

Title of research project,

マレーシア・セランゴール川に沿った薬剤耐性菌のプロファイリング
Antimicrobial resistance profiling along Selangor River, Malaysia

Names of project members (including affiliation),

Associate Professor Dr Lee Choon Weng (Universiti Malaya)

Associate Professor Dr Bong Chui Wei (Universiti Malaya)

Wong Yi You (Universiti Malaya)

Professor Dr Kozo Watanabe (Ehime University)

Professor Dr Tatsuya Kunisue (Ehime University)

Assistant Professor Dr Rumi Tanoue (Ehime University)

Purposes

1. To quantify levels of selected antibiotic residues and antibiotic resistance genes in the Selangor River Basin
2. To determine the impact of anthropogenic and antibiotic pollution on bacterial assemblage and antibiotic resistance

Methods*Quantification of selected antibiotic*

Water samples were collected from upstream, middle stream and downstream of Selangor River Basin in Peninsular Malaysia. *In situ* physicochemical parameters [temperature, salinity, pH, and dissolved oxygen (DO)], dissolved inorganic nutrients [nitrate (NO₃), nitrite (NO₂), ammonium (NH₄), phosphate (PO₄), and silicate (SiO₄)], chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solid (TSS) were measured. Water samples collected were kept frozen, and to be transported to Center for Marine Environmental Studies (CMES), Ehime University. CMES. The concentration of the selected antibiotics (Macrolides, fluoroquinolones, tetracyclines, sulfonamides, Trimethoprim, beta lactam) will be measured using the HPLC and LC-MS/MS in CMES.

Bacterial community profile

Water samples were filtered on 0.2µm pore size Isopore membrane. We are now optimizing the bacterial DNA extraction from these filters. After optimization, we will complete the DNA extraction and before sending it out for sequencing. DNA samples will be sent for both metagenomic and 16S metabarcoding analysis via high throughput sequencing (e.g., Illumina Miseq)..

Quantification of antibiotic resistance genes in the metagenomic reads

Quantification of antibiotic resistance genes in the metagenomic reads will be carried out with the Comprehensive Antibiotic Resistance Database (CARD) database.

Activities

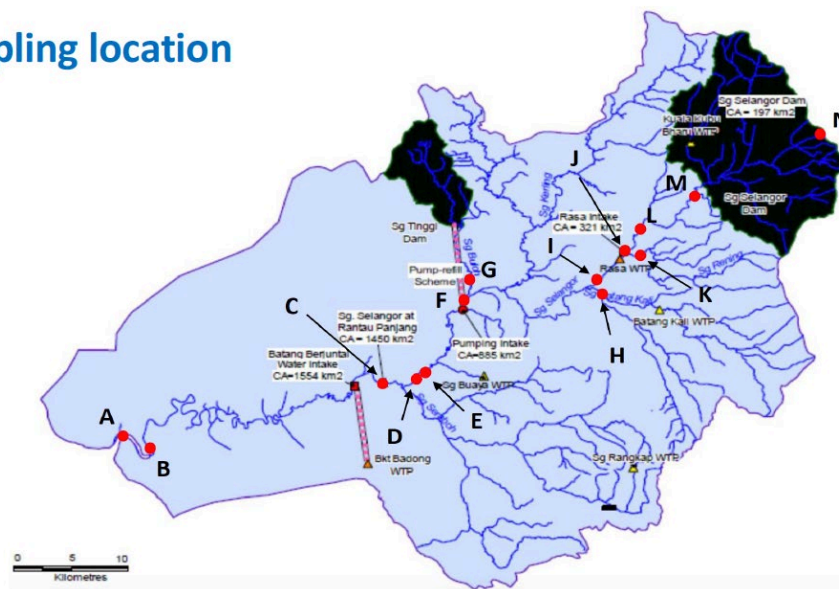
Online meetings via Zoom platform

Date	Attendees
18 May 2022 (2.30 pm)	Prof Kozo Watanabe, Dr Lee Choon Weng, Dr Bong Chui Wei
01 July 2022 (2.00 pm)	Prof Kozo Watanabe, Prof Tatsuya Kunisue, Dr Rumi Tanoue, Dr Lee Choon Weng, Dr Bong Chui Wei, Kyle Yang
13 Jan 2023 (9.00 am)	Prof Kozo Watanabe, Dr Lee Choon Weng, Dr Bong Chui Wei, Kyle Yang

Throughout the meetings to discuss work to be carried out, we have managed to sign and complete the Collaborative Research Agreement between Ehime University and Universiti Malaya completed. However before we could send samples over to Ehime University, we are now in the process of signing a Material Transfer Agreement between Ehime University and Universiti Malaya.

Sample collection

Sampling location



Sampling was carried out at selected stations along the Selangor River transect. The map above shows all the stations in the study whereas the table below shows the month and year of sampling.

Sampling location	Nov	Jan	Mar	Apr	Jul	Aug	Sept
A	1 (2019) 1 (2021) 1 (2022)	1 (2020) 1 (2022)	1 (2021) 1 (2022)	1 (2021) 1 (2022)	1 (2022)	1 (2020) 1 (2022)	1 (2020) 1 (2022)
C	1 (2019) 1 (2021) 1 (2022)	1 (2020) 1 (2022)	1 (2021) 1 (2022)	1 (2021) 1 (2022)	1 (2022)	1 (2020) 1 (2022)	1 (2020) 1 (2022)
G	1 (2021) 1 (2022)	1 (2022)	1 (2021) 1 (2022)	1 (2021) 1 (2022)	1 (2022)	1 (2022)	1 (2020) 1 (2022)
K	1 (2021) 1 (2022)	1 (2022)	1 (2021) 1 (2022)	1 (2021) 1 (2022)	1 (2022)	1 (2020) 1 (2022)	1 (2020) 1 (2022)
M	1 (2021) 1 (2022)	1 (2022)	1 (2021) 1 (2022)	1 (2021) 1 (2022)	1 (2022)	1 (2022)	1 (2020) 1 (2022)

Sampling:

Seasonal monsoons (2-3 times)

- Northeast monsoon (November-March)
- Inter-monsoon (March-May)
- Southwest monsoon (May-September)

Selected photos of river transect



Station C



Station G



Station J



Station L



Station N

END OF REPORT
