A Pilot Study on the Identification of Resistant *E. coli* in a Southern Malaysia River

Kwa, Y. C.*, Ho, J. Y., Ng, Y. M., Baldwin, I. C. and Othman, M.
150015248 – Y.Kwa1@newcastle.ac.uk – BSc (Honours) Biomedical Science
Supervised by Dr Mukhrizah Othman

Introduction

- *Escherichia coli (E.coli)* is a Gram-negative bacteria from the *Enterobacteriaceae* family that is commonly found in river water. *E.coli* causes intra-abdominal infections, urinary tract infection, sepsis, pneumonia and various other diseases[1,2].
- Resistant bacteria including *E.coli* can produce extended spectrum beta lactamase enzymes (ESBLs) capable of hydrolysing the structure of β-lactam antibiotics including penicillin and cephalosporin causing the loss of functions[3] .
- Early detection of ESBLs-producing bacteria aids treatments of infection, keep further widespread of bacterial resistance in check and thus improve the living quality of residents adjacent to or those who use the water source.

Aim

- To detect preliminarily the presence of bacteria in the river water samples and classify the bacteria into Gram-positive and Gram-negative.
- To determine antibiotic susceptibility among the bacterium present in the river water samples and hence detect any resistance to β-lactam antibiotics.

Methods

- Sample collection: Surface water samples were collected from Skudai river in Johor, Malaysia using sterile 50ml Falcon tubes and were stored in cold box for transportation to the laboratory.
- Bacteria isolation*: Spread plate method (Figure 1) was carried out. Serial dilutions of water samples were plated onto HiCrome Coliform (HC) agar (Sigma-Aldrich, USA) selective of *E.coli* and various Gram-negative coliforms.

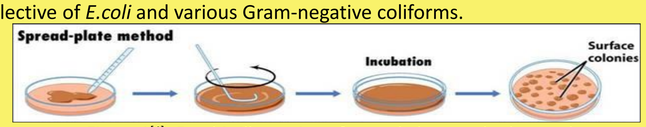

Figure 1[4]. Graphic illustration of spread plate method procedure.

- Gram staining[5].
- Antibiotic susceptibility test*: Disk diffusion method (Figure 2) was carried out using Mueller-Hinton agar with starting inoculum of 10^8 cells/ml. Antibiotics tested were as listed in Table 1. Inhibition zones were measured using a calliper.
- Control strain for susceptibility test was *E. coli K12*[3].

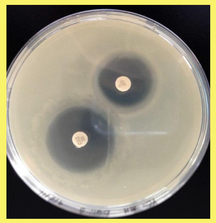

Figure 2. Zones of inhibition (halo rings) following disk diffusion susceptibility test

Table 1. Antibiotic discs used in the susceptibility test

Category	Antibiotics	Dosage
Cephems	Ceftazidime Cefotaxime	30µg 30µg
Penicillins	Ampicillin	10µg
β-lactam/β-lactamase inhibitor combination	Ampicillin/Sulbactam	10µg/10µg
Fluoroquinolones	Ciprofloxacin	5µg
Folate pathway inhibitors	Sulfamethoxazole/ Trimethoprim	23.75µg/ 1.25µg

Results

❖ Bacteria isolation:

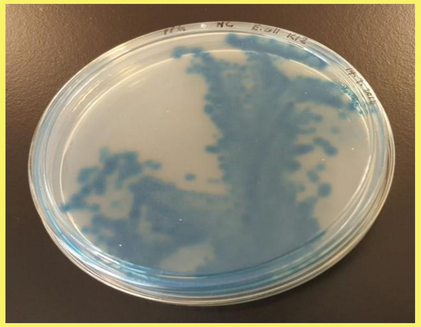

Figure 3. Blue colonies observed on the surface of HC agar spread with *E. coli K12.*

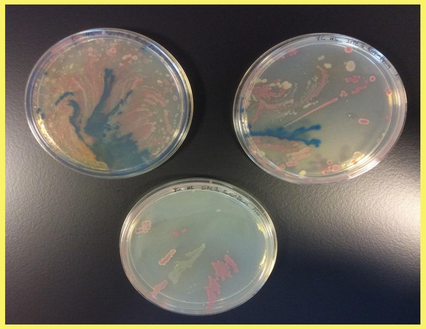

Figure 4. Blue, red, pink, white, translucent and yellowish red colonies grown on the surface of HC agar plated with river water samples.

❖ Gram staining:

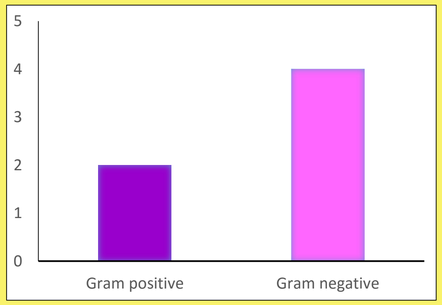

Figure 5. Gram staining results for colonies isolated from HC agar plate.

Table 2. Colonies on HC agar and their respective characteristics

Colony	Gram	Shape	Possible species
Blue	-	Cocci	*E.coli*
Red	-	Streptococci	*Citrobacter freundii*
Pink	-	Rod	*Klebsiella pneumoniae*
White	-	Streptococci	Further tests required
Translucent	+	Rod	*Shigella flexneri*
Yellowish red	+	Rod	Further tests required

❖ Antibiotic susceptibility test:

Table 3. Disk diffusion susceptibility of control *E. coli K12 and* isolated *E. coli* present in river water samples against antibiotics. (n = 3)

Antibiotics	Inhibition zone diameter (mm)					
	Control strain *E.coli K12*			Isolated *E. coli*		
	Complete	Partial	Total	Complete	Partial	Total
Ampicillin	8.8 ± 0.2	0.7 ± 0.7	9.5 ± 0.7	9.5 ± 0.7	0.0 ± 0.0	9.5 ± 0.7
Ceftazidime	28.0 ± 0.2	2.0 ± 2.0	30.0 ± 2.9	23.0 ± 0.0	6.0 ± 2.4	29.0 ± 2.4
Ciprofloxacin	17.0 ± 0.8	0.0 ± 0.0	17.0 ± 0.8	19.0 ± 1.2	7.8 ± 6.3	26.7 ± 5.3
Cefotaxime	28.4 ± 1.7	5.1 ± 3.6	33.5 ± 3.1	29.4 ± 1.7	1.9 ± 1.9	31.3 ± 4.3
Ampicillin/Sulbactam	14.3 ± 1.9	0.0 ± 0.0	14.3 ± 1.9	12.0 ± 0.0	0.0 ± 0.0	12.0 ± 0.0
Sulfamethoxazole/Trimethoprim	8.7 ± 0.5	10.7 ± 10.7	19.3 ± 15.3	9.0 ± 0.0	0.0 ± 0.0	9.0 ± 0.0

Coloured columns represent susceptibility based on CLSI (2014)[6]; red indicates resistant, yellow indicates intermediate susceptibility and green indicates susceptible. Values shown are average ± standard deviation. The complete inhibition zone diameter was used to determine antibiotics susceptibility as complete inhibition was more representative of the effect of antibiotics on *E. coli.*

Discussion and Conclusions

- ***E.coli* were present** in the river water samples as confirmed using selective HiCrome coliform agar.
- Other coliforms detected in the river water samples were presumed to be *Enterobacter cloacae, Citrobacter freundii, Klebsiella pneumoniae, Salmonella enteritidis, and Shigella flexneri* based on information sheet supplied by the manufacturer, Sigma-Aldrich.
- The river water samples contained **more Gram-negative bacteria than Gram-positive bacteria**. This is of concern because Gram-negative bacteria cause infections that are more challenging to treat due to the presence of a peptidoglycan cell wall.
- *E. coli* isolated from the river water samples were **not ESBLs-producer** because the bacteria demonstrated susceptibility to β-lactam antibiotics such as penicillins (Ampicillin), cephems (Ceftazidime and Cefotaxime), and were not resistant to β-lactam/β-lactamase inhibitor combination (Ampicillin/Sulbactam).
- The isolated *E. coli* were also **not multidrug-resistant (MDR)** because the resistance were similar to control *E. coli*.
- **Further molecular tests** such as PCR are needed to confirm the strains and species of the bacteria.

Acknowledgement

Many thanks for the ample assistances and supports from the NUMed laboratory staff, colleagues and supervisors. I would like to thank Global Excellence Fund for giving me the opportunity to undertake this summer research.

References

[1] Mahdi Yahya Mohsen S, Hamzah HA, Muhammad Imad Al-Deen M, Baharudin R. Antimicrobial Susceptibility of Klebsiella pneumoniae and Escherichia coli with Extended-Spectrum beta-lactamase associated Genes in Hospital Tengku Ampuan Afzan, Kuantan, Pahang. The Malaysian journal of medical sciences : MJMS. 2016 Mar;23:14-20.
[2] Paterson DL. Resistance in Gram-Negative Bacteria: Enterobacteriaceae. The American Journal of Medicine.119:S20-S8.
[3] Adesoji AT, Ogunjobi AA. Detection of Extended Spectrum Beta-Lactamases Resistance Genes among Bacteria Isolated from Selected Drinking Water Distribution Channels in Southwestern Nigeria. BioMed research international. 2016;2016:7149295.
[4] Image taken from: MAIN METHODS, PRINCIPLES and steps OF ISOLATING OF BACTERIA's PURE CULTURES. [cited 2016 4/9]; Available from: http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes_stud/en/pharm/prov_pharm/ptn/Microbiology%20with%20basis%20immunology/2/Lesson%203.%20The%20main%20methods,%20principles%20and%20steps%20of%20isolation%20of%20bacteria%E2%80%99s%20pure%20cultures.htm
[5] Shabbir S, Jamil S, Hafiz S. Pattern of Polymicrobial Isolates and Antimicrobial Susceptibility from Blood. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP. 2016 Jul;26:585-8.
[6] Clinical and Laboratory Standards Institute. Zone Diameter and Minimal Inhibitory Concentration Interpretive Standards for Enterobacteriaceae. 2014. ISBN: 1-56238-898-3(Electronic).