

Introduction

- Antibiotic resistance is becoming more prevalent worldwide.
- Almost every infectious bacterial species is resistant to at least one or more types of antibiotic.
- The World Health Organisation (WHO) in 2000 stated that antibiotic resistance in bacteria had become a global threat.
- Poor wastewater treatment, can expose whole populations to new strains of antibiotic resistance if they fail in filtering out infectious resistant bacteria.
- Some of the new antibiotic resistance developing is towards our last resort antibiotics – the Carbapenems

Aims

- To assess whether antibiotic resistant bacteria are present in Malaysian domestic wastewater.
- Focusing especially on whether the bacteria that produce resistance towards Extended Spectrum B-Lactamases (ESBL) antibiotics (e.g. Ampicillin) and Carbapenem (CRE) antibiotics (e.g. Meropenem) are present.

Methods

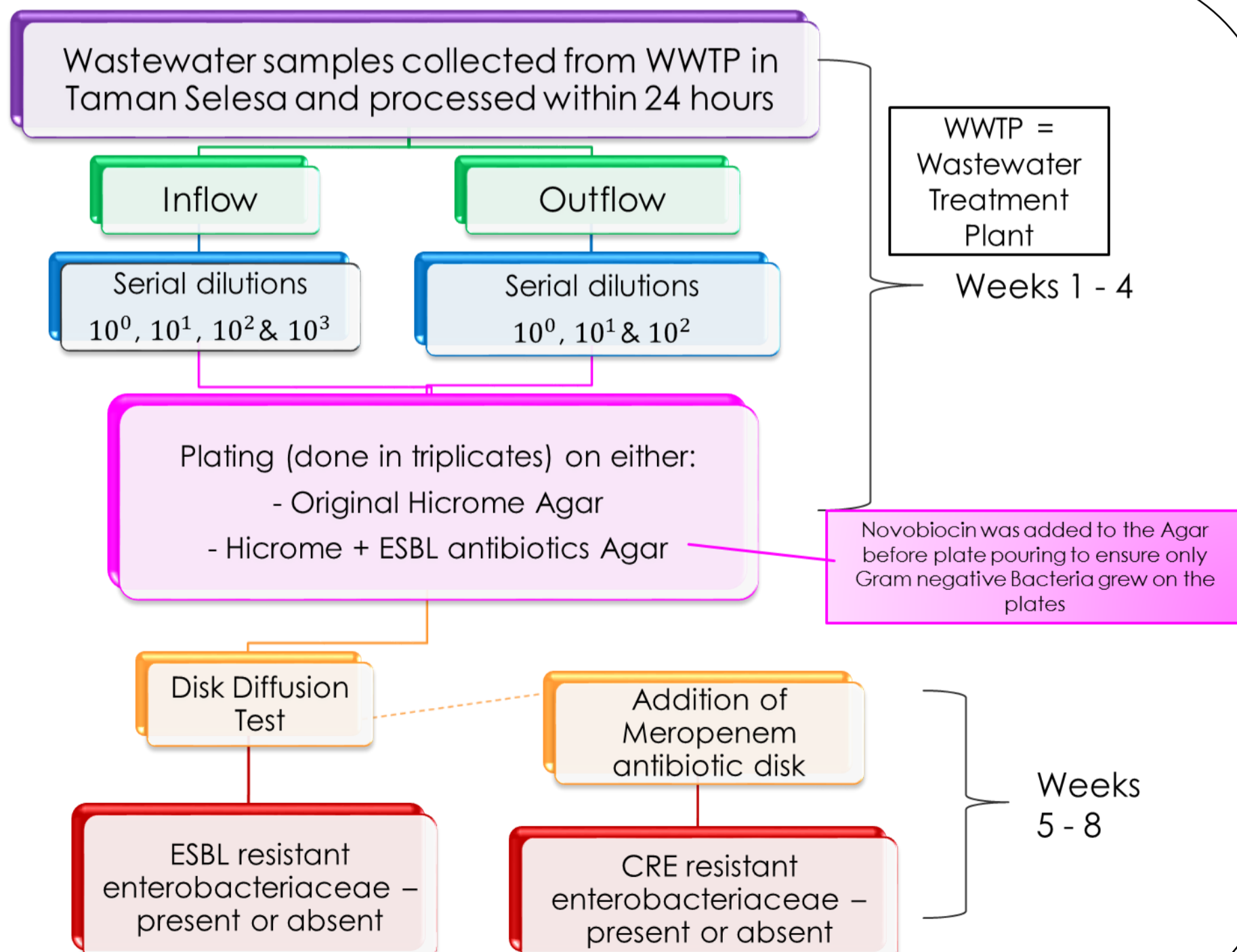


Figure 1: Flow chart to show the methods used to collect and analyse the data during this summer research project

Results

Results from Week 1-4 (in conjunction with figure 1 in methods)

The colours on the pie chart represent the 5 different colours of bacteria colonies found in the outflow. The colours can be used to distinguish the likely potential species of each bacteria on HiCrome Coliform Agar.*

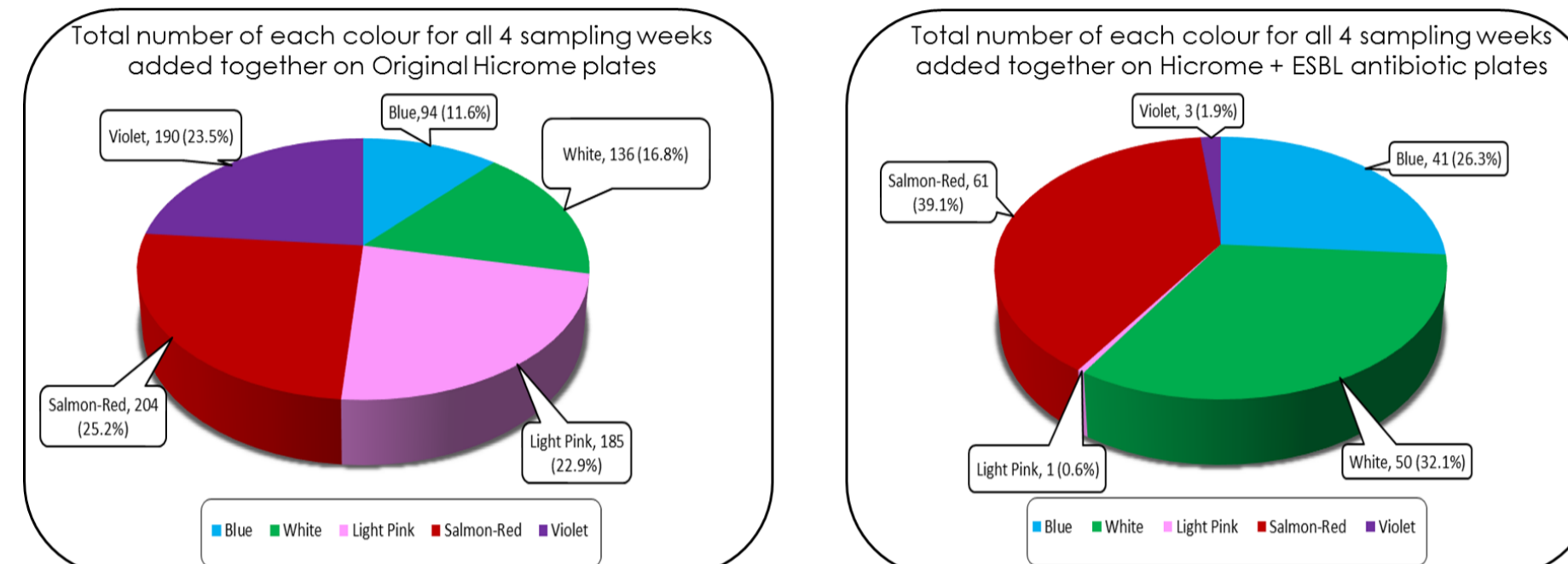


Figure 2: Graph showing results from 4 weeks sampling on Original (plain) HiCrome Agar, with each coloured section representing that specific colour of bacterial colonies.

Figure 3: Graph showing results from 4 weeks sampling on ESBL (antibiotic) HiCrome Agar, with each coloured section representing that specific colour of bacterial colonies.

Bacteria Strain – Blue Colonies	Potential Bacteria Strains – White Colonies	Potential Bacteria Strain – Pink Colonies	Potential Bacteria Strains – Salmon-Red Colonies	Bacteria Strain – Violet Colonies
• <i>Escherichia coli</i> (<i>E.coli</i>)	• <i>Salmonella enteritidis</i> • <i>Shigella flexneri</i>	• <i>Klebsiella pneumoniae</i>	• <i>Citrobacter freundii</i> • <i>Enterobacter cloacae</i>	• <i>Escherichia coli</i> (<i>E.coli</i>)

Figure 4: Tables showing the potential different bacteria for each colour of colony linked to the above Figures 2 and 3
*Colour descriptions and potential bacteria species are based on the product information sheet supplied by Sigma-Aldrich, USA

Results from Week 5-8 (in conjunction with figure 1 in methods)

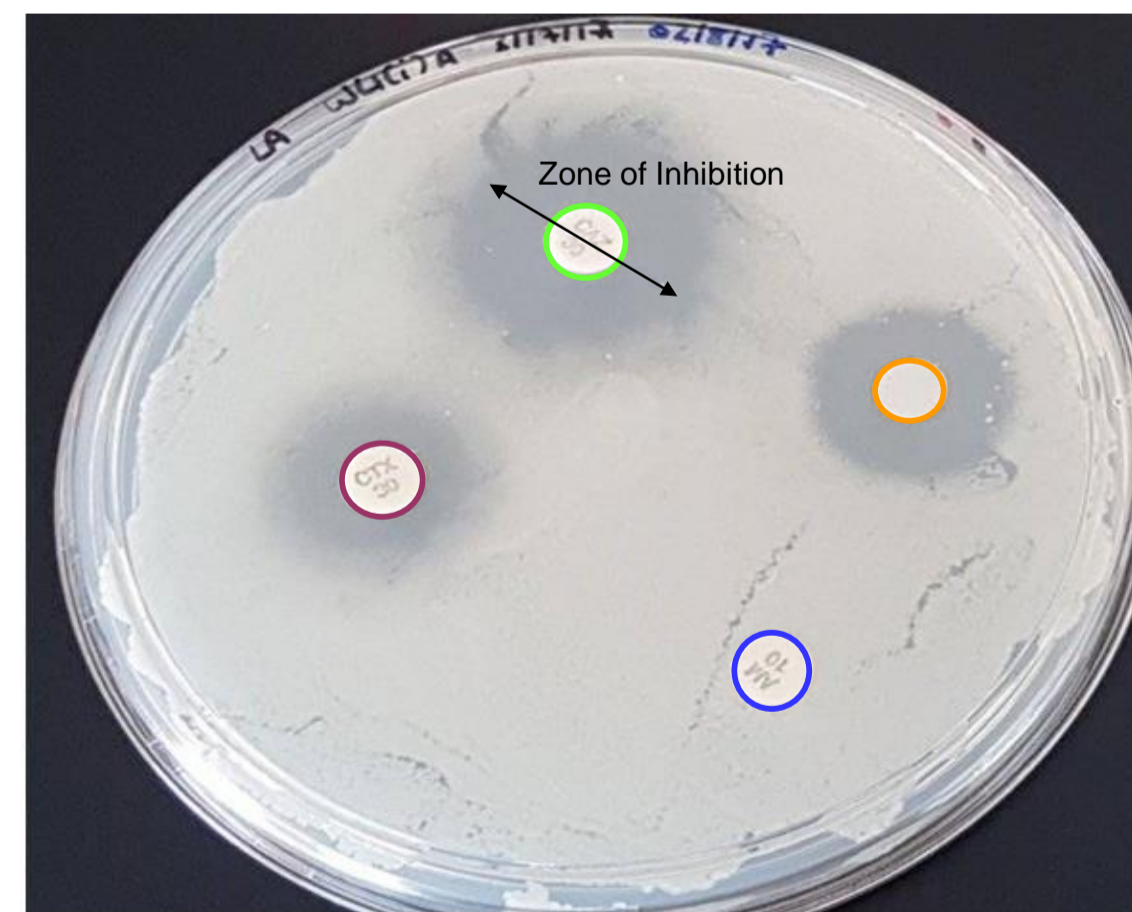


Figure 5: Muller-Hinton Agar plate used for one of the disk diffusion susceptibility tests. The Zone of Inhibition determines how effective an antibiotic is against a strain of bacteria. The bigger the Zone of Inhibition, the more effective the antibiotic is against that particular strain

Antibiotic	Zone of Inhibition (mm)		
	Susceptible	Intermediate	Resistant
Meropenem (10 μ g)	≥ 23	20 – 22	≤ 19
Ampicillin (10 μ g)	≥ 17	14 – 16	≤ 13
Cefotaxime (30 μ g)	≥ 26	23 – 25	≤ 22
Ceftazidime (30 μ g)	≥ 21	18 – 20	≤ 17

Table 1: Zone of Inhibition diameters for the 4 different tested antibiotics.
* The colour of the name of the antibiotic relates to the coloured circle around the antibiotic disks on Figure 5. Please read in conjunction with Table 2 (conclusion)

Conclusion

- As can be seen in Figure 1 and Figure 2, the violet *E. coli* and the potential *Klebsiella* strains have a very low abundance on the ESBL (antibiotic) plates, compared to the Original (plain) plates providing us with an interesting result.
- Health organisations state that, the natural gut bacteria *E. coli* and *Klebsiella* are the most common types to develop antibiotic resistances, especially for β -lactam and Carbapenem antibiotics. However, in our project we only found them in small quantities and therefore further investigation will be needed to see if there is a reason these strains in Malaysia seem to be more susceptible to ESBL antibiotics.
- These figures also show that the potential *Citrobacter* or *Enterobacter* (strains of bacteria), and *Salmonella* or *Shigella* strains were surprisingly high in abundance. These bacteria are naturally occurring within our gut's environment, and are excreted from the human body through stools, which could explain their abundance in our wastewater samples.

Colony	Zone of Inhibition (mm)							
	Mero	S/I/R	Am	S/I/R4	CTX	S/I/R2	CAZ	S/I/R3
V10 (A)	32	S	0	R	11	R	18	1
V10 (B)	31	S	0	R	10	R	25	5
V10 (1)	0	R	0	R	7	R	20	1
V10 (2)	26	S	0	R	8	R	18	1
W4(i) (A)	13	R	0	R	13	R	21	5
W4(i) (B)	17	R	0	R	12	R	22	5
W4(i) (1)	18	R	0	R	13	R	18	1
W4(i) (2)	11	R	0	R	15	R	17	R
B4(i) (A)	28	S	10	R	11	R	11	R
B4(i) (B)	28	S	0	R	11	R	15	R
B4(i) (1)	27	S	0	R	7	R	11	R
B4(i) (2)	25	S	0	R	8	R	11	R
P5(i) (A)	29	S	0	R	12	R	22	S
P5(i) (B)	31	S	0	R	15	R	29	S
P5(i) (1)	25	S	0	R	7	R	17	I
P5(i) (2)	27	S	0	R	7	R	18	I
S4(i) (A)	42	S	7	R	11	R	8	R
S4(i) (B)	43	S	7	R	14	R	9	R
S4(i) (1)	43	S	7	R	12	R	9	R
S4(i) (2)	43	S	8	R	13	R	8	R

Table 2: Table showing the results from the first set of disk diffusion tests. Figure 5's results are the ones highlighted with the colours representing the antibiotic disk colour associated in Figure 5 and Table 1.

- As can be seen in Table 2, the potential *Salmonella* or *Shigella* strain that showed Meropenem resistance (highlighted by an orange box) was a significant find for us as it shows that **resistance is occurring in our last line of defence antibiotics**. As all 4 plate repeats showed the resistance and the samples were taken from different colonies on the same plate, it helps consolidate that this is a Carbapenem antibiotic resistant bacterial strain.

Future Works

- The future prospects for this project is using DNA and PCR techniques for further analysis on ESBL and CRE bacteria, such as ribosomal RNA sequencing which can be used to clarify the bacteria's species and determine the genes that are causing ESBL and CRE resistance.

Acknowledgements

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