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Isolation, Identification and Comparison between some Physicochemical Characteristicsof E.Coli from Alur Ilmu, Ukm in Malaysia

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Abstract : A study was conducted to idenitify*E.coli*in the existing water samples at five different sites of AlurIlmu, UKM from March 2015 to May 2015, 3 replications from each sample points. The microbiological quality and the hygienic conditions of these collected water samples were compared. *E.coli*was identified by Pouring method. The result showed that, there are some area that showed a clear growth of *E. coli* colonies and there are also some location that did not show any growth of *E. col*i colonies. This is because *E. coli* is one of the subgroups of fecal bacteria that need the source of fecal such as sewage and others for their growth. So this proof that the existence of these sources will lead to the growth of *E. coli* and vice versa. Besides, others phytochemical parameters such as, turbidity, temperature, salinity and pH were also measured to determine their contribution to the growth of *E. coli*. So from the study, it showed that AlurIlmu contain high growth of E. coli. Thus, it is recommended for the population there to avoid sources that lead to contamination by E. coli.
Keywords : Isolation, Identification And Comparison Between Some Physicochemical Characteristics Of E.Coli From Alur Ilmu, Ukm In Malysia.

Introduction

Escherichia coli (*E. coli*) are microscopic bacteria and single-celled organisms that are the most numerous organisms on earth. They are small in size that over five million could be placed on the head of a pin. Bacteria can live in various environments and perform many complex actions, some of which are beneficial and some harmful. *E. coli* is one of the most important Enterobacteriacea species, which are normal flora (can live in the gut without causing any health problems), gram negative rode, motile, produce polysaccharide capsule, positive tests for indol, lysine decarboxlase and mannitol fermentation and produces gas from glucose[1,2,3].

E. coli is one subgroup of fecal coliform bacteria. Even within this species, there are numerous different strains which some of them can be very harmful. However, the release of these naturally occurring organisms into the environment is generally not a cause for alarm. But, other disease causing bacteria, which can include some pathogenic strains of *E. coli* may also be present in these wastes and pose a health threat. The use of an organism that can serve as a surrogate for another is called an indicator organism. Testing for pathogens requires high cost and large volumes of water and the pathogens can often be difficult to grow and isolate in the laboratory. Bacteria in water can be originated from the intestinal tracts of both humans and other warm-blooded animals, such as pets, livestock and wildlife. Human sources include failing septic tanks, leaking

sewage lines, wastewater treatment plants, combined sewage overflow, etc. Animal sources of faecal coliform bacteria include manure spread on land and improper disposed of farm animal wastes, pet wastes (dogs, eats), wildlife (deer, elk and raccoons), bird, ducks and gulls[4,5].

Polluted water runoff from the land is the leading cause of water quality problems nationwide. Fecal materials as well as other pollutants can be transported to waterways through runoff and how quickly they are transported partially depended on the type of land use[6]. Land that supports domestic animals, such as, cattle or hogs or horses, can also be a source of bacteria, particularly if animals enter the water for wash manure from the land into receiving waters. Another source of bacteria pollution to stream water routes from Combined Sewer Overflows (CSOs) because some sewer and storm water pipes are not separated[7]. When drop large amounts of rain water, the wastewater treatment plants unable to support the excess volume of water that being pumped into the plant. As a result, untreated sewage along with storm water is directly dumped into rivers and streams. Although the presence and levels of *E. coli* in a stream cannot give an indication of the source of the contamination, however, it can be a good starting point in investigating the watershed for potential domestic water sources[8]. The problem statementwas for this study *E.Coli* is a bacterium that causes of many diseases such as diarrhea, vomiting, fever and intestinal diseases. The spread of this bacterium has recently led to the contamination of water quality such as unpleasant odors, color, turbidity, pH, etc. Furthermore, polluted water can give negative impacts to aquatic species such as fish, aquatic plants and other organisms in AlurIlmu, UKM. It can also cause human health damage if get contact to the polluted water in the AlurIlmu. In addition, using this water as agriculture irrigation will affect the soil properties and plants production and thus cause significant environmental problems are harmful to human health.

The significance of the research*E.coli* may constitute great harm to human health through its presence in AlurIlmu UKM because of damage caused by the marine plants in the water, and damage to fish and some organisms. When humans are exposed to polluted water with *E. coli*, they can affected with many diseases such as fever, abdominal cramps, chest pain and inflammation of the liver and other related diseases. Many strains expressing haemolysin sand *E. coli* strains isolated from the extra intestinal infection is more than hemolytic isolated from healthy human feces. *E. coli* can cause urinary tract infect in chronic otitis media and sinusitis[9]. This study can be used to assess the situation of water quality and comparing current results with previous studies of water quality that have been done in AlurIlmu, UKM. This study is expected to help in the identification and isolation of *E. coli* in AlurIlmu so that it can determine the current status of water quality and the abundance of *E. coli* that can lead to damage to human life through their presence in waters.The objective for this study to isolate and identify *E. coli* organisms from AlurIlmu, UKM.that lead to determine the level of *E.coli* contamination in AlurIlmu, UKM.As well as determine physicochemical characteristics related with *E.coli* that can influence the number of colonies in the water body.

Materials and Methods

Introduction

The study was conducted in AlurIlmu, Bangi campus, UniversitiKebangsaan Malaysia (UKM). Figure 1 shows the map of the sampling stations. In the early 1970s, AlurIlmu has been modified with cement-based concrete and natural drainage since the construction of the campus building complex in UKM Bangi. AlurIlmu was built to control the flow of rain water, ground water and any fluid from UKM Bangi campus that flows to Sungai Langat. When it rain, water run off occurs and brings materials to flow into the channels available and directly into the AlurIlmu. There is possibility that sewage and sullage are channeled into AlurIlmu without prior treatment then release into the Sungai Langat[10].

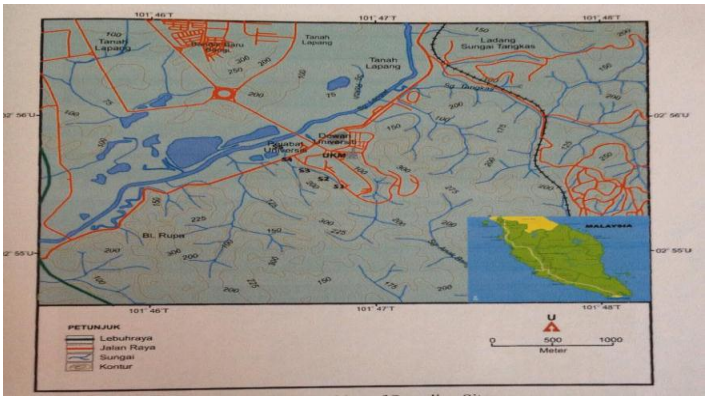


Figure1 Map of sampling stations
Source: Geography Department of Faculty of Social Science and Humanities, UKM (2014).

Sampling Stations

In this study, sampling activities were conducted three times and five sampling stations were chosen located along the AlurIlmu. All the five stations of AlurIlmu cover the upstream, middle stream and downstream. The distance between each sampling station is approximately 150 m. Table 1,2 explain the Laboratory Instruments and Manufacturing Company to cover our need foe isolation and identification *E.coli*

Table 1 Laboratory Instruments and Manufacturing Company

	Instrument	**Company**
	Sensitive Electronic Balance	A and D, Japan
	Autoclave	Stermite, Japan
	Incubator	Memmert, Germany
	Distillator	GFL- Germany
	Centrifuge	Hermle, Japan
	Oven	Memmert, Germany
	Refrigerator	Concord, Italy
	Milipore Filter	Satorius membrane filter GmbH, W. Germany
	Light Microscope	Olympus, Japan
	Micropipette	Oxford, USA
	pH Meter	Hoeleze and Cheluis, KG, Germany
	Water Bath	Memmert, Germany

Table 2 Chemical and Biological Materials

Material	**Company**
A- Chemical Materials	
K_2HPO_4 , NaCl, $MgSo_4$, HCL, $CaCl_2$,	Merk-Darmstadt.
alphα-nephthol amine, Tetramethyl-p-paraphylenediaminedihydihydrochloride, trichloroacetic acid, Amyl alcohol, Methyl red, Chloroform, gelatin, ,galactouronic acid, pto	B.D.H
H_2O_2, Glucose, 99% alcohol.Carbazole, Gram stain (crystial violates, alchole, iodine,andsufranin) 0.85% saline solution Paraffin oil	Flukachemika-Switzerland

B- Culture media	
MacConkey agar, Eosin methylene blue (EMB) agar, Agar-agar, Muller-Hinton agar, Pepton water medium, Nutrient agar media, Nutrient broth, Cimmoncitrat, Kligler iron agar.	Hi medium
C-Other Material and glass Bottles (number of weight 4), Glass Flask (number of weight 4), Plates (plastic) (number of weight 500), Sterile tube (plastic) (number of weight 100), Slides (number of weight 100), Pasteur pipette (plastic) (number of weight 100), transport swabs (plastic) (number of weight 100)	Hi medium

Serial Dilution for Macconkey Agar and Eosine Methylene Blue (EMB) Agar

Water samples from AlurIlmu, UKM were collected in properly washed and sterilized bottles. The sample was made into 5 serial dilutions (Figure 2). Five vials containing 9 ml of distilled water were prepared. Then, 1 ml of sample was placed into the first vial and this step was repeated until the fifth dilution.

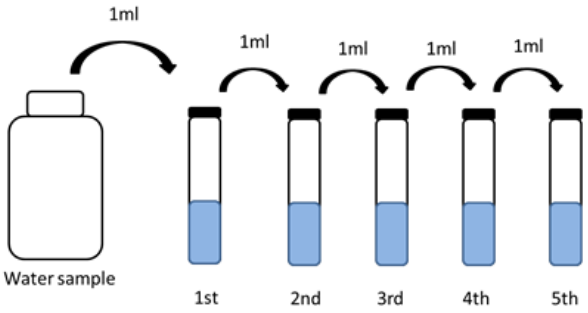


Figure 2 Serial dilution

Source:[11,12]

MacConkey agar and Eosine Methylene Blue (EMB) agar were used in this experiment. 0.1 ml was pipetted from the third dilution and then the MacConkey agar was added by using pouring method. Then, the step was repeated by pipetting the 0.1 ml from the fourth dilution. Same goes to fifth dilution. Then, they were then incubated at 37°C for 24 hours. The steps were repeated using Eosine Methylene Blue (EMB) agar.

Isolation Identification and Numerated of *E. Coli* Isolate

Garnish metallic sheen colonies on EMB which are characteristic of *E.coli*. Bright garnish metallic sheen colonies on EMB numerated between range of colonies (35-335 colony) in optimum dilution and Factorialin dilution inverted = (number of colony forming unite per ml (CFU).For example: the plate at 10-4 range of colonies (30-300, colony) approximately 100 colony**.** The number of colony at1000 in plate =100 * 1000 = 100000 CFU per ml[15](Figure 3).

Preparation of the Reagent and Solutions

a) Oxidase Preparation

This reagent was prepared by dissolving 1 gm of (tetramethyl-paraphenylene-diamine-dihydrochloride) into 100 ml of D.W and was immediately used for identification the bacteria[13].

b) Catalase Preparation

This reagent was prepared in (3%) using H_2O_2 dilute by D.W and stored in a dark container that was used for identification the bacteria[13].

c) Methyl Red Reagent

Methyl Red Reagent was prepared by dissolving 0.1 gm of methyl red in 300 ml of 95% ethanol and then the volume was completed to 500 ml by D.W[14]. It was used to detect the complete glucose hydrolysis.

d) VogusProskaur Reagent

It is composed of two solutions as below:-

a- A-Naphthol-reagent was dissolved in 100 ml of 99% ethanol.

b- Potassium Hydroxide (KOH) solution: - 40 gm of KOH was dissolved in 100 ml of D.W. and it was used to detect the partial glucose hydrolysis[16].

e) Kovac's Reagent

It was prepared by dissolving 5 gm of P-dimethylaminebenzylaldehyde in 75 of amyle-alcohole and 35 ml of concentrate HCl acid was added. It was used to detect the Indol production[13].

f) Phosphate buffer solution

80 g of NaCl, 0.34 g of $KH_2 PO_4$ and 1.12 g of $K_2 HPO_4$ were dissolved in 1000 ml of D.W. The pH is 7.3 aand the solution is sterilized in autoclave. It was used to detect the Haemagglutination Test[13].

Preparation of the Culture Media

The general culture media described below were prepared by the routine methods and used in appropriate experiments:-

a) MacConkey Agar Medium

MacConkey agar medium was prepared according to the method recommended by the manufacturing company and it is used for the primary isolation of most Gram- negative bacteria and differentiation of lactose fermentative from the non-lactose fermentative .

b) Eosin methylene blue (EMB)Agar Medium

Eosin methylene blue agar medium was prepared according to the method recommended by the manufacturing company and it is used for the primary isolation of most Gram- negative bacteria and differentiation of *E.coli*[16].

c) Nutrient Agar Medium

Nutrient agar medium was prepared according to the manufacturing company. It has been used for general experiment isolate culture, cultivation and activation of bacterial isolates when it is necessary[14].

d) Muller- Hinton Agar

Muller- Hinton agar was prepared according to the method recommended by[18] and it is used in anti-microbial susceptibility testing.

e) Kligler Iron Agar

Kligler Iron agar was used for determining glucose and lactose fermentation and possible hydrogen sulfide H 2S production as a first step in the identification of Gram- negative bacilli[14].

f) Pepton Water Media

Pepton Water Media was prepared and used to detect the ability of bacteria to produce Indole according to the method described by [1].

g) MR-VP Medium

MR-VP medium has been prepared and used to detect the partialandcomplete hydrolysis of glucose[13].

h) Simmons' Citrate Medium

Simmon's Citrate Medium has been used for determining the ability of bacteria to utilize citrate as the sole carbon source [14].

i) M9 Media

6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl and 1 g of NH_4Cl; were dissolved in 950 ml of distill water with 2% agar and then sterilized by autoclave. After cooling, 2 ml of 1M of $MgSO_4$, 10 ml of 20% glucose and 0.1 ml of 1M of $CaCl_2$ (sterilized separately by filtration) were added then the volume was completed to 1000 ml. This media was used for the detection of the Siderophores and Extracellular Proteases production[17].

Laboratory Diagnosis

According to the diagnostic procedures recommended by[14] manual for determinative bacteriology[16,1]. The isolation and identification of gram positive and gram negative bacteria in the otitis media of patients were performed as follows.

a) Microscopy Examination and Colonial Morphology

A single colony was taken from each primary positive culture and its identification depended on the morphology properties (colony size, shape, color and natural of pigments, translucency, edge, elevation and texture) and then colonies that are suspected to be pathogens were selected and investigated by gram-stain to observe a specific shape, color, aggregation and specific intracellular compounds. After staining the bacteria by gram stains, specific biochemical tests were done to reach the final identification.

Physiological and Biochemical Tests

a) Oxidase Test

A filter paper circle was placed into a sterile plastic disposable petridish and was moistured with several drops of the freshly prepared oxidase reagent then a small portion of the colony to be tested was removed and rubbed on the filter paper changing in the color to blue or purple within 10 seconds indicated for a positive result [13].

b) Catalase Test

Catalase test was done by streaking the nutrient agar medium with the selected bacterial colonies and incubated at 37 ºC for 24 hours and then transfer the growth by the steak and put indicated on the surface of a clean slide and add a drop of (3% H_2O_2). The appearance of gas bubbles indicates the positive result[13].

c) Citrate Utilization Test

The surface of simmon's citrate slant medium was incubated with colony of the tested bacteria at 37 °C for 1-3 days. Conversion of the indicator's color from green to blue indicates that the organism was able to utilize citrate as a sole carbon source[18].

d) Kligler's Iron Agar Test for H_2S Production

Only the colonies growing on MacConkey agar were touched by a straight wire and inoculated on the media by stabbing the bottom of the tube and streaking the slant. Fermentation was detected by a change in the indicator phenol red to yellow. The pH changes in the bottom and the slant of medium were recorded after 18 - 24 hours of incubation of gas formation which is usually visualized as bubbles in the medium caused by the gas formed in the agar. Organisms can produce H_2S form black precipitate in the bottom[13].

e) Indol Test

Tubes containing a peptone water medium were inoculated with the colony of the tested bacteria and incubated at 37 °C for 18 hours then several drops of kovac's reagent were added to the broth medium. After shaking, the appearance of the red ring on the surface was regarded as a positive result[18].

f) Methyl Red Test

The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at 37^{o}C for 24 hours then 5 drops of methyl red reagent were added to it. The appearance and observation of red colour indicates a positive result and a complete analysis of glucose[14].

g) VogusProskaur Test

The tubes of (MR-VP broth) were seeded with the specific bacterial culture and were incubated at 37°C for 48 hours then the result was obtained by adding (0.6 ml of α- naphthol reagent) and (0.2 ml of 40% KOH solution); appearance of red colour after 15 min. Positive result due to partial analysis of glucose, which produce acetone or (Acetyl methyl-carbinol) [14].

Isolation, identification and numeration of *E. coli*

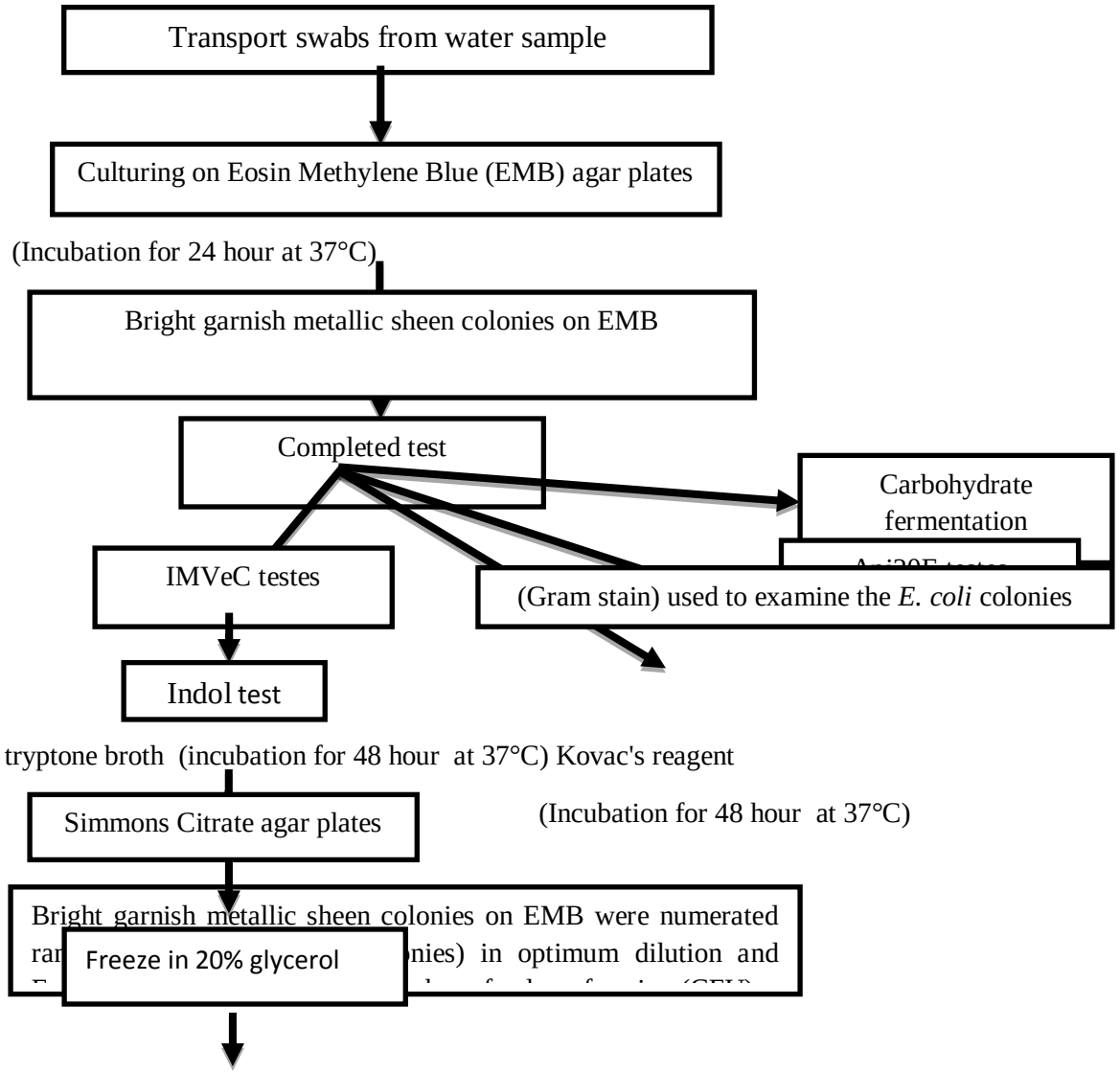


Figure 3: Method of isolation, identification and numeration of *E. Coli*

h) Motility test

This test was carried-out as mentioned in[14]. Figure 3.4 shows the flowchart and method of isolation, identification and numeration of *E. coli*.

Physicochemical Characteristics

Parameters involved

The temperature, salinity and pH is measured directly by YSI 556 MPS devices and water clarity measured by the secchidisk(Table 3). As well as the secchi disk was slowly lowered into the water until it is no

longer visible and the depth was recorded. Then, the secchi disk was slowly raised until the pattern clearly seen and the depth was recorded. The both value was used to get the average value for secchi depth. This procedure follows[19] as guidelines.

Figure 4: Secchi disk

Table 3 List of parameters, method and instrument used

Test Method	Parameters	Instruments used	Unit
In-situ	Secchi depth	Secchi disk	M
	pH	YSI 556 MPS	-
	Temperature	YSI 556 MPS	°C
	Salinity	YSI 556 MPS	mg/L

Statistical Analysis

Data were expressed as the means values ± standard deviation. Mean of minimum three measurements were compared by the analysis of variance (ANOVA). Significant differences between means were determined by Duncan ($P<0.05$). The SPSS ver. 19 software was used for this statistical analysis[20].

Results and Discussion

Introduction

Eosin Methylene Blue (EMB, also known as "Levine's formulation") is a selective stain for Gram-negative bacteria. EMB contains dyes that are toxic for Gram positive bacteria and bile salt which is toxic for Gram negative bacteria other than coliforms. EMB is the selective and differential medium for coliforms. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1. A common application of this stain is in the preparation of EMB agar, a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., *E. coli*) and those that do not *(e.g.,Salmonella, Shigella*). Organisms that ferment lactose display nucleated colonies, colonies with dark centers [21].

This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time[22].

a. Rapid lactose fermentation produces acids, which lower the pH. This encourages dye absorption by the colonies, which are now colored purple-black.
b. Lactose non-fermenters may increase the pH by deamination of proteins and ensures that the dye is not absorbed. The colonies will be colorless.

On EMB, *E. coli* that is grown will give a distinctive metallic green sheen (due to the metachromatic properties of the dyes, *E. coli* movement using flagella and strong acid end-products of fermentation). Some species of Citrobacter and Enterobacter will also react this way to EMB. This medium has been specifically designed to discourage the growth of gram positive bacteria. EMB contains the following ingredients: peptone,

Lactose, Dipotassium phosphate, Eosin Y (dye) and Methylene blue (dye) Agar. There are also EMB agars that do not contain lactose[21].

E.coli is a single celled organism that is the most numerous organism found on earth. *E. coli* is one of the most important *Enterobacteriacea* species that is gram negative rode, usually motile, produce polysaccharide capsule, positive tests for indol, lysine decarboxlase and mannitol fermentation and produces gas from glucose. *E. coli* is one subgroup of fecal coliform bacteria. Even within this species, there are numerous different strains, some of which can be harmful.

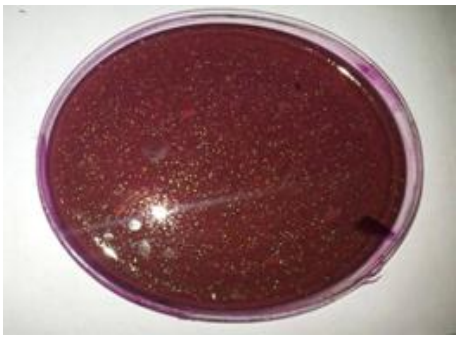
*E.coli*produces gas from glucose and it is considered as one of the most important *Enterobacteriaceae* species and typical colonial morphology with an iridescent sheen on differential media such as Eosin-methylene blue (EMB) agar. They grow on non-selective media with most strain ferment lactose producing large red colony on macconkey agar and Bright garnish metallic sheen colonies on EMB which is characteristic of *E. coli*.

Isolation and Identification of *E.coli*

Figure 7 shows the colonies of coliform bacteria that were found for five sampling stations along the AlurIlmu, UKM. It was found that the highest colonies of*E.coli* were found at sampling station 2(Close to the Faculty of Science and Technology and Cafeteria) ranging from 315 colonies to 328 colonies and the average values of 322.6 colonies. Colonies of coliform bacteria values at the sampling station 1 ranges between 300 colonies to 330 colonies, with the average values of 316.6 colonies. Colonies of coliform bacteria at the sampling station 3were found between 210 colonies to 290 colonies, with the average value of 260 colonies.Based on one-way ANOVA, there was significant difference in *E.coli* reading between sampling stations ($P < 0.05$) for all three samplings. Table 6 shows the results of the ANovA analysis for the *E.coli* between stations in three samplings.

No colonies of *E.coli* are found at the sampling stations 4 and 5. There is no growth due to the lack of the wastewater, drainage and facilities because of the speed of the water runoff in those locations. Thus, the water becomes unstable, leading to difficulty in obtaining *E.coli*[23]. Figures 5 and 6 show the growth and no growth of colonies, respectively.

[24]studied isolation and identification of pathogenic bacteria from drinking water of Khairpur, Sukkur and Rohri and found that the range of *E.coli* colonies were between 275 to 300, consistent with the values of *E.coli* recorded in this study. According to the[26], the value of *E-coli* colonies is high because the sampling was taken in the dry season. Furthermore,[27] studied antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho located at the north-west province of South Africa. They found that a total of 230 *E. coli* isolated were obtained following biochemical characterization. High value reading due to increasing amount of wastewater, manure and sewage located at the surrounding areas[23].



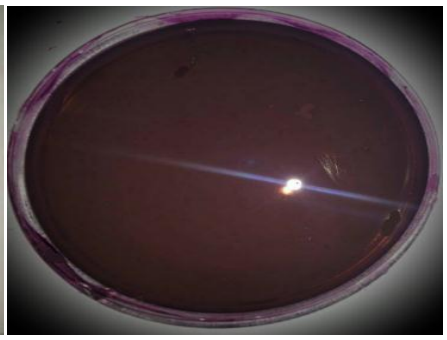


Figure 5: Growth of the colonies **Figure 6 : No growth of the coloies**

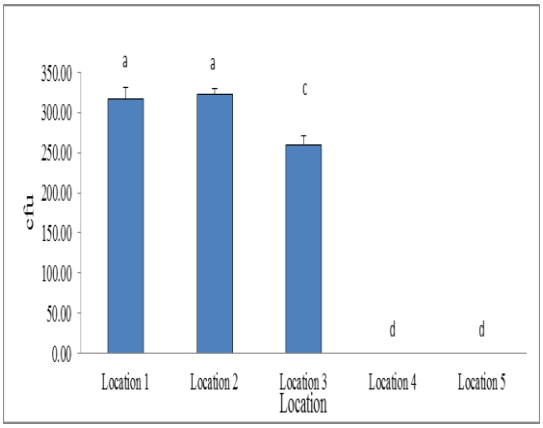


Figure 7:E.coli colonies at five sampling stations in AlurAlmu, UKM

Diagnosis of *E. coli* Bacteria

This group of bacteria was identified by conventional method according to[14] using staining and culturing methods. The gram negative bacteria represented by *Escherichia coli* was identified as short rods shape, indol positive produces tryptophanase, thus able to degrade the amino acid tryptophan into pyruvic acid, ammonia and indole. It was also identified as methyl red positive (production of acid from complete fermentation of glucose), Voges-Proskauer negative, utilization of Simmons citrate was negative and colonies on eosin methylene blue (EMB) agar which appeared as green metallic sheen and catalase test was positive for this organism (able to catalyse hydrogen peroxide H2O2). Table 4 shows that the biochemical tests such as (indole, catalase, coagulase, oxidase, methyl Red and Api- 20E technique, etc.) which are used to recognize the *E. coli.*

Table 4Diagnosis by biochemical tests for gram negative bacteria.

Biochemical tests	***E. coli***
Gram stain	-ve Rod
Coagulase	-
Mannitol fermentation	-
Motility	-
Catalase	+ve
EMB	Appear green metallic sheen
Indole	+ve
Methyl red	+ve
Voges Proskauer's	-ve
Citrate utilization	-ve
Urease	-
Hemolysis Api- 20E technique	- +ve

Indol Test

Figure 8 shows the positive result of *Escherichia coli* in indole test. This test was done by inoculating tubes containing a peptone water medium with the colony of the tested bacteria and incubated at 37 °C for 18 hours with several drops of kovac's reagent were added to the broth medium. A positive result *Escherichia coli* was found when the appearance of the red ring on the surface after the tube was shaked[18].

Figure 8: Positive results of*Escherichia coli* in indole test

Catalase test

The catalase test was used to detect the ability of bacteria to produce catalase enzyme. It was carried-out by mixing a single isolated colony transferred by a woody stick with a drop of hydrogen peroxide (30% H_2O_2). Figure 9 show that the production of gas bubbles indicates a positive result[18].

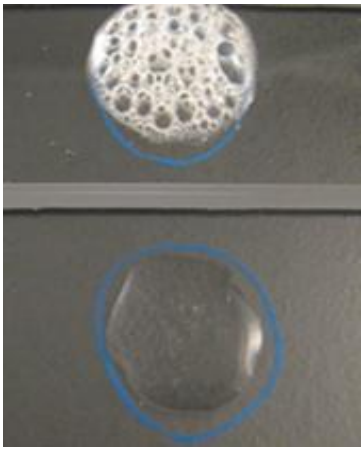


Figure 9: Positive results of *Escherichia coli* in catalase test

Coagulase test

The coagulase test was used to detect the ability of an organism to clot plasma by the action of the enzyme coagulase. Coagulase slide method was applied to detect the bound coagulase that is found on the surface of cell walls. After emulsifying *E.coli* colony with a drop of sterilized normal saline on a clean slide, one drop of human plasma was added and mixed gently. Later coagulase positive organisms became clumped after a few seconds. The comparison of the result control test was done by mixing saline and bacteria without plasma to ensure that the organisms do not clump spontaneously (Figure 10)[27].

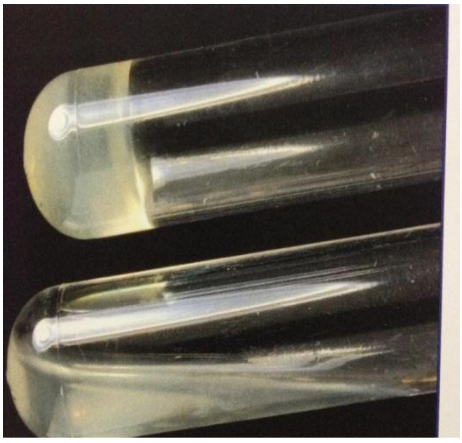


Figure 10: Negative results of *Escherichia coli* in coagulase test

Oxidase test

The oxidase test was performed by transferring a part of bacterial growth by sterilized woody sticks into filter paper saturated with recently prepared oxidase reagent (TetramethylP-Paraphenylenediamine hydrochloride). Turning the color from white to dark purple indicated the positive result, as shown in the Figure 11[14].

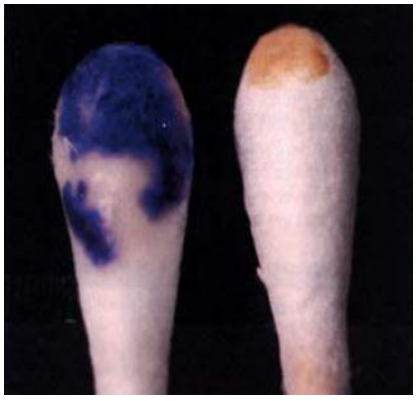


Figure 11 Negative results of *Escherichia coli* in oxidase test

Methyl Red Test

The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at 37 ^{o}C for 24 hours, then (5 drops) of methyl red reagent were added to it. The appearance and observation of red colour means a positive result and a complete analysis of glucose (Figure12) [14].

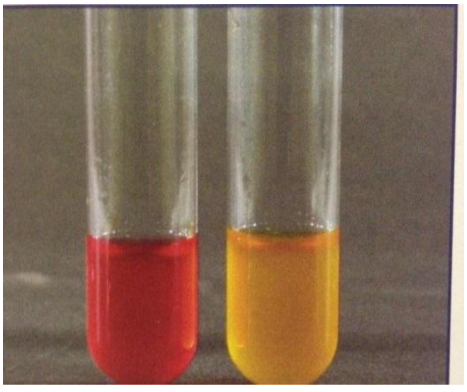


Figure 12 Positive results of *Escherichia coli* in methyl Red Test

Citrate Utilization Test

Figure 13 shows the surface of simmon's citrate slant medium that was incubated with colony of the tested bacteria at 37 °C for 1-3 days. Conversion of the indicator's color from green to blue indicates that the organism was able to utilize citrate as a sole carbon source[18].

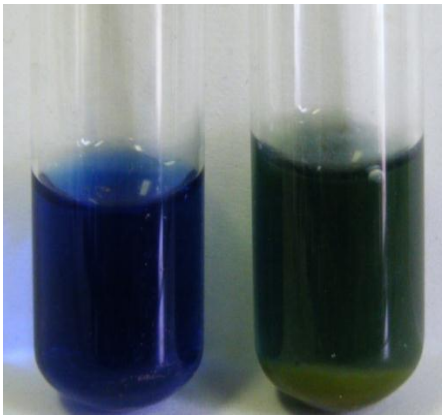


Figure 13 Negative results of *Escherichia coli* in Citrate Utilization Test

VogusProskaur Test

Figure 14 shows the tubes of MR-VP broth that were seeded with the specific bacterial culture and were incubated at 37°C for 48 hours then the result was obtained by adding 0.6 ml of α- naphthol reagent and 0.2 ml of 40% KOH solution and red colour was appeared after 15 min. Positive result due to partial analysis of glucose, which produce acetone or Acetyl methyl-carbinol[14].

Figure 14 Positive results of *Escherichia coli* in VogusProskaur Test

Analytical Profile Index (Api 20E) technique

Api 20-E system consists of 20 micro tubes containing dehydrated substrates. Micro tubes were inoculated with a bacterial suspension; metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading (Table 4.2) and the identification is obtained by referring to the analytical profile[24,25].

The identification of Gram-negative bacteria was based on Api-20E-system (Table 5) and the cultural characteristics, biochemical properties of these bacteria revealed that these isolates belonged to the bacterial *E. coli* (Figure 4.11).

Figure 14 Positive results of *Escherichia coli* in Api 20ETest

Table 5 Biochemical properties of *E. coli* that isolated form AlurIlmu, UKM

Bacterial isolates	Catalase	oxidase	gelalatinase	lipase	capsule	hemolysin	motility	spore	Growth on KIA media
E. coli 1	+	-	-	-	+	ß	+	-	A/A G
E. coli 2	+	-	-	-	-	ß	-	-	A/A G

Physico-Chemical Characteristics of *E. coli*

There are some physicochemical characteristics related with *E.coli* that can influence the number of colonies in the water body**.**

Water clarity

The water clarity was analyzed using secchi disk and it was found that the values for five sampling stations range between37.00 cm to 62 cm (Figure 15). The sampling station 3 found to have the largest value ranges between 56 cm to 62 cm. The lowest value for water clarity was found at sampling station 5 ranges between 38 cm to 40 cm.

Based on one-way ANOVA, there was significant difference in water clarity reading between sampling stations ($P < 0.05$) for all three samplings.Table 6 shows the results of the ANovA analysis for the Water clarity parameter between stations in three samplings.According to [29] that studied water quality monitoring of an urban stream for total maximum daily load assessment, the range of water clarity were between 42cm to 71 cm, where this values similar to the values of water clarity recorded in this study. Various factors influence the turbidity in AlurIlmu, UKM such as the rate of suspended solids which consist of organic and inorganic matters like bacteria, sediment, algae and other contaminants. In addition factor of weather, particularly during heavy rainfall can also affect water flow, which in turn affects turbidity. Rainfall can increase stream volume as well as stream flow that subsequently suspend settled sediments and erode riverbanks[28].

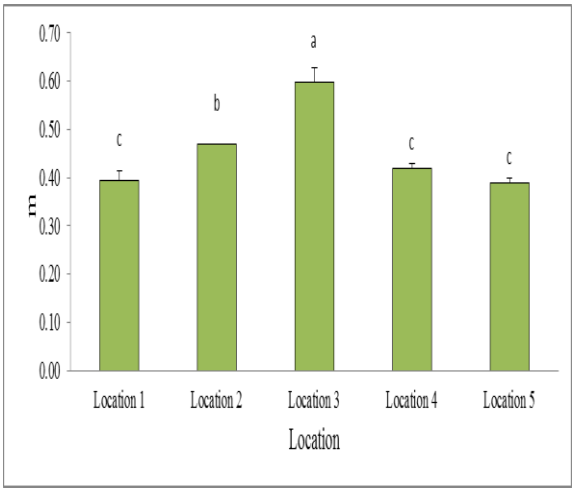


Figure 15: Water clarity at five sampling stations in AlurIlmu, UKM

pH of sample station:

Figure 16 shows the pH values at five sampling stations in AlurIlmu, UKM. pH values at the five sampling stations ranging between 3.1 to 7.8 with the highest pH was recorded at the sampling station 3, while the lowest pH was found at the sampling station 5. Based on one-way ANOVA, there was significant difference in pH reading between sampling stations ($P < 0.05$) for all three samplings. Table 6 shows the results of the ANovA analysis for the pH parameter between stations in three samplings.

Optimum pH value for the growth of *E.coli* is between 5 to 9. In this study, it was found that the pH values for sampling stations 1, 2 and 3 are within this range that resulted in high number of colonies found for these three sampling stations. However, the pH values for the sampling stations 4 and 5 are found to be lower preventing the growth of the bacteria due to the high water level and flow velocity. This line of reasoning is consistent with[32]explaining the relationship between the growth of bacteria with the value of pH.[31] studied pH in AlurIlmu and the range of pH were between 5.44 to 8.84, where this values similar to the values of pH recorded in this studied, where the value reading high because the structure of the surface water pathway which is built from cement and concrete, which cause the alkaline for water in AlurIlmu. There are many factors that can affect pH in AlurIlmu, UKM, such as fluctuation in pH due to precipitation (especially acid rain) and wastewater. In addition, CO_2 concentrations can also influence pH levels, the presence of these factors will cause increase pH level and the absence will cause decrease pH level [30].

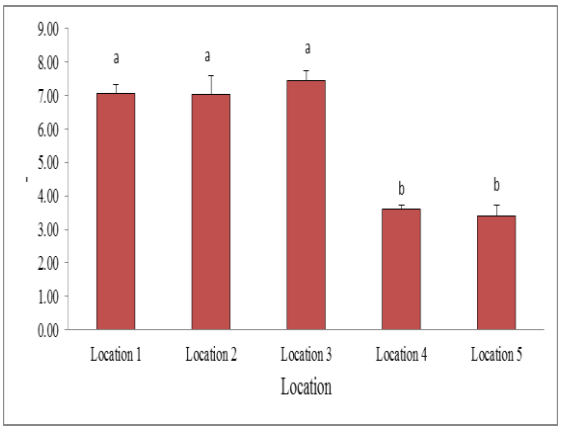


Figure 16: pH at five sampling stations in AlurIlmu, UKM

Temperature

The temperature values for five sampling stations were taken in-situ at three different points. The temperature for all selected sampling stations range between 24. 1 ^{o}C to 32.3 ^{o}C as shown in Figure 17.Based

on one-way ANOVA, there was significant difference in temperature reading between sampling stations (P <0.05) for all three samplings.Table 6 shows the results of the ANovA analysis for the temperatureparameter between stations in three samplings.According to [31]study in AlurIlmu, the range of temperature was recorded between 26.75 ^{o}C to 31.35 ^{o}C, there is little change in the temperature compare with this study where the temperature reading is high because the waterway bottom surface constructed from concrete, as well as the sunlight can penetrate directly because of less obstruction from the tree, while the lower temperature reading as the sunlight was being blocked by trees and faculty building.

Optimum value of temperature for the growth of *E.coli* is at a temperature of 37 ^{o}C but it can grow in any temperature ranges between 28 ^{o}C to 40 ^{o}C. The reason of bacterial growth in the first three sampling stations is due to the suitable value of temperature at these stations that encourage the growth of the colonies (Sampson 2006). Water temperature in AlurIlmu, UKM can be affected by various ambient conditions. These elements include sunlight or solar radiation, heat transfer from the atmosphere, stream confluence and turbidity. Shallow and surface waters are easily influenced by these factors as compared to the deep waters [30].

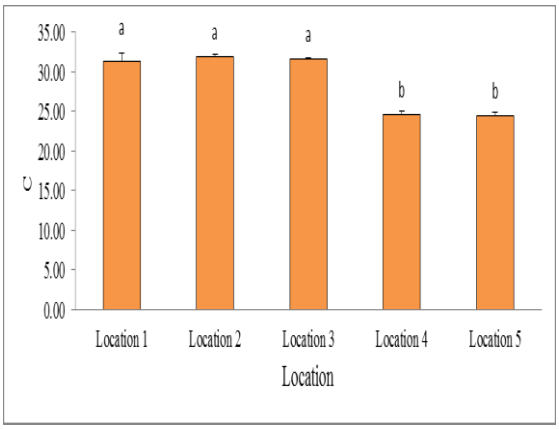


Figure 17: Temperature at five sampling stations in AlurIlmu, UKM

Salinity

The value of salinity at three different points for five sampling stations in AlurIlmu, UKM were obtained and shown in Figure 18. The salinity ranges from 0.01 mg/L to 0.04 mg/L. The lowest value for salinity was recorded for both sampling stations 1 and 2, while the highest value was found for the sampling stations 4 and 5.Based on one-way ANOVA, there was significant difference in salinity reading between sampling stations (P <0.05) for all three samplings. Table 6 shows the results of the ANovA analysis for the salinity parameter between stations in three samplings.

Optimum value of salinity for the growth of *E.coli* is at a salinity of 0.01 mg/L - 0.03 mg/L).In this study, it was found that the salinity values for sampling stations 1, 2 and 3 are within this range that resulted in high number of colonies found for these three sampling stations. However, the salinity values for the sampling stations 4 and 5 are found to be higher preventing the growth of the *E.coli*.According to[34], response of biotic communities to salinity changes in a Mediterranean hypersaline stream and the range of salinity value 0.35 mg/L to 0.42 mg/L found in their study shows where this values similar to the values of salinity recorded in this study.The low of salinity was recorded because of there is no intrusion and source of saltwater that flows into the AlurIlmu, UKM like dissolved salts, such as chloride, sodium, magnesium, sulfate, calcium and potassium[30].

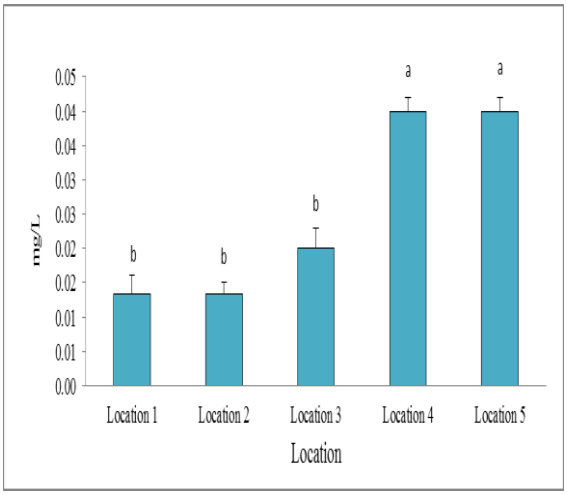


Figure 18: Salinity at five sampling stations in AlurIlmu, UKM

Table 6ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ecoli	Between Groups	330694.400	4	82673.600	189.647	.000
	Within Groups	4359.333	10	435.933		
	Total	335053.733	14			
Turbidity	Between Groups	.089	4	.022	66.470	.000
	Within Groups	.003	10	.000		
	Total	.092	14			
Ph	Between Groups	49.097	4	12.274	109.162	.000
	Within Groups	1.124	10	.112		
	Total	50.221	14			
Temperature	Between Groups	183.420	4	45.855	142.690	.000
	Within Groups	3.214	10	.321		
	Total	186.633	14			
Salinity	Between Groups	.002	4	.001	7.636	.004
	Within Groups	.001	10	.000		
	Total	.003	14			

Conclusions

The isolation and identification of *E.coli* from AlurIlmu, UKM was carried-out in this study in order to evaluate the distribution of *E.coli* in the water body. Results of the study show that the number of *E.coli* is increasing during dry season compared to the wet season in addition, the number of *E.coli* also depending on the several factors such as sedimentation rate, drainage system and sewage waste water system that can affect the growth of colonies in AlurIlmu.

Besides, others physico-chemical parameters such as temperature, salinity and pH were also measured to assess the correlation between these factors with the growth of *E. coli*. In this study, it was found that high number of *E.coli* is recorded between the ranges of temperature between 28 ^{o}C to 40^{o}C. For pH, high number of *E.coli* is found between pH 5 to 9. The results show that the number of *E.coli* is high for salinity within a range of 0.01 to 0.03. In addition, the results of the study also show that the number of *E.coli* is high from the sampling stations located near to the FST building and cafeteria compared to sampling stations located near to the entrance of UKM. These stations have high number of *E.coli* maybe due to a various number of sources that coming from the leakage from the sewage systems that make them prone to the contamination.

In order to reduce the number of *E.coli*level in AlurIlmu, proper wastewater systems with totally function are needed. The wastewater systems built nearby AlurIlmu are not fully under maintenance which causes the increasing level of *E.coli* that was found at some sampling stations. High level of precipitation could result in decreasing number of the *E.coli* level in the water body. Construction or maintaining the sewage treatment plants could help to prevent the leakage to the AlurIlmu. Although the construction and operation of such systems are expensive, they are necessary to protect water quality and public health. Besides having regularly water quality assessment and upgrading the wastewater system located nearby AlurIlmu, a series of awareness campaigns and programmes should be conducted to educate students and local communities about the importance of conserving AlurIlmu that serves as a symbol of UKM.

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