan bakteria patogenik.

(microbiological quality, aquatic, Pulau Langkawi)

INTRODUCTION

Increasing human population introduces a high percentage of untreated sewage into the sea or other aquatic environments and this form of pollution is probably responsible for the greatest number of human morbidities and mortalities [1]. In particular, waterborne infections caused by *Salmonella* spp., *Vibrios* spp. and *Shigella* spp., which are commonly transmitted by the faecal-oral route, pose a major public health hazard. They are commonly present in sewage effluents that can contaminate recreational waters. Waterborne diseases can be prevented by appropriate monitoring through regular inspection of sanitary and hygienic aspects for raw water sources, treatment facilities and distribution networks. However, it is practically impossible to test the water for each of the wide variety of pathogens that may be present. Hence, microbiological water quality is primarily based on tests for indicator organisms [2, 3].

Indicators most commonly used are of faecal or sewage origin such as the total coliforms, thermotolerant coliform bacteria and faecal streptococci. *Salmonella, Shigella* and *Vibrio* are some of the principal pathogenic microorganisms causing severe gastroenteritis in humans. It is commonly present in sewage effluent that can contaminate recreational waters. Water microbiological quality standards of recreational waters are based on coliform indicators as predictors of the presence of pathogenic microorganisms. The reliability of the indicator-based standards to predict the presence of pathogenic microorganisms such as *Salmonella* is still a matter of debate. There is a general agreement that *Salmonella* is present at high densities of indicator organisms. However, detection of *Salmonella, Shigella* and *Vibriso* in the absence of indicators of faecal pollution has also been reported. The objective of this short study was to determine the microbiological quality of selected rivers based on the presence/absence of the total coliforms, faecal coliforms, faecal streptococci, *Salmonella, Shigella spp.* and *Vibrio spp..*

MATERIALS AND METHODS

Sampling sites
Field samplings were conducted during the dry period on April 2004 in Northeast Langkawi (6° 20'S and 99° 50'E). Duplicate water samples were collected from 15 sites along Sg Kilim, Sg Ayer Hangat, Sg Kisap, Sg Batu Asah, Sg Korok, Sg Ulu Melaka, S. Telaga Tujuh, Sg Temurun and well water on Langgun Island. The stations were selected on the basis of site accessibility, existing use as potable drinking water, pristine or marginally disturbed condition and located at hill streams and the lower reaches. The sites were identified by using a Geographical Positioning System-Personal Navigation Garmin GPS 12XL (Table 1).

Sampling procedures and physical parameters
Mineral water bottles (1 L) were used to collect the water samples. The bottles were washed by rinsing with the water samples and subsurface samples were taken. The samples were kept in the dark in ice during transport and all the samples were examined within 6 hours of collection. Water parameters such as temperature, pH, salinity and dissolved oxygen were measured (HACH DR/2000 Direct Spectrophometer) at sites.

Isolation and detection of total coliforms, faecal coliforms and faecal streptococci
100 ml of water sample was filtered through a 0.45 µm-pore size membrane filter and then placed on m-Endo agar for the detection of total coliforms. All organisms that produce red colonies with metallic sheen within 24 hours of incubations were considered members of the coliform group. Representative colonies were then selected for verification by a two-step process. The first step, called the presumptive test, was an enrichment procedure in Lauryl Tryptose broth (LTB). LTB is designed to promote a rich growth and copious gas production from coliforms while aerobic sporing bacteria are inhibited. The second step, the confirmed test involved the transfer of the inoculum from the positive LTB to tubes of Brilliant Green Bile Broth (BGBB) followed by incubation at 35°C for 24-48 hrs. All BGBB tubes that produced a positive (gas) response within 48 hrs were considered to be of coliform origin. The enumeration of faecal coliform and *Escherichia coli* was carried out on chromogenic coliform agar (CCA). CCA allows rapid detection of coliforms but inhibits other Gram positive and gram-negative flora. The verification step was as described earlier but EC medium was used instead of BGBB. Faecal streptococci (FS) were enumerated on membrane enterococcus agar (MEA). MEA contains sodium azide as the selective agent to suppress the growth of gram-negative organisms. All presumptive FS are indicated by maroon, light and dark red colonies. The presumptive FS was further confirmed by subculturing to bile asesculin agar (BAA) and incubated at 44°C for 16-2 hrs.

Isolation of pathogenic bacteria
Salmonella spp., *Vibrios* spp. and *Shigella* spp. were also determined by the membrane filtration techniques followed by plating on selective media and standard biochemical tests. For *Salmonella* isolation, buffered peptone water was used as pre-enrichment broth to recover any injured *Salmonella* spp. The broth was then transferred to Rappaport- Vassiliadis (RV) broth to encourage the growth of Salmonellae. Presumptive Salmonellae were determined by using Xylose-Lysine Deoxycholate (XLD) and *Salmonella–Shigella* agar (SSA). For *Shigella* spp., after the initial enrichment step in BPW, the cultures were plated directly onto XLD and SSA.

For *Vibrios* spp., the membrane filters were pre-enriched in alkaline peptone water and then streaked directly onto TCBS and CHROMagar Vibrio agar (Paris, France).

All the presumptive pathogenic bacteria identified based on typical colony morphology and biochemical reactions on selective or differential media were further characterized and identified using the API 20E system (BioMerieux, Paris, France).

RESULTS AND DISCUSSION

Duplicate water samples were collected from nine different rivers in the North Eastern part of the Langkawi Island during the study period in April 2004. The three main rivers, Sg Air Hangat, Sg Kisap and Sg Kilim form part of the estuarine ecosystem and are well connected and continuous with each other and have direct influence from the sea. The pH of these estuarine rivers ranged from 6.88 to 8.08, salinity ranged from 32 to 36 ppm while temperatures ranged from 29.7 to 30.8° C (Table 1). For the freshwater rivers (Sg Batu Asah, Sg Korok, Sg Ulu Melaka, Sg Telaga Tujuh and Sg Temurun, the water pH ranged from 6.50 to 6.98 while the temperature ranged from 26.6 to 28.8 °C. The dissolved oxygen was more variable, ranging from 3.1 mgL^{-1} in Sg Kilim to 7.5 mgL^{-1} in Sg Telaga Tujuh (Table 1). Total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) were detected in only 22%, 11% and 22% of the marine samples, respectively (Table 1). The mean concentrations of TC, FC and FS were 8.9 $X10^2$, 3 $X10^1$ and 4.2 $X10^3$ cfu/100ml respectively (Table 1). On the other hand, all the samples from the freshwater sites had 100 % TC, FC and FS, with a mean concentration of 7.9 $X10^3$, 4.9 $X10^3$ and 4.4 $X10^3$ cfu/100ml, respectively. The maximum allowable limits for no risk in rivers are 1 $X10^4$ cfu/100ml, 2 $X10^3$ cfu/100ml and 5 cfu/100ml for TC, FC and FS respectively. All the freshwater rivers passed through human habitations. Hence, it is not surprising that a higher percentage of indicator organisms and pathogenic bacteria were detected in these freshwater rivers.

Salmonella was detected in one marine site (no.7) along Sg Kisap and in all the freshwater sites except Sg. Temurun. *Vibrio* spp was more abundant in the marine environment (8/9 samples or 89%) while it was detected in only two freshwater sites (Sg Ulu Melaka and Sg Korok).

As saltwater organisms, *Vibrio* bacteria are resistant to alkaline environments but are acid sensitive, *Vibrio* spp. die rapidly in solutions below pH 6. Some of the other identified bacterial pathogens include *Salmonella arizonae*, *Salmonella* spp., *Aerohydrophila gr*, *Vibrio arabinon*, *Vibrio fluvialis*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio cholerae* and *Citrobacter youngae*. No *Shigella* spp. was detected.

Spearmen correlation coefficient was applied to determine the association of indicator organisms with enteric pathogens. However, due to the small sample size, no significant correlation was determined. Moreover, the duration of the study was too short for meaningful conclusions to be made whether there is any correlation between the indicators and pathogenic bacteria. Only four sites (no. 10, 11, 13, 14) had positive results for both the indicators and *Salmonella* spp. In another two sites (no. 12, 15), indicators were present in the absence of *Salmonella* spp., while at another site at Sg Kisap, *Salmonella* spp. was detected in the absence of all three indicator organisms. Hence, the reliability of the indicator organisms to predict the presence of pathogenic microbes such as *Salmonella* has been questionable [4]. WHO (2004) recommends the use of *Escherichia coli* (or, alternatively, thermotolerant coliforms) as an index for these enteric pathogens in drinking water [5]. The occurrence of pathogens and indicator organism in the environmental waters depends on a number of factors, including intrinsic physical and chemical characteristics of the area, the magnitude and range of human activities and animal sources that release pathogens to the environment. Salmonellae have been isolated in higher numbers from the bottom sediments than from the overlying water [6].

Most of the freshwater rivers had a higher bacterial count for indicator organisms, *Salmonella* spp. and *Vibrios* spp. as compared to that found in the estuarine rivers. The maximum acceptable concentrations for bacteriological quality of public drinking water systems are no coliform detected per 100 ml.

These rivers meander through the housing areas and human activities are certainly a contributory factor. Potential pathogen sources include municipal sewerage, and contaminated runoff

from agricultural areas and areas with sanitation through latrines [5].

Overall, the microbiological quality of the natural, marine waters of NE Langkawi is marginal as the bacterial counts for the total coliforms, faecal coliforms were below the maximum acceptable level but the faecal streptococci count was slightly higher than the permissible limit. This is especially so for the freshwater rivers. No correlation between the indicator organisms and pathogenic bacteria was determined. However, with the increased interest in ecotourism to our pristine Malaysian habitats, there is a higher risk of contamination of our aquatic environment by pathogenic microorganisms and appropriate measures should be considered to reduce the anthropogenic source.

REFERENCES

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APPENDIX

Table 1. Physico-chemical properties and microbiological indicators and bacterial pathogens of selected rivers in NorthEast Langkawi Island, Kedah

	Site	Location	pH	Salinity (ppt)	Temp (°C)	Dissolved Oxygen (DO-mg/L)	Total Coliforms (TC)	Faecal Coliforms (FC)	Faecal Strept (FS)	Salmonella	Vibrio	Shigella
1	Sg. Kilim	N 33°59'59" E 68°54'22"	7.44	35	29.7	4.3	-	-	-	-	+	-
2	Sg. Kilim	N 06°25'56" E 99°50'20"	6.90	35	28.7	3.1	+	-	-	-	+	-
3	Sg. Hangat Ayer	N 06°25'49" E 99°49'51"	6.88	35	29.8	3.4	-	+	-	-	+	-
4	Sg. Hangat Ayer	N 06°27'21" E 99°49'55"	7.48	35	30.2	4.6	-	-	-	-	+	-
5	Sg. Hangat Ayer	N 06°25'04" E 99°52'30"	8.03	36	30.5	6.3	-	-	-	-	+	-
6	Sg. Kilim	N 06°23'46" E 99°51'39"	7.29	34	29.7	4.1	-	-	+	-	+	-
7	Sg. Kisap	N 06°23'11" E 99°53'43"	7.45	34	30.4	5.8	-	-	-	+	-	-
8	Sg. Kisap	N 06°23'13" E 99°53'43"	7.28	32	30.4	5.2	+	-	+	-	+	-
9	Sg. Kisap	N 06°25'56" E 99°51'09"	8.08	34	30.8	7.0	-	-	-	-	+	-
10	Well from Langgun Island	-	6.80	2.0	26.6	6.0	+	+	+	+	-	-
11	Sg. Batu Asah	N 06°20'16.4" E 99°48'43.6"	6.98	2.0	27.3	5.0	+	+	+	+	-	-
12	Sg. Korok	N 06°20'46.8" E 99°47'23.1"	6.50	1.0	27.5	6.7	+	+	+	-	+	-
13	Sg. Ulu Melaka	N 06°21'46.0" E 99°47'23.1"	6.50	0.5	26.8	5.3	+	+	+	+	+	-
14	Sg. Telaga Tujuh	N 06°21'46.5" E 99°47'23.0"	6.63	0.5	28.8	7.5	+	+	+	+	-	-
15	Sg. Temurun	N 06°21'46.5" E 99°47'23.1"	6.56	0	-	5.6	+	+	+	-	-	-