Title of research project,

Metagenomic profiling of Bacterial Community and their antimicrobial resistance in anthropogenic impacted Selangor River, Malaysia

Names of project members (including affiliation),

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Purposes

1. To analyse the bacteria community structure, the distribution of potential waterborne bacteria pathogens, their antibiotic resistance genes and virulence factors in Selangor River
2. To determine the impact of anthropogenic activities and antibiotic pollution on the potential waterborne bacteria pathogens and their antibiotic resistance genes in Selangor River

Methods

Sample collection

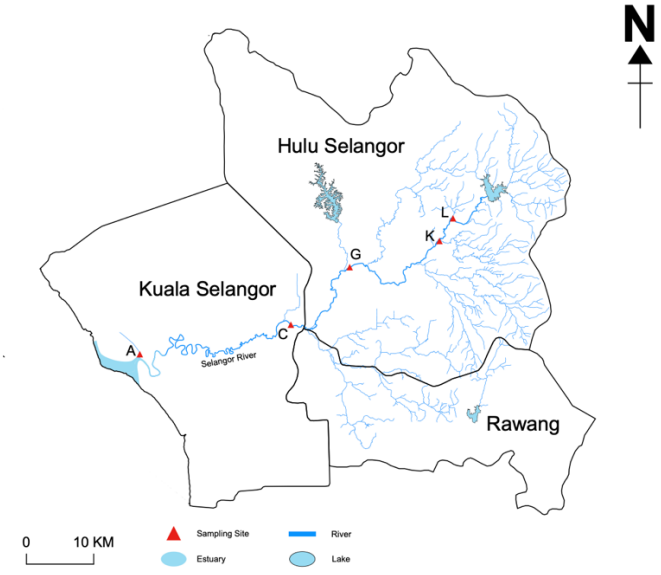

Figure 1. Map showing the five sampling sites along the Selangor River Basin.

A total of 12 water samples were collected from upstream (Site L), middle stream (Site C, G and K) and downstream (Site A) of Selangor River Basin in Peninsular Malaysia (Figure 1). The map above shows all the stations in the study whereas the table below shows the month and year of sampling (Table 1).

Table 1. Details of the samples collected along the Selangor River Basin.

Sample name	Site	Month	Year
A0120	A	January	2020
C0120	C	January	2020
A0722	A	July	2022
C0722	C	July	2022
G0722	G	July	2022
K0722	K	July	2022
A1122	A	November	2022
G0923	G	September	2023
K0923	K	September	2023
A1023	A	October	2023
C1023	C	October	2023
L1023	L	October	2023

In situ physicochemical parameters [temperature, salinity, pH, and dissolved oxygen (DO)], dissolved inorganic nutrients [nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), and silicate (SiO_4)], chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solid (TSS) were measured.

Quantification of selected antibiotic

Water samples collected were kept frozen, and transported to Center for Marine Environmental Studies (CMES), Ehime University for antibiotics analyses. The concentration of selected antibiotics from six different classes (Macrolides, fluoroquinolones, tetracyclines, sulfonamides, Trimethoprim, beta lactam) were measured using HPLC and LC-Ms/Ms in CMES.

Bacterial community profile

A volume of 200-500 mL of water samples were filtered on 0.2µm pore size Isopore membrane, depending on the water turbidity. Bacterial DNA extractions were carried out using DNeasy PowerWater kit and checked for concentration using Qubit 3.0 fluorometer. DNA samples were sent for whole genome metagenomic analysis via high throughput sequencing (Illumina Novaseq), with library preparation. Raw data were screened and trimmed for quality reads using fastp software (Chen, 2023). The paired reads were combined to form contigs for classification using kraken software (version 1.1.1).

Quantification of antibiotic resistance genes in the metagenomic reads

The processed metagenomic reads were also screened for antibiotic resistance genes (ARGs) using ARGs-OAP tools with the Comprehensive Antibiotic Resistance Database (CARD) database. The quantification of antibiotic resistance genes was carried out using the normalized reads.

Results/Activities

A total of eight antibiotics (Trimethoprim, Lincomycin, Clindamycin, Clarithromycin, Ciprofloxacin, Fluconazole, Metronidazole and Florfenicol) ranging from 0.14–5 ng/L were detected from the 12 samples.

On the other hand, raw data from the 12 metagenomic sequencings were successfully received. From the raw data, there were 0.85–1.26 × 10^8 trimmed reads. Among the reads, approximately 81.27–88.90% were classified as bacteria. The heatmap (Figure 2) below shows 49 major bacteria Family taxa classified, which contributed ≥1% of the whole bacterial community in at least one of the samples. The minor bacteria taxa that accounted for <1% of the bacterial community, contributed 7.12–24.32% among the samples. On the other hand, the unclassified bacteria family taxa were ranging from 11.1% to 19.5% among the samples. The most dominant bacteria found among the 12 samples belongs to *Comamonadaceae*, which contributed an average of 23.28±14.64% of the bacteria community, followed by *Sphaerotilaceae* (3.07±4.73%) and *Burkholderiaceae* (9.02±4.03%).

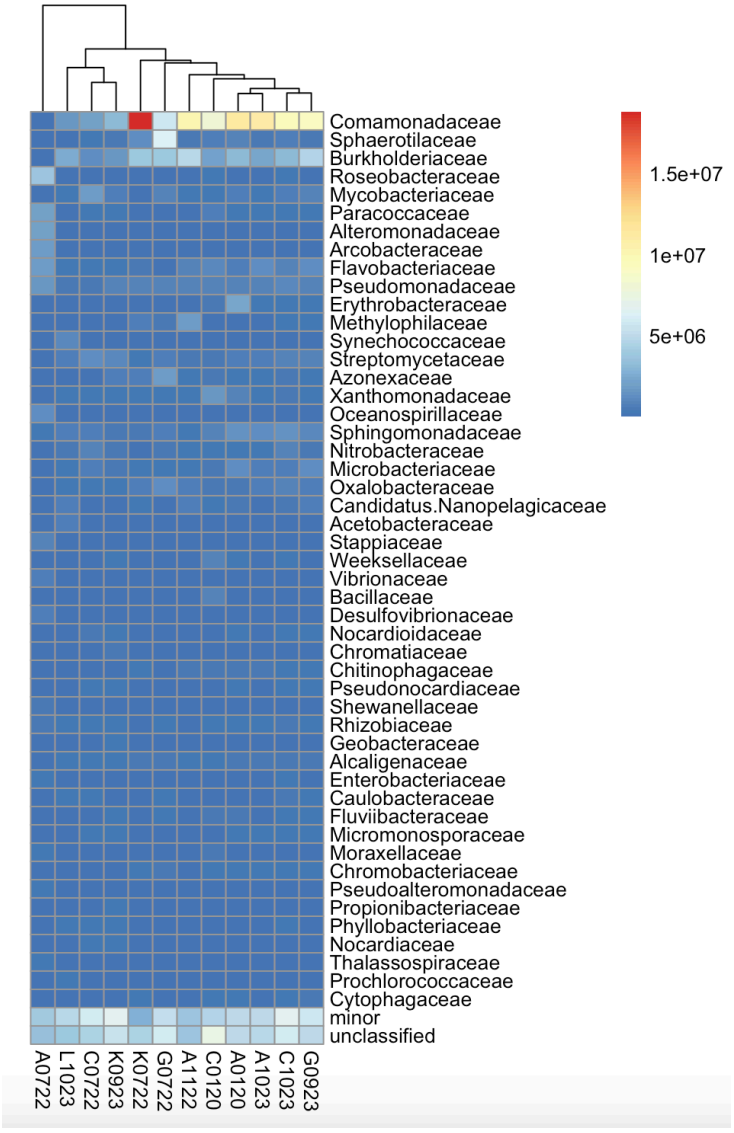

Figure 2. Heatmap showing the distribution of major bacterial taxa (≥1%) detected among the whole bacterial community, shown in number of reads.

Figure 3 is a heatmap showing the ARGs (normalized to reads per 16S rRNA) distribution among the 12 samples. All the counts represent ARG copies per 16S rRNA gene copies. There are 27 types of ARGs detected among the samples, among which the ARGs against bacitracin (0.08±0.04), polymyxin (0.02±0.01), sulfonamide (0.02±0.02) and multidrug (0.03±0.01) were frequently detected. Notably, the ARGs distribution at downstream site A in the wet season (A0120, A1122 and A1023) may be different from those detected in the dry month, July 2022 (A0722).

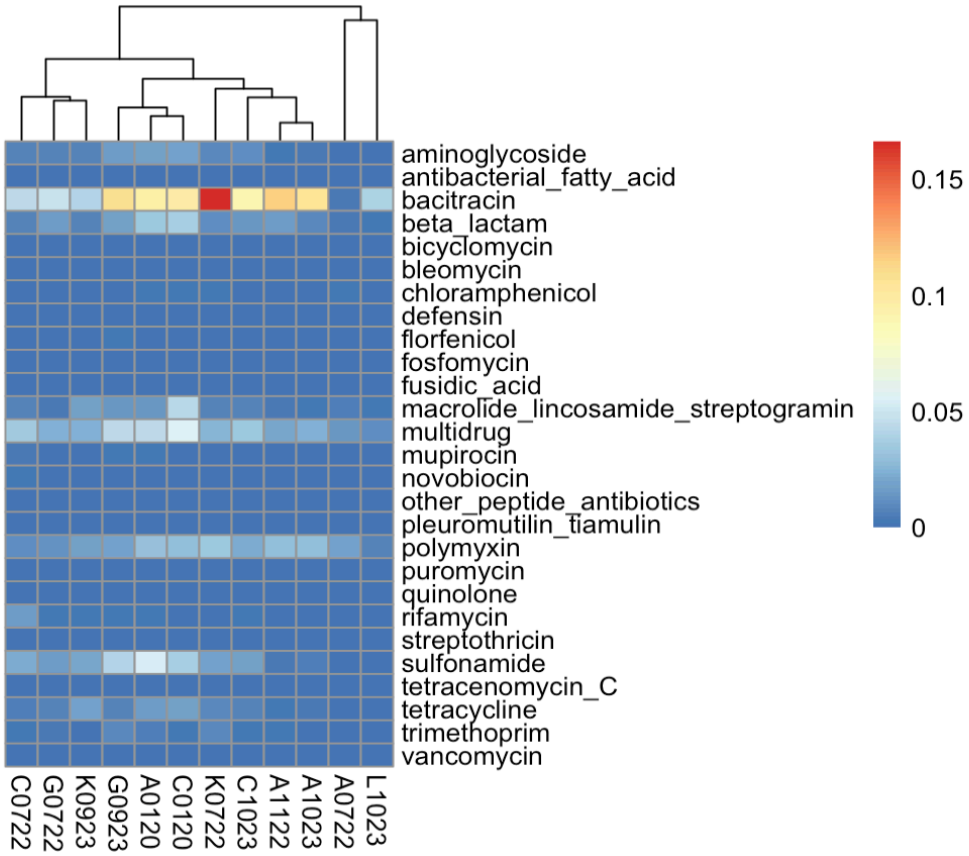

Figure 3. Heatmap showing the ARGs type (normalized to 16S reads) detected.

Currently, analyses using bioinformatic tools for the bacterial community profile and antibiotic resistance genes are ongoing for the contigs.


N
Hulu Selangor
Kuala Selangor
Rawang
A
C
G
K
L
Selangor River
0
10 KM
Sampling Site
River
Estuary
Lake