Isolating antibiotic resistant bacteria from river water

Presence of Multidrug-Resistant and Extended-Spectrum Beta-Lactamases (ESBLs) Producing *E. coli* – A pilot study of a Johorian River, South Malaysia

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INTRODUCTION

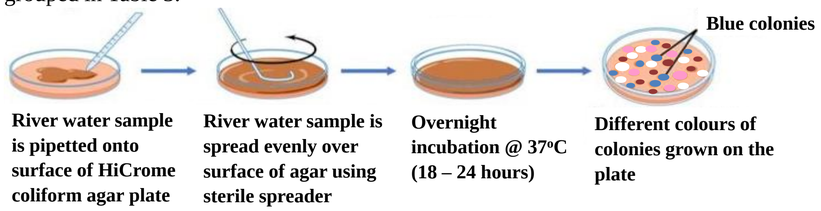
- Gram negative bacteria including *Escherichia coli* (*E. coli)* are becoming increasingly resistant to the existing drugs. The prescription of antibiotics to human and animals can result in drug-resistant bacteria developing in the gut, in particular *E. coli.*
- Extended-spectrum beta lactamases (ESBLs) are enzymes that break down the chemical component named β-lactam ring of penicillin and cephalosporin antibiotics. Bacteria that produces ESBLs are hence resistant to penicillin, cephalosporin and potentially to other types of antibiotics.
- The genera *Citrobacter, Enterobacter, Escherichia* and *Klebsiella* are Gram negatives in the coliform group and members of the family Enterobacteriacae. They are indicator organisms used as a sign of quality or hygienic status in food, water, or environment.

AIMS

- To determine the presence of Gram positive and Gram negative in the water sample.
- To determine the presence of coliforms in the water sample from the river.
- To detect the presence of multidrug-resistance and ESBLs producing *E. coli* in the water sample from the river.

METHODS

Water sample was collected from Ulu Pulai river in Johor, South Malaysia. Part of the water sample was frozen at -20 ˚C for DNA extraction for future studies. The river water samples were spread on nutrient agar and HiCrome coliform agar (Sigma-Aldrich, USA) (Scheme 1) in multiple dilution. Colonies grown on HiCrome coliform agar can be grouped in Table 3.



Scheme 1. Spreading river water sample on HiCrome coliform agar. [4]

Bacteria grown on the plate were Gram stained (Table 1 & 2). *E. coli* which appeared as blue colony on HiCrome coliform agar were then tested for resistance to a panel of antibiotics (Table 4,5 & 6) using disc diffusion on Müeller-Hinton agar.

RESULTS

Table 1. Classification of colonies grown on nutrient agar based on Gram stain.

Colony	Gram stain*	Shape
Yellow	Negative	Cocci (Round shape)
White	Negative	Rod
Translucent	Negative	Cocci (Round shape)
	Positive	Rod
Purple	Positive	Rod

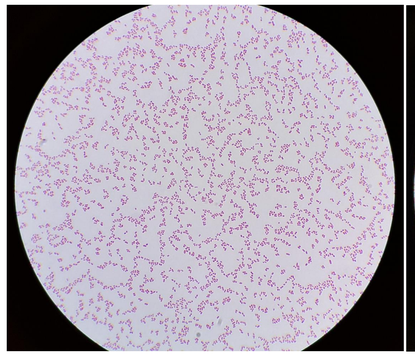


Figure 1. Gram staining of white colony grown on nutrient agar indicating Gram negative bacteria. (Gram stained bacteria looks pink in colour: Gram negative bacteria)

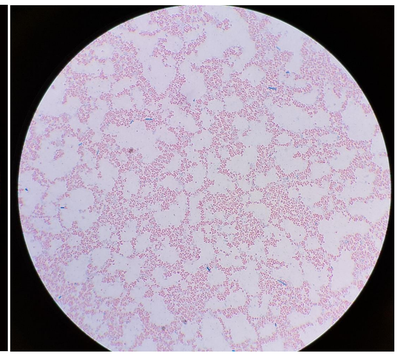


Figure 2. Gram staining of blue *E. coli* colony grown on selective HiCrome coliform agar.

Table 2. Classification of colonies grown on HiCrome coliform agar based on Gram stain.

Colony	Gram stain*	Shape
Red	Negative	Rod
Orange	Negative	Rod
Blue	Negative	Rod
Pink	Negative	Rod
White	Positive	Rod
Grey	Positive	Rod

*Gram stain – Pink: Gram negative, Purple: Gram positive

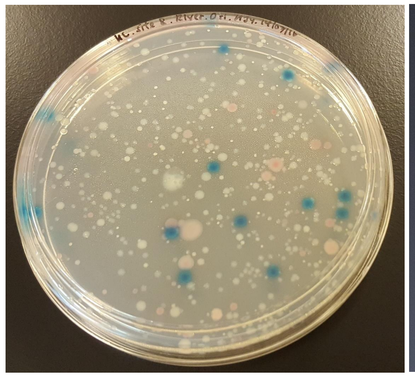


Figure 3. Various colonies grown on HiCrome coliform agar (see description in Table 3).

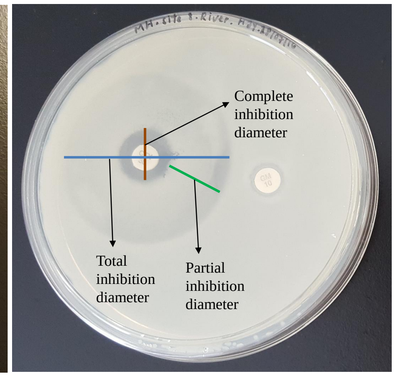


Figure 4. One of the Müeller-Hinton agar plate used in disc diffusion susceptibility test.

Table 3. Different colour of colonies grown on HiCrome coliform agar and the species of the organisms.*

Colour of colony on HiCrome coliform agar	Species
Blue	*Escherichia coli*
Red	*Enterobacter cloacae / Citrobacter freundii*
Light pink	*Klebsiella pneumoniae*
Colourless (White)	*Salmonella enteritidis / Shigella flexneri*

*Description is based on product information sheet supplied by Sigma-Aldrich, USA.

Table 4. Average inhibition diameter (mm) of complete, partial and total inhibition, and susceptibility for *E. coli* isolated from the river water sample. (n = 3).

Antibiotics (dosage)	Average inhibition diameter (mm)			Susceptibility*
	Complete	Partial	Total	
CAZ – ceftazidime (30 µg)	27.10 ± 2.85	2.83 ± 4.91	29.93 ± 2.90	S
CIP – ciprofloxacin (5 µg)	11.93 ± 3.35	0.00 ± 0.00	11.93 ± 3.35	R
CTX – cefotaxime (30 µg)	27.10 ± 1.01	8.97 ± 3.23	36.07 ± 3.58	S
SAM – ampicillin (10 µg)/sulbactam (10 µg)	11.10 ± 2.15	3.90 ± 3.38	15.00 ± 4.00	R
SXT – sulfamethoxazole (23.75 µg) with trimethoprim (1.25 µg)	8.33 ± 1.15	0.00 ± 0.00	8.33 ± 1.15	R

Table 5. Average inhibition diameter (mm) of complete, partial and total inhibition, and susceptibility for control strain *E. coli* K 12 (n = 3).

Antibiotics (dosage)	Average inhibition diameter (mm)			Susceptibility*
	Complete	Partial	Total	
CAZ – ceftazidime (30 µg)	28.00 ± 1.00	2.00 ± 3.46	30.00 ± 3.61	S
CIP – ciprofloxacin (5 µg)	17.00 ± 1.00	0.00 ± 0.00	17.00 ± 1.00	R
CTX – cefotaxime (30 µg)	28.43 ± 2.11	5.07 ± 4.41	33.50 ± 3.77	S
SAM – ampicillin (10 µg)/sulbactam (10 µg)	14.33 ± 2.31	0.00 ± 0.00	14.33 ± 2.31	I
SXT – sulfamethoxazole (23.75 µg) with trimethoprim (1.25 µg)	8.67 ± 0.58	10.67 ± 18.48	19.33 ± 18.77	R

Table 6. Average inhibition diameter (mm) of complete, partial and total inhibition, and susceptibility for control strain *E. coli* B. (n = 3)

Antibiotics (dosage)	Average inhibition diameter (mm)			Susceptibility*
	Complete	Partial	Total	
CAZ – ceftazidime (30 µg)	27.83 ± 1.26	1.17 ± 2.02	29.00 ± 1.00	S
CIP – ciprofloxacin (5 µg)	15.67 ± 0.76	1.33 ± 2.31	17.00 ± 1.80	R
CTX – cefotaxime (30 µg)	29.67 ± 2.08	3.33 ± 5.77	33.00 ± 5.57	S
SAM – ampicillin (10 µg)/sulbactam (10 µg)	13.33 ± 0.58	0.00 ± 0.00	13.33 ± 0.58	I
SXT – sulfamethoxazole (23.75 µg) with trimethoprim (1.25 µg)	8.67 ± 0.58	10.67 ± 18.48	19.33 ± 18.77	R

Values shown in Table 4, 5 & 6 are average ± standard deviation; *susceptibility is determined using average total inhibition diameter based on CLSI (2014) [1], S – susceptible, I – intermediate, R – resistant.

DISCUSSION

- Three Gram negative and two Gram positive bacteria were detected on nutrient agar.
- Two Gram positive bacteria and four Gram negative coliforms were detected on selective HiCrome coliform agar as indicated by different colour of colonies.
- The presence of Gram positive bacteria on selective HiCrome coliform agar is probably due to the absence of novobiocin antibiotic supplement in the agar to supress the growth.
- The complete inhibition diameter against CIP and SXT for isolated *E. coli* are smaller than the inhibition diameter for control *E. coli.*
- Partial inhibition observed might be due to the action of antibiotics which halted bacterial growth (bacteriostatic) but not killing them (bactericidal), and prolong incubation time.

CONCLUSIONS

- The blue *E. coli* colony isolated from HiCrome coliform agar is resistant to most of the antibiotics tested. However, it is susceptible to third generation cephalopsorins antibiotics, ceftazidime and cefotaxime.
- This work warrants further investigation into molecular detection for antibiotic resistance genes in isolated *E. coli* through quantitative polymerase chain reaction (qPCR).

ACKNOWLEDGEMENT

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