Antibiotic Resistance in Domestic Wastewater

Screening of Extended-Spectrum Beta-Lactamases (ESBLs)-Producing and Carbapenem-Resistant *Enterobacteriaceae* (CRE): A study of a sub-urban wastewater treatment system in Johor, Malaysia

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Introduction:

- Poor wastewater management can lead to the transmission of infectious diseases and environmental antibiotic resistance via contaminated water.
- In 2017, World Health Organization (WHO) declared antibiotic resistant *Enterobacteriaceae* as one of the critical resistant bacterial group.
- *Enterobateriaceae* are Gram negative, rod-shaped bacteria commonly found in human gut, for example: *Escherichia coli* and *Enterobacter cloacae.*
- ESBL-producing *Enterobacteriaceae* able to hydrolyze third generation antibiotic: cephalosporins and the antibiotic becomes inefficient.
- Carbapenem is “last line of defense”.
- ESBL-producing *Enterobacteriaceae* and CRE could pose threat to the human health.

Aim & Objectives:

The goal of this study is to determine the presence of the ESBL-producing and CRE in the inflow of a local wastewater system in Southern Malaysia.

- To examine the *Enterobacteriaceae* populations in domestic wastewater.
- To isolate ESBL-producing *Enterobacteriaceae* using ESBLs selective agar.
- To purify ESBL-producing *Enterobacteriaceae* for further screening of Carbapenem (Meropenem) resistance traits using a modified 96-Microbroth Screening (96-MS).

Sampling location:

- The study site is a small-scale wastewater treatment plant located in sub-urban Southern Malaysia (Taman Selesa).
- Inflow water sample was collected from the primary clarifier after the grit chamber and transported on ice to the lab.

Figure 1. Flow chart of wastewater treatment at Taman Selesa.

Acknowledgement:

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Methodology:

Wastewater sample processed in 24 hours.

ESBL Screening

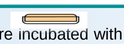
- Using HiCrome Coliform Agar

10-fold dilution

Plate Spreading

- 100 µl of diluted water samples or less were pipetted onto HiCrome Coliform agar plates.

Incubation



- Agar plates were incubated with lid inverted in the incubator for overnight at 37 °**C**.

Calculation

- After incubation, colony-forming units were calculated = number of colonies x 1/dilution factors x correction units

Plate Streaking

- Suspected ESBL-producing isolates were purified.

CRE Screening

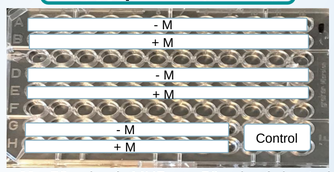
- Using 96-well plates

Pure cultures were used

Overnight growth method

Incubated in Mueller Hinton Broth (MHB)

Each well contained 100 µl of mixture



- **-M**: 50 µl of MHB + 50 µl of bacteria suspension
- **+M**: 50 µl of Meropenem + 50 µl of bacteria suspension. 11 isolates with triplicate.
- **Control**: 3 wells x 100 ul of MHB; 3 wells x 100 µl of Meropenem.

Absorbance was taken

Figure 2. Flow chart of the ESBL-producing *Enterobacteriaceae* and CRE screening test.

Discussions:

- 10^5 of *Enterobacteriaceae* concentrations were consistently detected in the inflow water samples across 4 weeks of screening with minimal variations.
- Up to 10^3 of ESBL-producing *Enterobacteriaceae* were present in the water samples.
- 96-MS was modified from Broth Microdilution method (modified from CLSI) in order to screen for CRE.
- Multiplex polymerase chain reaction (PCR) can be used to confirm the presence of genes, for example: *bla*CTX-M and *bla*KPC genes.
- Genome sequencing could be used to confirm the species of potential *Enterobacteriace*ae.

Conclusions:

- *Enterobacter cloacae* or *Citrobacter freundii* (salmon to red) were the most abundant residents in the wastewater samples.
- *E.coli* (blue) and *Salmonella enteriditis* or *Shigella flexneri* (white) were common ESBL-producing *Enterobacteriaceae* during the screening test.
- 96-MS was optimizing protocol but it was accurate and could help to minimize the wastage and screen 11 isolates at one time.
- Potential ESBL and potential CRE exist in the Johorian domestic wastewater.

Results:

Figure 3. (A) Five different colour colonies grew on the HiCrome Coliform agar plates. **(B)** Fewer colour colonies grew on the antibiotic-containing HiCrome Coliform agar plates.

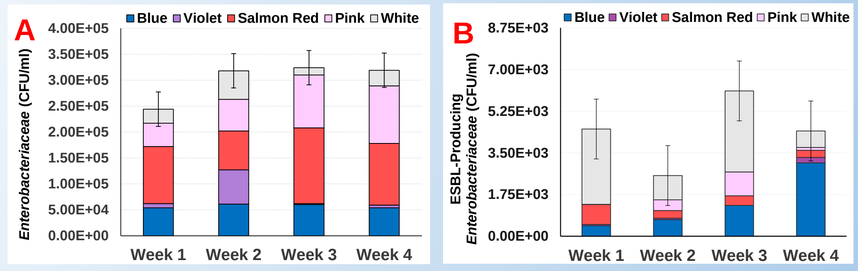


Figure 4. *Enterobacteriaceae* isolates abundances in the inflow, distinguishable by different colony colours on the HiCrome Coliform agar plates across 4 weeks of screening. Overlapped error bars indicated the difference is not statistically significant. **(A)** *Enterobacteriaceae* Coliform Forming Units (CFU/mL) concentrations on the HiCrome Coliform agar without antibiotic. **(B)** ESBL-producing *Enterobacteriaceae (*CFU/mL) concentrations on the HiCrome Coliform agar with the ESBL supplement.

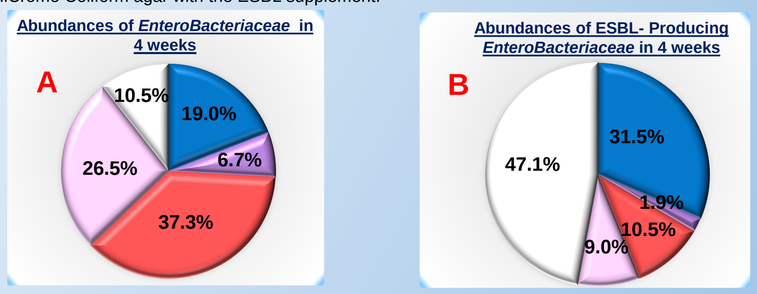


Figure 5. (A),(B) Blue and violet referred to ***Escherichia coli***. Salmon to red referred to either potential ***Enterobacter cloacae*** or ***Citrobacter freundii***. Pink referred to potential ***Klebsiella pneumoniae.*** White referred to potential ***Salmonella enteriditis*** or ***Shigella flexneri***.
[Description is based on product information sheet provided by Sigma-Aldrich, USA.]

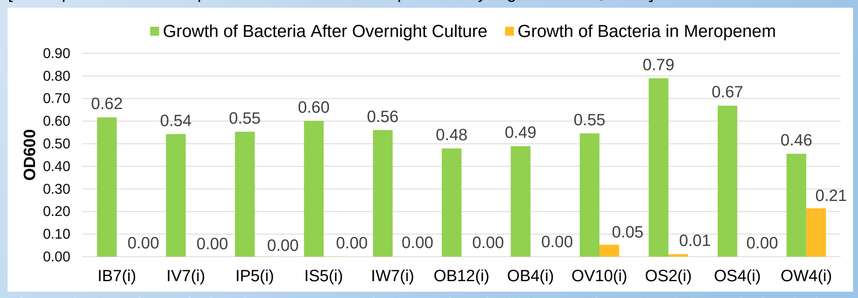


Figure 6. Bar chart of absorbance measured at wavelength 600nm against 11 ESBL-producing isolates selected for CRE screening using the 96-MS method. **OV10(i)**, **OS2(i)** and **OW4(i)** showed bacteria growth in antibiotic broth.

References:

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