Microbiome of an artificial inland water body (Dhanmondi Lake)

A Project

Submitted by

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This work is dedicated to my parents and elder sister for their love and constant support…

Certification statement

This is to certify that this project titled “Microbiome of an artificial inland water body (Dhanmondi Lake)” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Mohd. Raeed Jamiruddin, Assistant Professor, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

_____

Countersigned by the supervisor,

_____

Acknowledgement

All praises are solely due to Almighty God whose mercy enabled me to complete this research work and preparation of this thesis successfully.

I express my wholehearted feeling of appreciation, earnest gratefulness, massive obligation and significant respect to my honorable supervisor Dr. Mohd. Raeed Jamiruddin, Assistant Professor, Department of Pharmacy, BRAC University for his untiring direction, important proposals, warm support, accurate remarks and nonstop supervision all through the time of the exploration work and planning of this manuscript. I am also thankful to my honorable teachers, the chairperson of the pharmacy department, Professor Dr. Eva Rahman Kabir for their inspiration and astounding instructions.

At long last, I acknowledge my most profound feeling of regard to my dearest guardians and wishes to express the deepest love and affection to my elder sister, relatives, companions, and well-wishers for their steady endeavors, friendly emotions, favors and co-activity in all periods of my academic interest.

Abstract

Water is essential for life but it can also be a source of various human related diseases if it is contaminated by microscopic organisms. The study was conducted to isolate and characterize gram-negative bacteria isolates present in lake water used by humans. Water sample was collected from Dhanmondi Lake of Dhaka in a 250 ml of Duran bottle. After the sample collection 20ml of water sample was mixed in nutrient broth for the pre-enrichment of microorganisms. When culture was observed in nutrient broth a loopful of enriched culture was streaked into MacConkey agar plate for the separation of gram-negative bacteria. Total twenty-four bacterial colonies were selected from the MacConkey plate that were further characterized by observing their morphology and by performing biochemical tests which included "Indole test, Methyl Red test, Voges-Proskauer test, Citrate test, Kligler's Iron agar test and Motility test". After performing the entire test, it was found that among twenty-four isolates 41.66% was *E. coli*, 29.16% was *Klebsiella*, 8.33% was *Pseudomonas*, 8.33% was *Shigella*, 8.33% was *Citrobacter* and only 4.16% was *Enterobacter*. PCR method can be used for the precise detection of the species of this organism. Following the study, the desire is to become aware about the presence of these bacteria in water and ensure a safe use of water.

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List of Symbols

ºC	Degree Celsius
%	Percentage
psi	Pound per square inch
µl	Micro liter
µm:	Micrometer

List of Abbreviation

UV	Ultraviolet
EPS	Extracellular polymeric substances
e.g.	For example
g	Gram
g/l	Gram per liter
hrs	Hours
MAC	MacConkey agar
IMVIC	Indole production, Methyl Red test, Voges-Proskauer test andCitrate utilization test
MR	Methyl Red test
VP	Voges-Proskauer test
KIA	Kligler's Iron Agar (KIA) Test
ml	Milliliter
mg	Milligram
pH	Negative logarithm of hydrogen ion
L	Liter
NB	Nutrient broth
rpm	Revolution per minute

1. Introduction

Water is used as a medium for the circulation of microscopic organisms. Drinking safe water is a key right and on the off chance that it is defiled with different pathogenic ecological tiny living beings then it may cause genuine medical problems. Human wellbeing ought to be secured by anticipating microbial pollution of water that is expected for utilization. In rural areas people use polluted river, lake and stream water for drinking and other local purposes. These unprotected environmental waters may be polluted by microorganisms via rainfall, overflow and rural information, blending with waste matter effluent and faces from wild-life, which make it unsatisfactory for utilization by people (Onuoha, 2017).

The accessibility of safe drinking water shows an overall test, and experts support significant endeavors in water amount and quality confirmation. The quality of water can be broken down because of the nearness of microorganisms in it as a few apparent demonstrates that the majority of the water-related infections are caused by microbial defilement (WHO 2008; Riley et al. 2011). The advancement of microorganisms in water can be portrayed by two ways: achievement and outside defilement, trailed by further growing. In a study it is found that, the percentage of bacterial number arranged in the surface and stage of water are about 95% and 5% and recognized by a standard procedure of internal control moderation (Fleming et al. 2002).

Bacterial pollution of drinking water is a noteworthy supporter of water-borne illnesses, for example, loose bowels, sickness, gastroenteritis, typhoid looseness of the bowels and other wellbeing related issues, particularly in youngsters and people with powerless safe systems. Wellsprings of fecal coli form present in water can incorporate release of effluent from the septic framework, agricultural spillover, invasion of household or wild creature waste and sewage discharge. (Yang et al., 2013). As indicated by “UNICEF” report, around 800 million individuals in “Asia” and “Africa” are not getting the purified water for drinking. As a result of this, numerous individuals endure from different infections. In any case, deficient amount, low excellence of water for drinking, and poor recycling of waste are the fundamental cause in occurrence and commonness of sicknesses in worldwide. (Bayatti et al., 2012). The radial nearness of pathogens presents in water and pollution of water can be detected by indicator organisms. Coliform microscopic organisms are probably not going to cause sickness. In any case, their

quality in water shows that sickness causing life forms (pathogens) that might be related with “intestinal diseases, looseness of the bowels, hepatitis, typhoid fever, cholera” and other ailments could be available in the water system. Their nearness in drinking water would thus be able to be viewed as a sign of fecal contamination and conceivable falling apart water quality (Mulamattathil, Bezuidenhout and Mbewe, 2015).

Lakes are superb frameworks for researching bacterial network elements since they have clear limits and solid natural angles. Microbial biology foresees bacterial network structure and it is a significant objective. Notwithstanding, we just have a shallow information of the variables that would enable us to foresee bacterial network elements. To describe the decent variety and progression of a biological system's bacterial community, testing a similar site various occasions is similarly as important as inspecting repeat environments (Linz, Crary et al, 2017). Because of the high growth rate, bacterial communities can go through a rapid change than the communities of macro-organisms. An examination of a period arrangement traversing 1 to 3 years discovered positive species-time connections, showing that more categories were seen as the term of testing increased, because of the deficient process, elimination and migration (Shade, Caporaso, Knight and Fierer, 2013). Bacterial time arrangement shows time decay, implying that the communities keeps on winding up more different from that present at the underlying inspecting occasion as the time from that occasion expands (Faust, Lathi, Vos and Raes, 2015). On the other hand, bacterial communities can likewise change continuously over prolonged stretch of time, as they are not ready to accept the changes in ecological equation, for example, supplement, accessibility and temperature. The amount and proportion of bacterial communities are not same in the different layer of water so when gathering tests for the investigation of bacterial networks water profundity acts as a critical factor that ought to be considered. (Garcia, 2013).

Lakes that has different layers, vertical zonation in the synthesis of bacterial communities is generally accounted for by changes in ecological conditions, for example, “UV radiation, temperature, concentration of dissolved oxygen and supplement components” (Wever, 2005; Corno, 2009). Bacterial reaction to temperature can surpass a metabolic impact and can be populace or network particular. Psychrophiles develop ideally at temperatures <15°C, with an upper temperature of 20°C (Morita, 1975), and are seen in an extensive variety of situations for instance, the shoreline front Arctic Ocean (Connelly et al., 2006), sea ice (Kottmeier and Sullivan, 1988; Huston, 2000) and

Antarctic saline lakes. Psychrotolerant organisms are in like manner found in cool living spaces, anyway have higher perfect development ("~temperatures 20°C") and can resist temperatures as low as - 10°C (Bakermans, 2006). At a given temperature, "Psychrophilic" and "Psychrotolerant" organisms respond particularly in perspective of useful impediments, for instance, layer smoothness and chemical structure (Feller, 1997; Russell, 2000). The gathering of these tiny creatures is seen in various areas, and their opposition in view of people specific advancement rates may in like manner be controlled by temperature, which is bringing about an evaluated temperature response that is network specific. While particular cells move their physiology to respond to temperature changes, the power of a people inside a network will change in light of contention between bunches that have assorted advancement rates at different temperatures or natural conditions (Adams, Crums, Kling, 2010). When favorable conditions wind up positive the microbes despite of being modestly latent or in low densities present in flawed temperature may increase in quality. In trademark networks, the optical perfect temperature for a network can move every so often (Tison, Pope, Boylen, 1980), showing that with different perfect temperatures and substrate affinities the accessibility of bacterial networks change (Ogilvie et al, 1997).

High mountain lakes (i.e. situated over the tree line) in comparison with lowland lake are harsh conditions notwithstanding for microscopic organisms in light of the fact that these biological communities experience moderately quick changes in natural conditions, for example, light, supplement concentration, UV exposure and temperature. Lakes situated highly are extremely delicate to natural changes and therefore especially fascinating with respect to radial difference in the bacterial network composition. In high mountain lakes the bacterial networks contrasts amongst various layers of water (Hortnagl, Perez, Zeder, Sommaruga, 2010). In the water layers of snow, slash and lake situated at high mountain pool of the Alps, Austria, and Hervas the bacterial networks show differences in their composition (Alfreider, 1996). Casamayor (2009) discovered subjective contrasts between bacterial network creation of a high mountain lake surface miniaturized scale layer and the underlying water situated in the "Pyrenees of Spain".

Then again, Soda lakes are seen as exceptional to all other maritime natural frameworks in the meantime showing higher rate of productivity (> 10g/cm²per day), and high pH (9.0– 12.0) and saltiness (up to submersion centers). Many pop lakes experience tremendous intermittent or enduring microbial blooms consistently achieving specific

tinge of the lake water. Development dependent and free outlines of pop lakes in the "East African Rift valley" realized the disengagement of a couple of hundred strains of vigorous, "heterotrophic" and "haloalkaliphilic" Bacteria and "Archaea" (Duckworth et al., 1996; assessed in Grant, 2011) and the recognizable proof of a couple of novel heredities of putative "Bacteria" and "Archaea" (Grant, 1999; Rees, 2004).

In case of dead sea, the expanded saltiness and the raised focus of divalent particles from the Dead Sea an outrageous situation that isn't endured by generally life forms. This is seen in a by and large low assorted variety and low plenitude of microorganisms. The general cell wealth in the Dead Sea water is low ($<5\times10^4$ cell/mL), with the exception of two blossoms in 1980 and 1992, when after serious winters the above meter of the water section was weakened by 15– 30%, floods gave a contribution of phosphate, and the cell focuses accomplished 20– 35×10^6cells/mL (Oren and Gurevich, 1995).

On the other hand, the arrangement of ocean ice bacterial networks is habitually unique in relation to that in seawater. Bacterial development in ocean ice is represented by the communication of saltiness, temperature, and supplements and in addition biotic factors, for example, grazing. Bacteria appear to end up entrained in ocean ice alongside phytoplankton while physical advancement alone seems to be incapable. Other conceivable clarifications for bacterial sediment in ocean ice are gas vesicle and connection with the micro gels in the continuous series of EPS (extracellular polymeric substances) (Deming, 2011). In recently formed ocean ice, bacterial movement could be repressed, yet as the ice joins together, bacterial action appeared to increment and psychrophilic microbes turn out to be more abundant. There is prove that ensuing advancement of the bacterial system is more subject to the accessibility and liability of natural issue than on temperature (Helmke et al, 1995).

Consumable water contains a wide assortment of microscopic organisms, a large number of which are ineffectively portrayed or have not yet been examined. A noteworthy extent of the cultivable heterotrophic populace in drinking water falls into a vast group referred to as gram-negative bacteria (Ward, Wolfe, Justice and Olson, 1986).

1.1 Objective

The objective of the study is to collect the lake water sample and perform primary study to isolate gram-negative bacteria present in it. The final objective of this study is morphological and biochemical characterization of this gram-negative bacteria.

2. Methodology

2.1 Experimental design

The schematic representation of the experimental procedures performed in my study is summarized below (Figure 2.1)

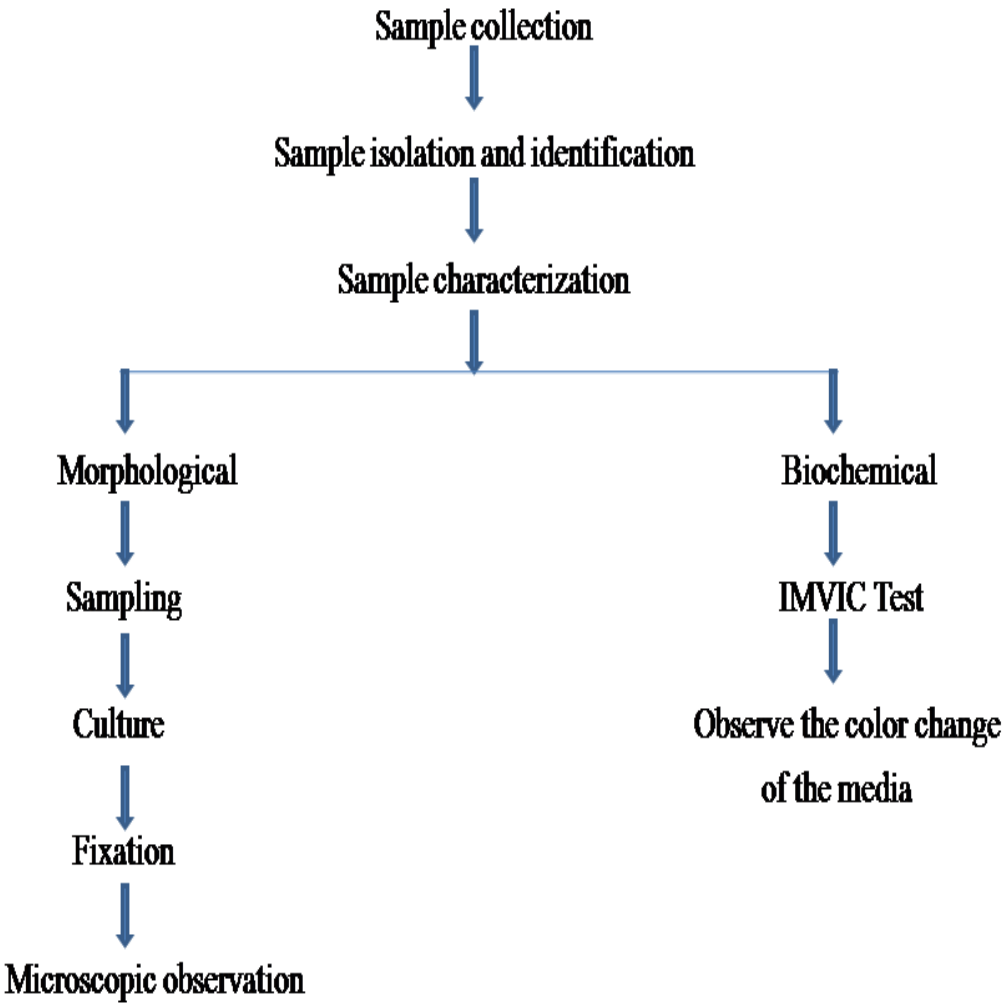


Fig 2.1: The schematic presentation of experimental workflow.

2.2 Sample collection and Inoculation of sample in Nutrient Broth

Water sample was collected from Dhanmondi Lake in a 250 ml Duran bottle that was previously autoclaved at 121ºC for 45 minutes. The sterile bottle was immersed one foot below the water surface. Confronting the heading of the current, the Duran bottle was filled with water by opening the top, conveyed to the surface and immediately closed its mouth with the lid. Then the sample was properly labeled by indicating the date, source and time of collection. For the growth of the sample microorganism's 80 ml nutrient broth was prepared. Nutrient broth was used for the pre-enrichment of the sample microorganisms. For the preparation of Nutrient broth 1.04gm of nutrient broth was dissolved in distilled water of an amount of 50ml and it was then transferred in another Duran bottle and autoclaved. 20 ml water sample was taken from the 250 ml and then it was transferred into 80 ml of the nutrient broth. It was then incubated at 37ºC for 24hrs for observing the growth.

2.3 Isolation and Identification of Gram-negative Bacteria

Loopful of enriched culture from the nutrient broth indicating the growth of microorganisms were streaked in MacConkey agar medium for the isolation of "gram-negative" bacteria and then incubated at 37ºC for 24hrs. "MacConkey" agar is a differential media use for the detection of the gram-negative bacteria and for the separation of bacteria that can ferment lactose (Allen, 2005). After the growth of "gram-negative" bacteria on MAC agar total twenty-four colonies from four MAC agar plate were identified and marked. The colonies were then picked and streaked onto another fresh MAC agar plate for further growth and incubated at 37ºC for 24 hrs. After the growth of the twenty-four gram-negative bacteria colonies they were further streaked and sub-cultured onto MAC agar plate and incubated at 37ºC for 24hrs for purification to possess a pure culture. By seeing morphological characteristics, using gram staining and biochemical tests ("Indole, Methyl Red, Voges-Proskauer, Citrate, Motility and KIA test") the pure cultures were then identified and potentially affirmed.

Table 2.1: Plate name and Isolates with their given ID

Plate Name	Isolate ID
A	A1, A2, A3, A4
B	B1, B2, B3, B4
C	C1, C2, C3, C4, C5, C6, C7, C8
D	D1, D2, D3, D4, D5, D6, D7, D8

2.4 Characterization of the selected bacterial isolates

2.4.1 Morphological Characterization

2.4.1.1 Growth on MacConkey media

Morphology of the twenty-four bacterial colonies and their appearance on the MAC agar plate were observed after the incubation at 37ºC for 24hrs. The color, shape, size and the surface of the colonies were observed and the appearance of the cultures was recorded.

2.4.1.2 Gram staining and Microscopic Observation

Bacterial isolates that were grown on MacConkey agar plate were stained to distinguish the Enterobacteriaceae a large group of gram-negative bacteria (under which there are both lactose fermenting and lactose non-fermenting bacteria) from gram-positive bacteria by observing their color and shape. In this procedure crystal violet (primary stain), gram iodine solution (mordant), 95% ethanol (decolorizing agent), safranin (counter stain) were used. For performing the gram staining smear were prepared by fixing the bacterial cells on the slide. Then heat-fixed smear of cells was stained with crystal violet reagent for 1 minute. After washing the reagent with distilled water slides were flooded with gram's iodine for 1 minute and form a complex between crystal violet and iodine. Then rinsed it gently with 95% ethanol to decolorize the slide. The outer membrane of the gram-negative bacteria was degraded and thin peptidoglycan layer of gram-negative bacteria cell could not retain the crystal violet-iodine complex and lost the color. After

that the slide were stained with safranin and left it for about 30 seconds to 1 minute. The slides were washed with distilled water and air dried and then observed the color under microscope. The cell wall of gram-negative bacteria holds a peptidoglycan layer that is thin. During the discoloring process the outer membrane of the gram-negative bacteria is degraded and thin peptidoglycan layer of gram-negative bacteria cell wall is no longer able to retain the crystal violet-iodine complex and lost the color of primary stain and retain the color of safranin (secondary stain) and thus a red color is appeared under the microscope (Smith & Hussey,2005)

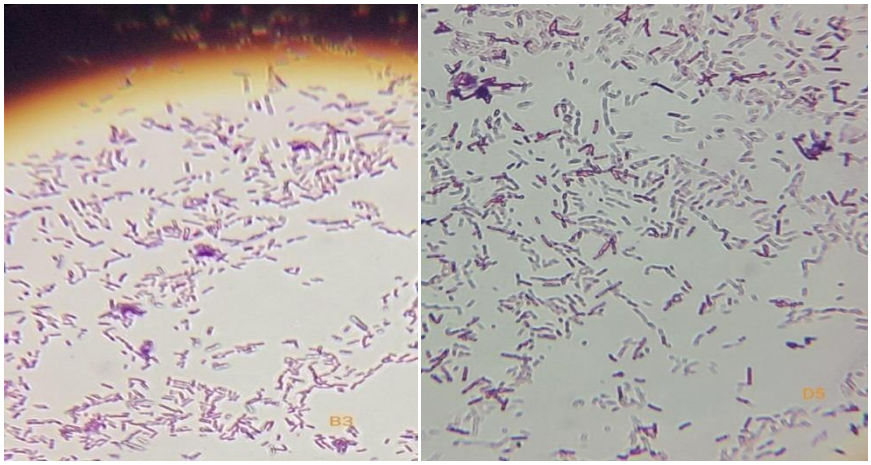


Figure 2.2: Gram-negative, rod shaped bacteria

2.4.2 Biochemical Characterization

Identification and differentiation of various groups of Enterobacteriaceae can be performed by biochemical test. The bacteria under this group are gram-negative, rod shaped bacilli and does not form spore. For this study Indole, Methyl Red, Voges-Proskauer, Citrate, KIA and motility test were performed to detect different gram-negative bacteria isolates.

2.4.2.1 Indole test

Indole test is utilized to decide the capacity of a microorganism to separate amino acid tryptophan to produce indole. Due to the hydrolyzation of tryptophan by the organism's the metabolic product indole is produced which serves as biochemical marker. By the addition of Kovac's reagent which contains 4 (p)-dimethyl amino benzaldehyde indoles produce a cherry red colored compound which indicate the positive result. Negative

result indicate that the tryptophan was not hydrolyzed and no formation of metabolic compound indole (Cappuccino & Sherman, 2014).

For the Characterization of isolates by using indole test nutrient broth was used as the media. Twenty-four test tubes were used for the isolates and each test tube contained a volume of 5 ml aliquots of the media and autoclaved at a temperature of 121ºC and the pressure was 15 psi. When the media was cooled, the test tubes were inoculated with selected isolates by loop inoculation. After that the test tubes were kept down in a shaking water bath (temp 5ºC-100ºC and speed range-20-200 rpm) for 24hrs to mix the isolates properly with the broth. Indole positive results were determined by seeing the cherry red colored layer when 10 drops of Kovac's reagent was added to the tubes. Indole negative result was found by the absence of cherry red colored layer.

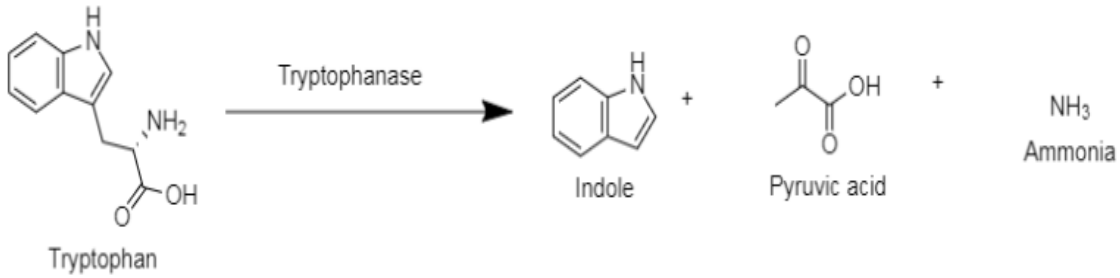


Figure 2.3: Enzymatic degradation of tryptophan

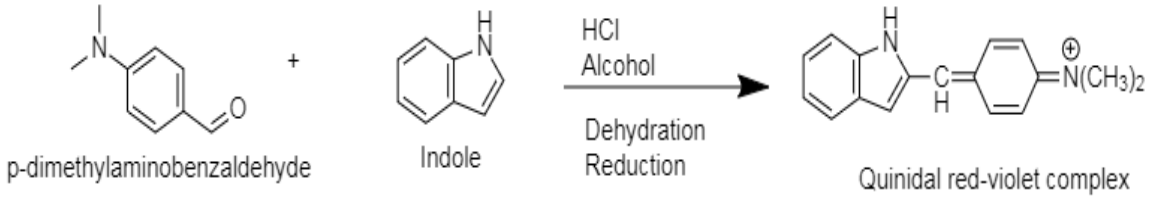
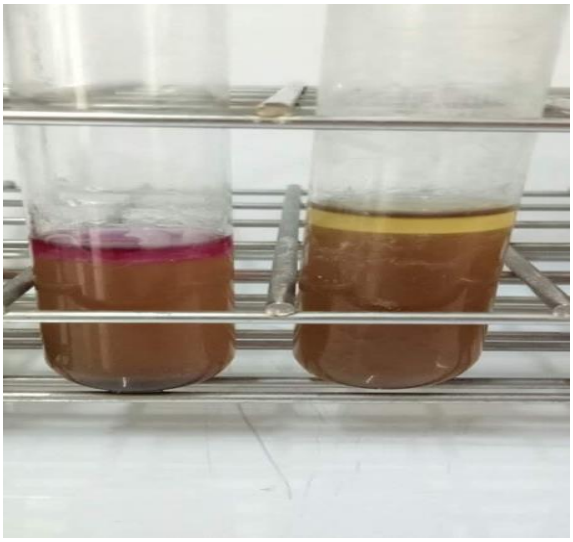


Figure 2.4: Indole reaction with Kovac's reagent



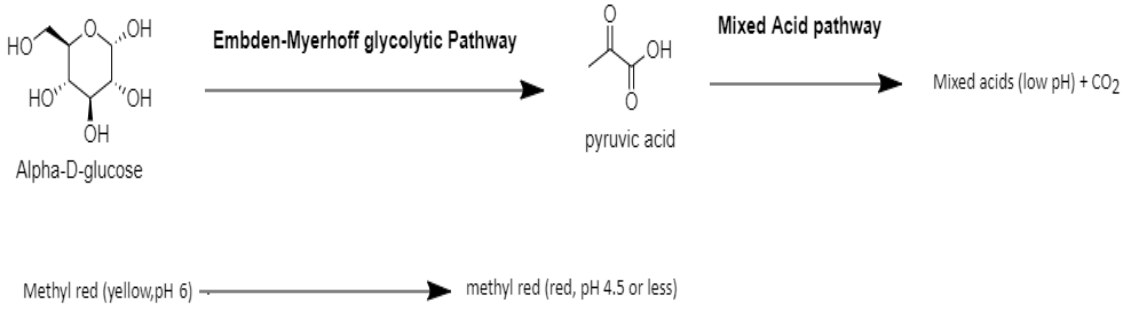
Indole positive **Indole Negative**

Figure 2.5: to the production of indole. The right sided test tube is indole negative and showing no red color due to Indole test result. The 1st test tube from the left side is indole positive and showing red color due the absence of indole.

2.4.2.2 Methyl Red Test

Methyl Red test is used to identify the organism that is capable of glucose fermentation with the production of organic acid end products during the process of energy production. Due to the low acidic pH (4) methyl red indicator will turn red and this red color will indicate that the organism is methyl red positive and absence of this color means the organism is MR negative (Cappuccino & Sherman,2014).

Reaction:



Methyl red test was performed by using MR-VP broth as the media. All the test tube was utilized for the selected isolates and each test tube contained a volume of 8 ml aliquots of the media and autoclaved at 121ºC and 15 psi. When autoclaving was done the media was cooled down and under the aseptic condition the selected isolated were inoculated

into the tube by a means of loop inoculation and kept it down in a shaking water bath (temp 5ºC-100ºC and speed range-20-200 rpm) for 24hrs to mix the isolates properly with the broth. After the incubation for performing the Voges-Proskauer test half portion of each inoculated media was transferred into another test tube. Methyl red indicator then added to the remaining cultured media. Methyl red positive organism showed red color and methyl red negative organism showed no red color in the test.

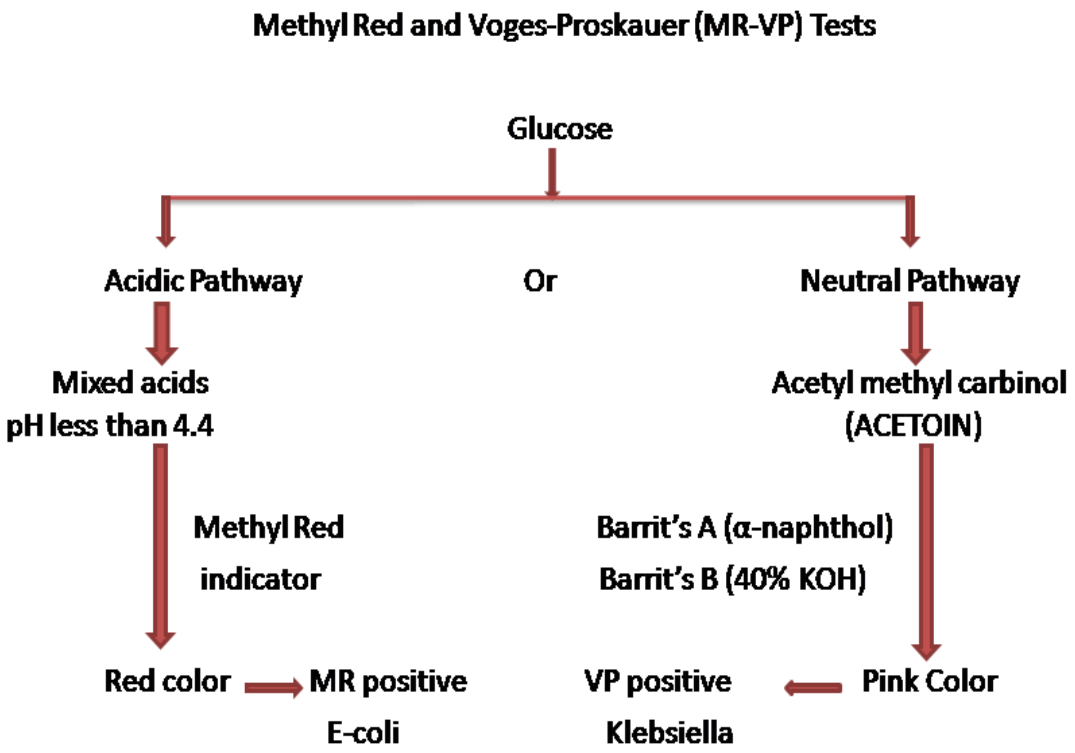
MR positive **MR negative**

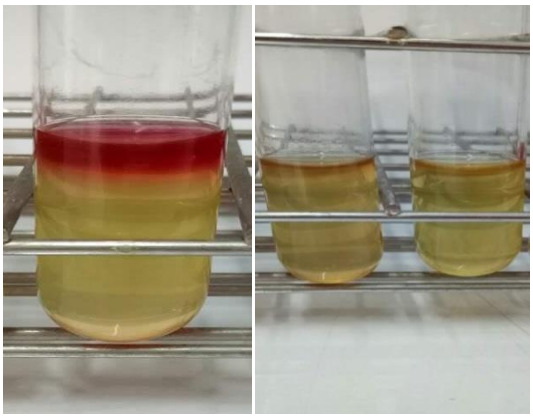
Figure 2.6: Methyl red test result. Here the left side image represents MR positive as "organic acid end product" produce in it and right-side image is MR negative and show yellow color.

2.4.2.3 Voges-Proskauer Test

Voges-Proskauer" test is used to identify the organisms that produce the "non-acidic products" such as acetylmethylcarbinol from the organic acid during glucose fermentation. Barritt's reagent contain α-naphthol that is used as a catalyst in addition with "guanidine" group that is present in peptone of "MR-VP" medium for the oxidation of acetylmethylcarbinol to "diacetyl" compound. Because of the reaction pink colored complex is formed and show color change of the media. This indicated the presence of acetylmethylcarbinol and shows a VP positive result (Cappuccino & Sherman, 2014). "Barritt's reagent A" (10 drops) was added to each the test tube that was separated during the MR test and then shake the tubes. After the addition of "Barritt's reagent A" 10 drops

of “Barritt's reagent B” was added immediately and waited for 15 minutes for the changing of color. Development of deep rose color indicated the VP positive result due to the presence of acetylmethylcarbinol. And absence of the color indicated negative VP result.





VP positive **VP negative**

Figure 2.7: MR and VP reaction pathway and right-side image indicate the VP positive and VP negative test result.

2.4.2.4 Citrate Test

The organisms that is not capable of ferment the glucose and lactose and use citrate as their carbon source for energy production, “citrate test” is performed for their detection. Citrate permease helps citrate to enter into the cell and citrase enzyme acted on citrate to produce oxaloacetate and acetate. These products then converted to CO_2 and pyruvic acid. The CO_2 then combine with Na and water in the medium to produce alkaline sodium carbonate. This alkaline sodium carbonate cause color change of “bromothymol blue indicator from green to blue. The organisms that are not able to utilize citrate will not show any color change of the medium (Cappuccino & Sherman, 2014).

Citrate test was done by using Simmons agar medium. At first the Simmons agar was boiled by heating in an oven during the preparation because it is a solid agar so heating was must. The cap tube was then taken for slant preparation and each of the test tube was poured with a volume of 6 ml aliquots of the Simmons agar media and finally they were sterilized at a temperature of 121ºC and pressure was 15 psi. The media was then cooled and settled down properly to prepare the slant. When the slant was prepared under the aseptic condition the selected organisms were inoculated into the media by streak inoculation and incubated at 37ºC for 24hrs. After 24 hrs all the slant was examined for the growth of organism and color change of the slant. Organism that was considered positive formed an intense Prussian blue color on the medium and their growth was visible on the slant surface. For the isolates that were citrate negative there was no change in color of the slant medium and even no growth on slant surface.

Citrate positive Citrate negative

Figure 2.8: Citrate test in Simmons agar medium. Here the left side image indicates the positive result by showing Prussian blue color due to the production of alkaline sodium carbonate. The right-side image represents negative result and do not show any color change.

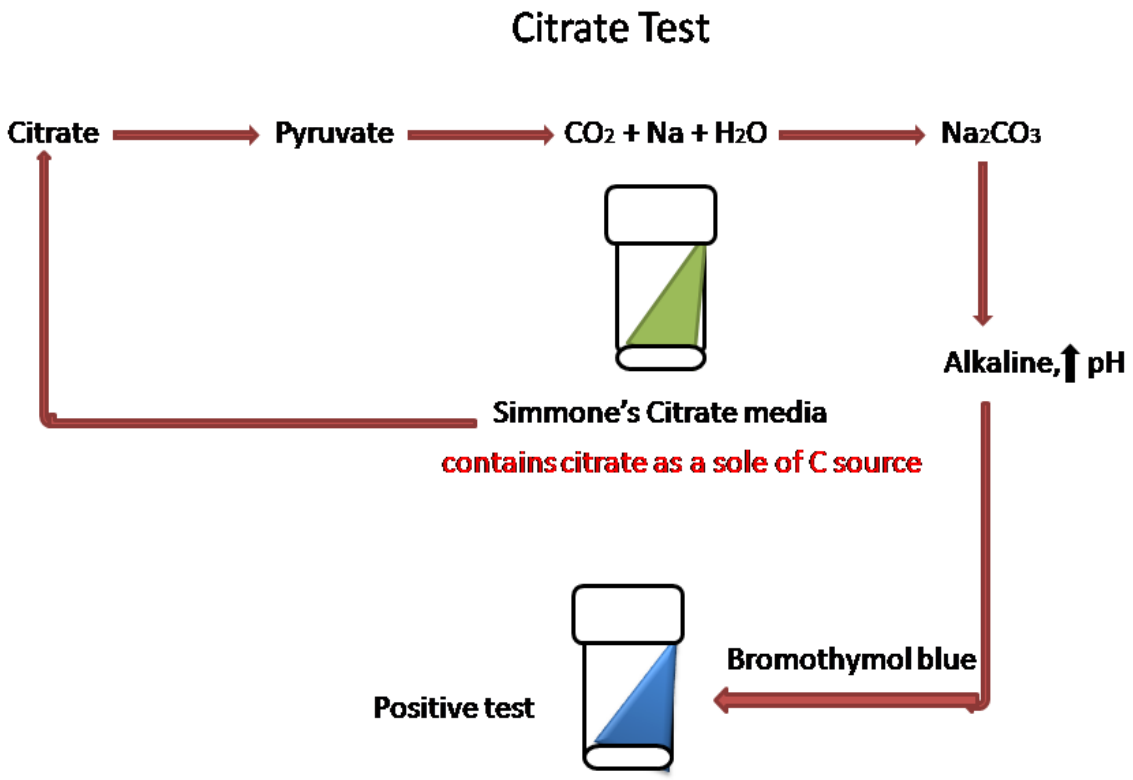


Figure 2.9: Citrate test pathway

2.4.2.5 Kligler's Iron Agar (KIA) Test

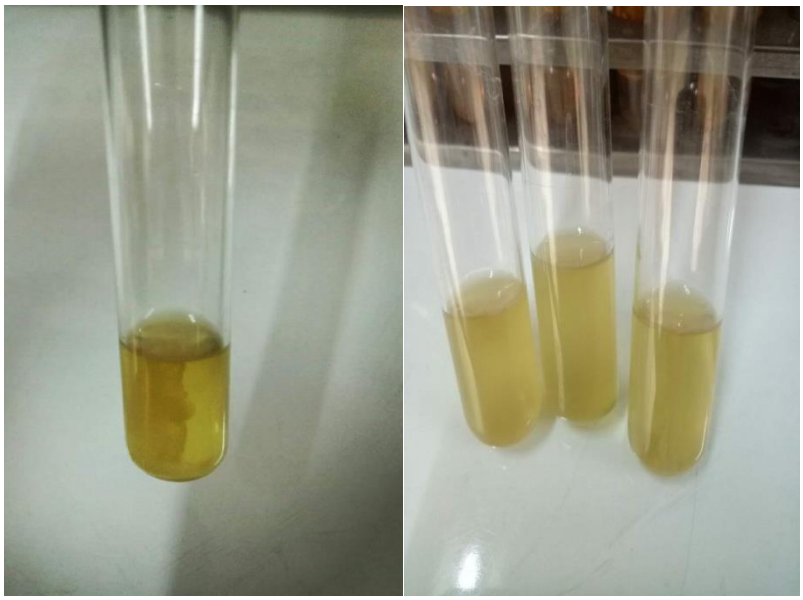
Characterization of isolates by KIA test was carried out using Kligler's iron agar media. Kligler media was first boiled by heating it in an oven to properly mix with purified water. About 8 ml aliquots of the media was poured in each cap tube for the selected isolate and sterilized at a temperature of 121ºC and pressure of 15 psi. After cooling and settling of the media using the aseptic condition the isolates were inoculated into the slant by streak inoculation and incubated for 24 hrs at 37ºC.The result was observed by following rules-

1. Acid slant (yellow, lactose fermentation) and Acid butt (yellow, glucose fermentation) with or without gas production.
2. Alkaline slant (red, no lactose fermentation) and Acid butt (yellow, glucose fermentation) with or without gas production- Alkaline ammonia is produced in the slant.
3. Alkaline slant (red, no lactose fermentation) and Alkaline butt (red, no glucose fermentation) with or without gas production-Only alkaline ammonia is produced (Cappuccino & Sherman, 2014).

Figure 2.10: Here the left side image represents the acid slant and acid butt because of the production of both lactose and glucose. Middle image indicates alkaline slant and acid but due to the production of only glucose and ammonia instead of lactose. Right side image represents alkaline slant and alkaline butt because of the absence of carbohydrate fermentation.

2.4.2.6 Motility Test

SIM agar was utilized to distinguish the motility of the isolates. When growth of the isolates containing flagella was not limited to the line of inoculation it considered as motile. For non-motile living being growth was kept to the line of inoculation. SIM agar media was first bubbled by warming in an oven to legitimately blend with distilled water. Then each of the test tubes was filled with a volume of 5 ml of aliquots of the media and autoclaved at 121ºC and 15 psi. Using the aseptic condition, the organisms were inoculated in the labeled tube media by stab inoculation and incubated at 37ºC for 24hrs.Motile bacteria gave a hazy and opaque appearance throughout the line of inoculation and growth were constrained to the stab line for non-motile bacteria.



Non-motile Motile

Figure 2.11: Here the left-side image shows negative motility test as the growth of organism is limited to the line of inoculation and that's why it is non-motile. Right-side image shows positive motility test because of hazy appearance throughout the line of inoculation and that's why it is motile.

3. Results

3.1 Sample Isolation

After the growth on nutrient broth the sample was streaked in the MacConkey agar plate for separation of gram-negative bacteria from gram positive bacteria and it was incubated at 37ºC for 24hrs.Various types of gram-negative bacteria colonies were seen on the solid MAC(MacConkey) plate. Different kinds of bacterial colonies were chosen by considering their color, texture, shape, size, margin, elevation etc. These colonies were further streaked into MAC plate to obtain a pure culture formation on solid MAC plate.

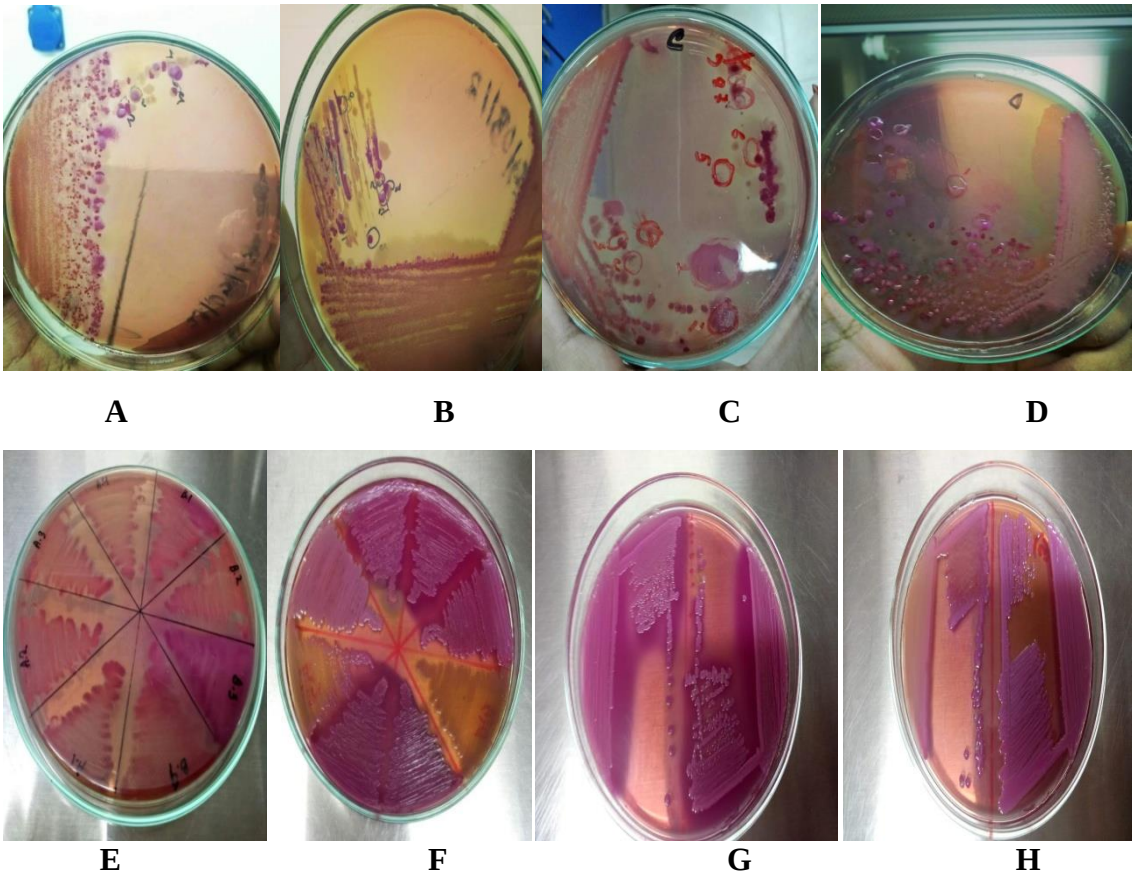


Figure 3.1: Isolation of gram-negative bacteria in MAC solid plate. Here A, B, C and D represent samples spread in MAC solid plate. E, F, G and H represent the series of pure culture.

3.2 Characterization of the selected isolates

3.2.1 Morphological Characterization

3.2.1.1 Growth on MacConkey media

The morphology of the selected isolates and their appearance on MAC agar medium was examined after incubating at 37ºC for 24 hrs. The shape, size, color, elevation, surface and structure of the colonies was recorded and it is shown in table 3.1

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

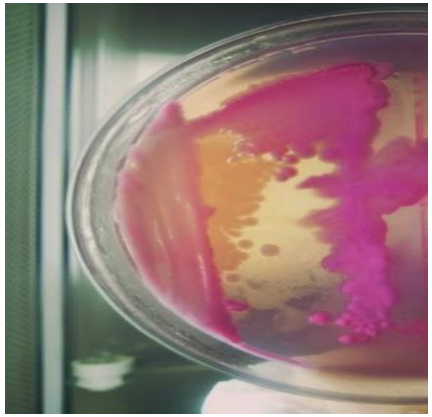
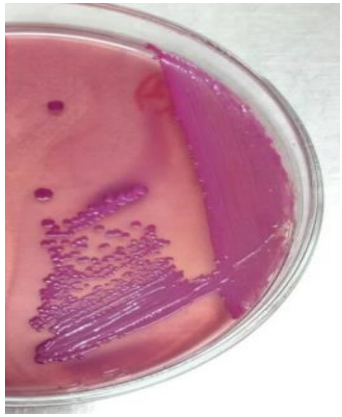
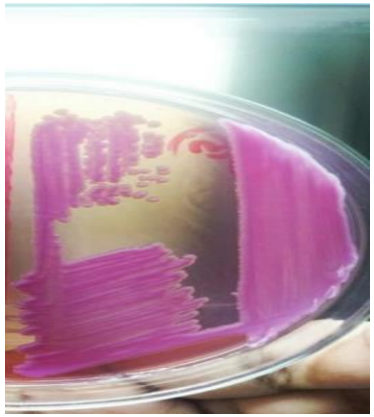
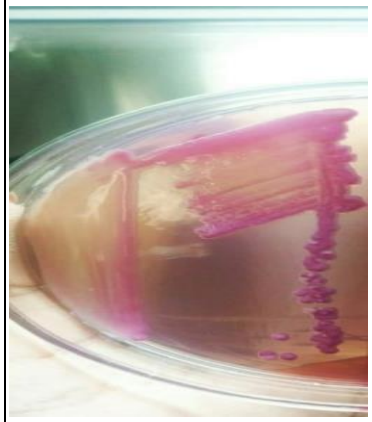
Isolate ID	A1	A2	A3	A4
Colony Characteristic	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Small 3.Color- Pink 4.Elevation- Convex 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Small 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque
Picture of the colonies				

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

Isolate ID	B1	B2	B3	B4
Colony Characteristic	1.Shape-Irregular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Circular 2.Size- Small 3.Color- Colorless 4.Elevation- Low Convex 5.Surface- Smooth 6.Structure- Transparent	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque
Picture of the colonies				

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

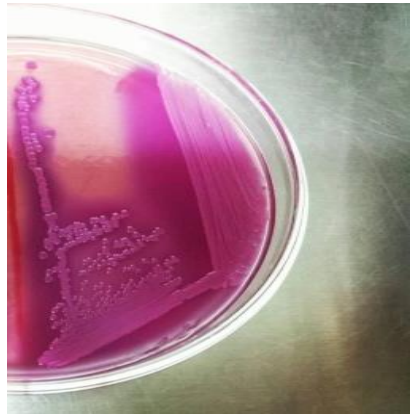
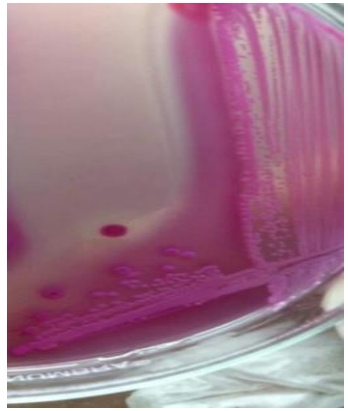
Isolate ID	C1	C2	C3	C4
Colony Characteristic	1.Shape- Irregular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opauqe	1.Shape- Circular 2.Size- Large 3.Color- Colorless 4.Elevation- Convex 5.Surface- Smooth 6.Structure- Transparent	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque
Picture of the colonies				

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

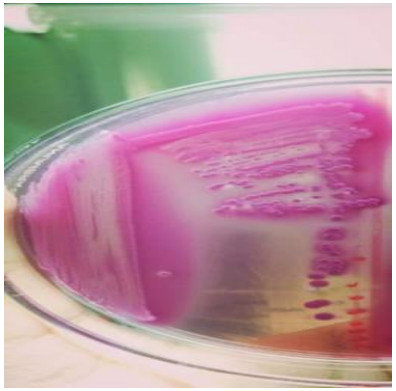
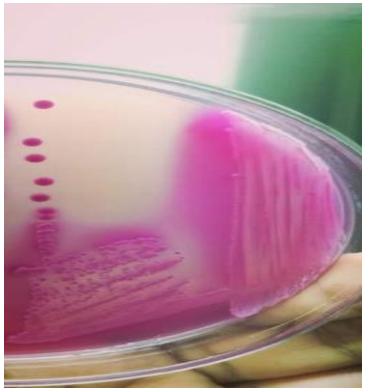
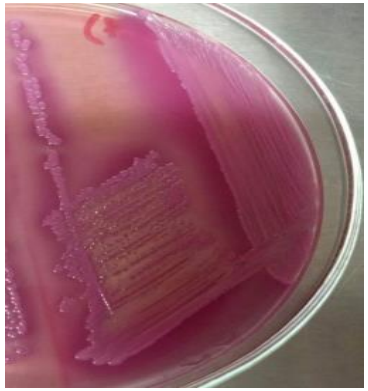
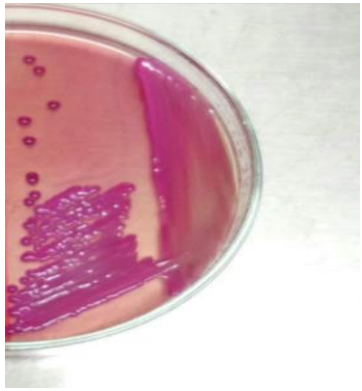
Isolate ID	C5	C6	C7	C8
Colony Characteristic	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque
Picture of the colonies				

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

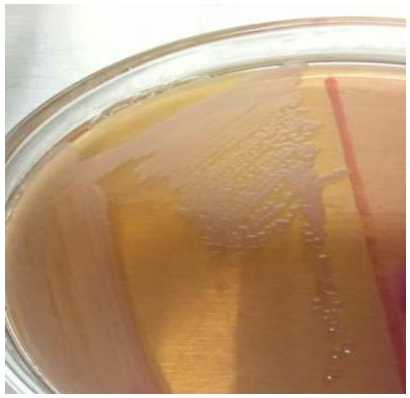
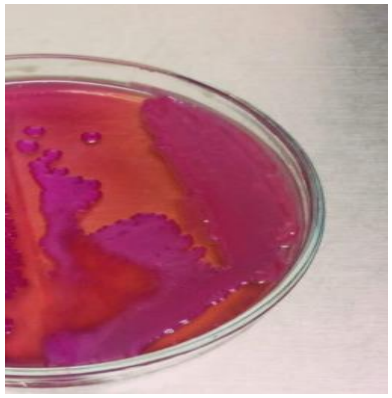
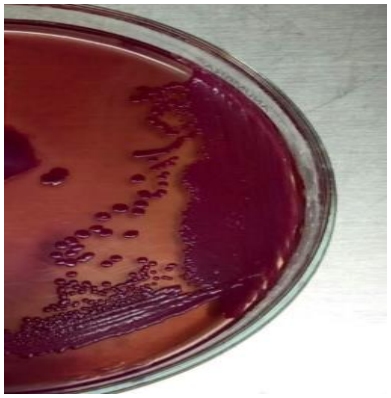
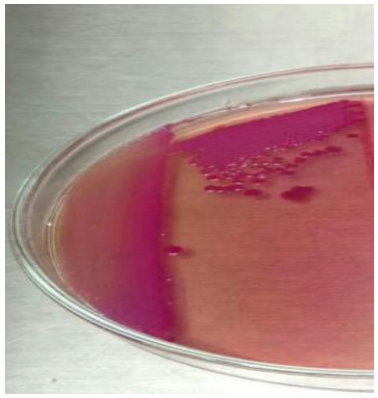
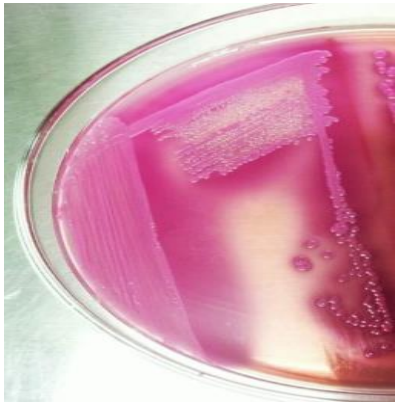
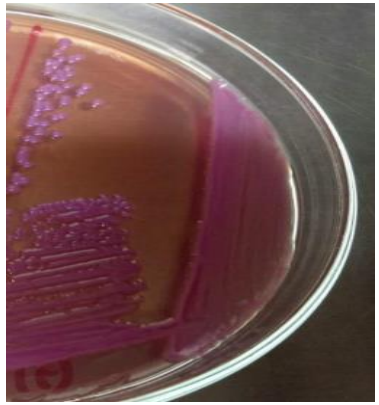
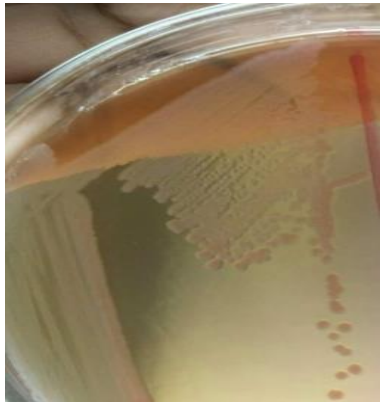
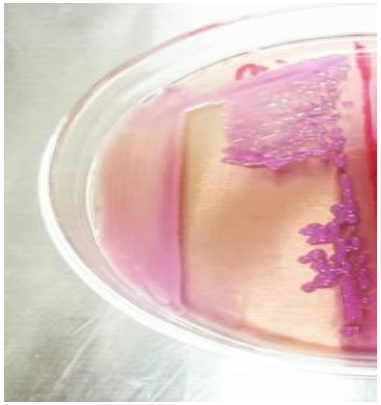
Isolate ID	D1	D2	D3	D4
Colony Characteristic	1.Shape- Circular 2.Size- Small 3.Color- Colorless 4.Elevation- Convex 5.Surface- Smooth 6.Structure- Transparent	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Irregular 2.Size- Small 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Irregular 2.Size- Small 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque
Picture of the colonies				

Table 3.1: Colony Characteristic of twenty-four isolates in MacConkey agar media

Isolate ID	D5	D6	D7	D8
Colony Characteristic	1.Shape- Irregular 2.Size- Small 3.Color- Pink 4.Elevation- Convex 5.Surface- Mucoid 6.Structure- Opaque	1.Shape- Circular 2.Size- Large 3.Color- Pink 4.Elevation- Convex 5.Surface- Smooth 6.Structure- Opaque	1.Shape- Circular 2.Size- Small 3.Color- Colorless 4.Elevation- Convex 5.Surface- Smooth 6.Structure- Transparent	1.Shape- Circular 2.Size- Small 3.Color- Pink 4.Elevation- Flat 5.Surface- Smooth 6.Structure- Opaque
Picture of the colonies				

3.2.1.2 Gram Staining and Microscopic Observation

Enterobacteriaceae is large group of gram-negative bacteria under which there are both lactose fermenting and lactose non-fermenting bacteria. For my study MAC agar was used to isolate the gram negative and enteric bacilli so to end up guarantee that the isolates were under Enterobacteriaceae group gram staining was performed. Enterobacteriaceae are rod shaped, non-spore forming bacilli and facultative anaerobes. Cells of all the isolates from fresh culture of MAC plate were taken for microscopic observation and slide preparation procedures described in materials and methods. The microscopic observation of selected isolates is given in table 3.2 below-

Table 3.2: Microscopic observation of twenty-four isolates

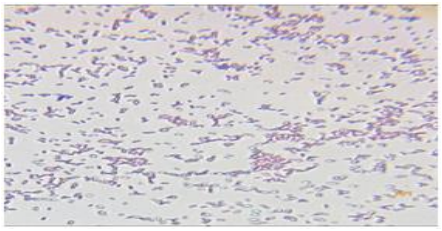
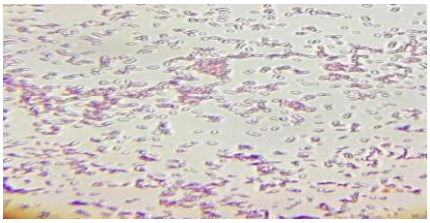
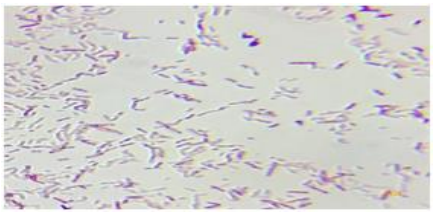
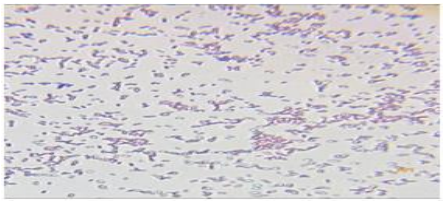
Isolate Id	Microscopic Observation	Comment
A1		Rod shaped, separate, some are streptobacilli, red in color.
A2		Straight rods, cells are singly arranged and some are in pairs, red in color.
A3		Straight rods, cells are singly arranged singly, red in color
A4		Rod shaped, cells formed clumps and some are singly arranged, red in color.

Table 3.2: Microscopic observation of twenty-four isolates

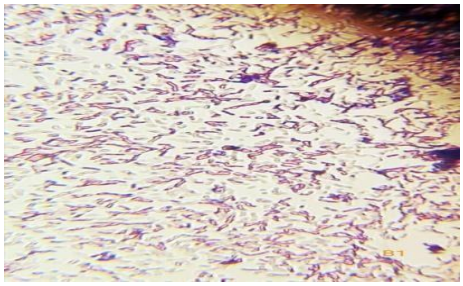
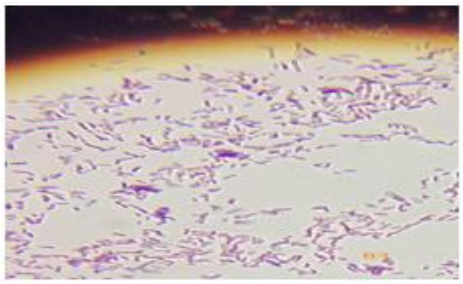
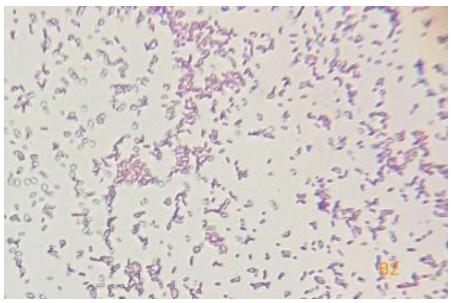
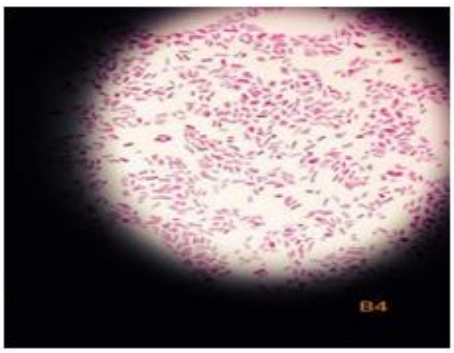
Isolate Id	Microscopic Observation	Comment
B1		Rod shaped, single in arrangement, red in color
B2		Rod shaped, single in arrangement, red in color
B3		Rod shaped, largely clumped in arrangement, some cells are single, red in color.
B4		Straight rod, single in arrangement, red in color.

Table 3.2: Microscopic observation of twenty-four isolates

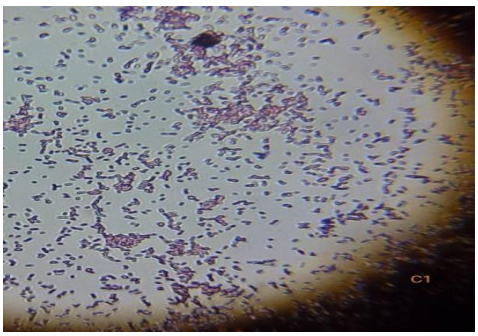
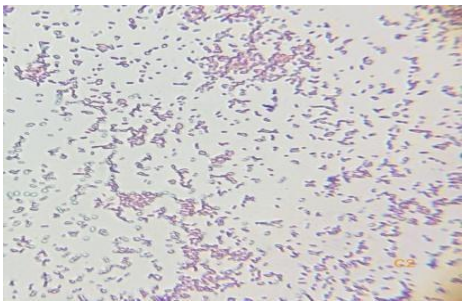
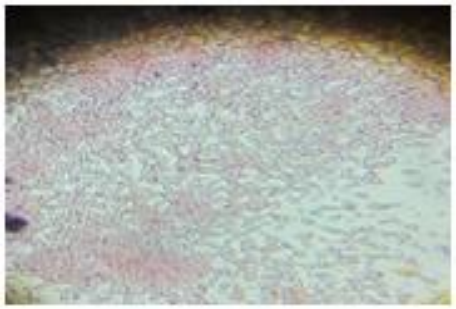
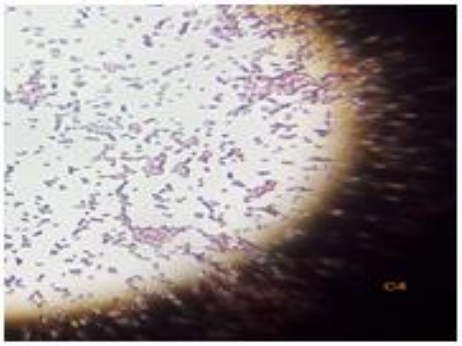
Isolate Id	Microscopic Observation	Comment
C1		Rod shaped, largely clumped in arrangement, some cells are single, red in color.
C2		Rod shaped, largely clumped in arrangement, some cells are single, red in color.
C3		Rod shaped, arranged in clumps, red in color.
C4		Rod shaped, largely clumped in arrangement, some cells are single, red in color.

Table 3.2: Microscopic observation of twenty-four isolates

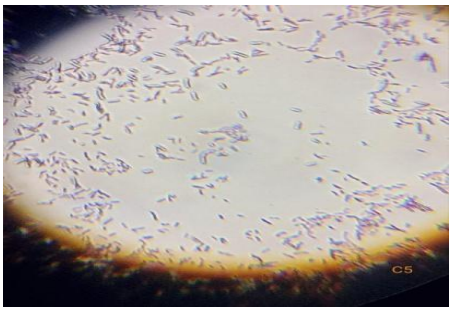
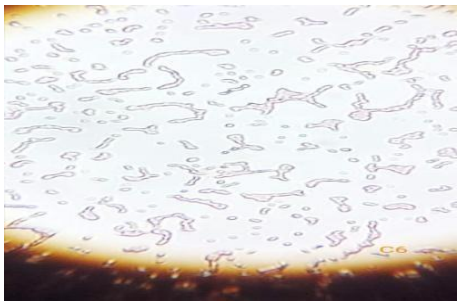
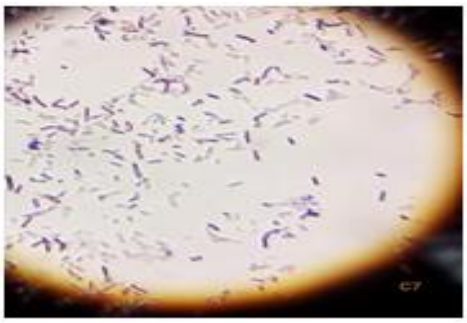
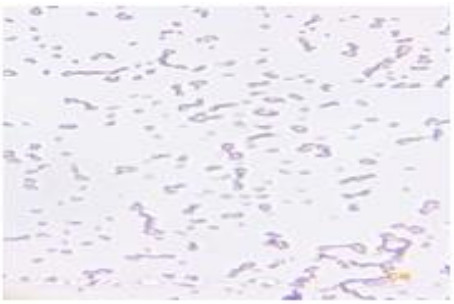
Isolate Id	Microscopic Observation	Comment
C5		Straight rod, single in arrangement, red in color.
C6		Straight Rod, single in arrangement, red in color.
C7		Rod shaped, largely clumped in arrangement, some cells are single, red in color.
C8		Rod shaped, largely clumped in arrangement, some cells are single in arrangement, red in color.

Table 3.2: Microscopic observation of twenty-four isolates

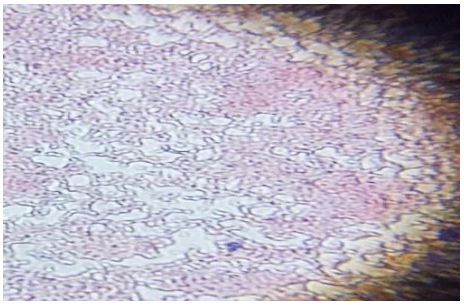
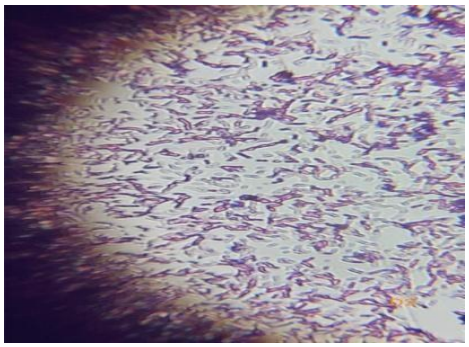
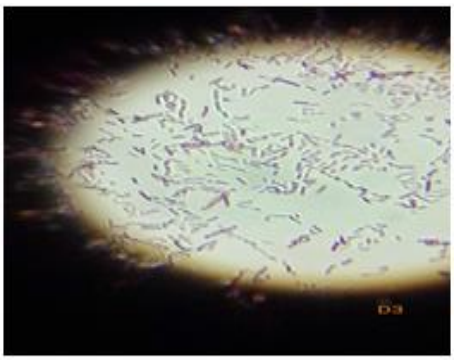
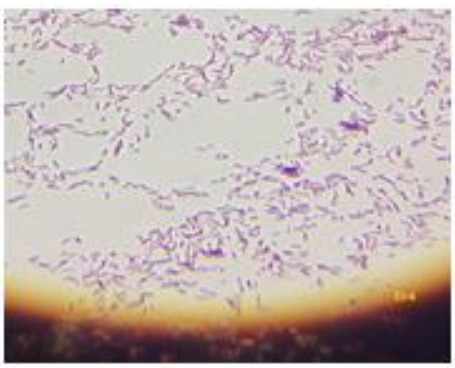
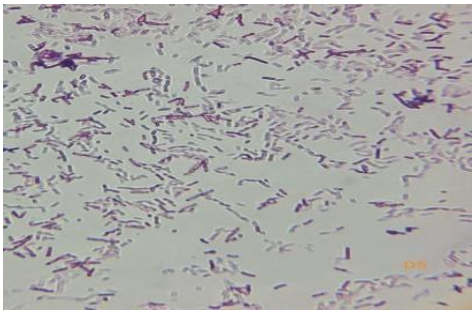
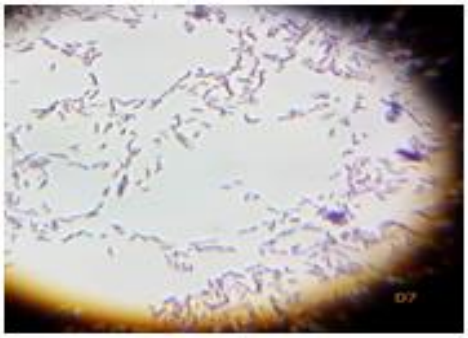
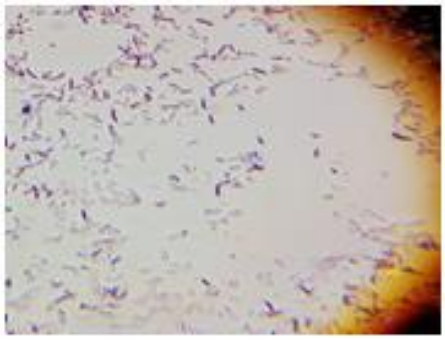
Isolate Id	Microscopic Observation	Comment
D1		Rod shaped, clumped in arrangement, red in color.
D2		Rod shaped, largely clumped in arrangement, some cells are single in arrangement, red in color.
D3		Rod shaped, single in arrangement, red in color.
D4		Straight Rod, single in arrangement, red in color.

Table 3.2: Microscopic observation of twenty-four isolates

Isolate Id	Microscopic Observation	Comment
D5		Straight Rod, single in arrangement, red in color.
D6		Straight rods, arranged in clump, red in color.
D7		Rod shaped, single in arrangement, red in color.
D8		Rod shaped, single in arrangement, red in color

3.2.2 Biochemical Characterization

3.2.2.1 Indole Test

Indole test was done to identify the organism that can separate amino acid tryptophan to produce indole. Indole serves as biochemical marker which forms a cherry red color when "Kovac's" reagent is added into it and show positive result. Among the twenty-four isolates only ten isolates showed cherry red color and were considered indole positive and the remaining fourteen isolates were indole negative.

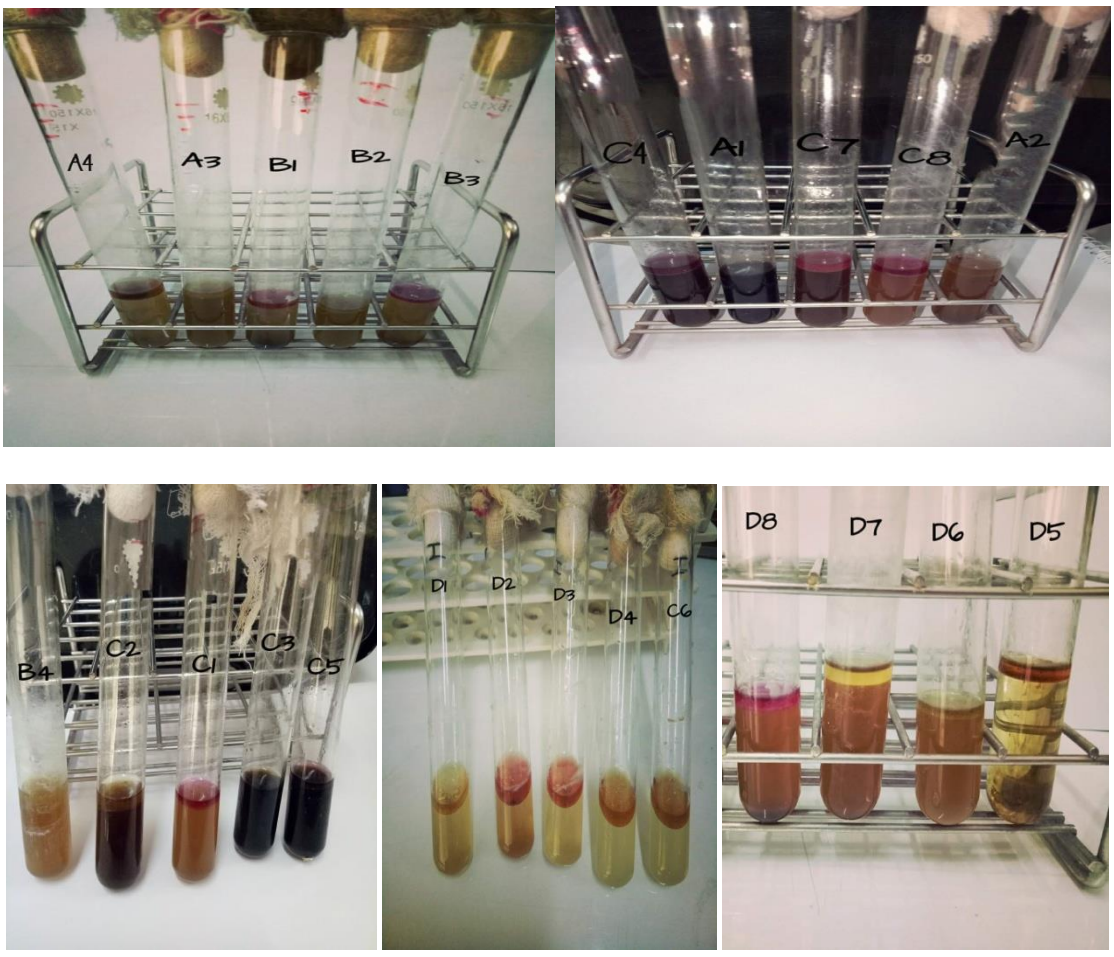


Figure 3.2: Indole test result of twenty-four isolates. Here A4, B1, B3, C1, C4, C7, C8, D2, D3 and D8 isolate are indole positive due to the presence of red color. These isolates separated tryptophan to produce the indole and in the presence of kovac's reagent they change the color of the medium.

3.2.2.2 Methyl Red Test

"Methyl Red" test was performed for the identification of the organism that ferment the glucose with the generation of organic end products. Methyl red indicator was added to the inoculated media tube and among twenty-four isolates nineteen isolates showed the positive result due to the production of organic acid. Due to low acidic pH (4) methyl red turned red for this isolates and absence of this low acidic pH the remaining isolates showed no red color and gave negative result.

Figure 3.3: Methyl Red Test results of twenty-four isolates. Here A2, B3, C1, C5, D1, D2, D7, D8, C4, C2, C6, D3, B1, C3, C7, C8, A4, D4, D5 isolates indicated red color due to the fermentation of glucose and produced organic acid end products. Other isolates did not produce any organic end product that's why they did not give any red color.

3.2.2.3 Voges-Proskauer (VP) Test

VP test was performed for the detection of the organisms that can yield "acetylmethylcarbinol" which is a non-acidic product. When "Barritt's reagent A" and Barritt's reagent B" were added to the inoculated media only eight isolates showed positive result and indicate deep rose color to the medium. That means those eight isolates produced non-acidic products and that's why changed in color. The remaining sixteen isolates showed no deep rose color.

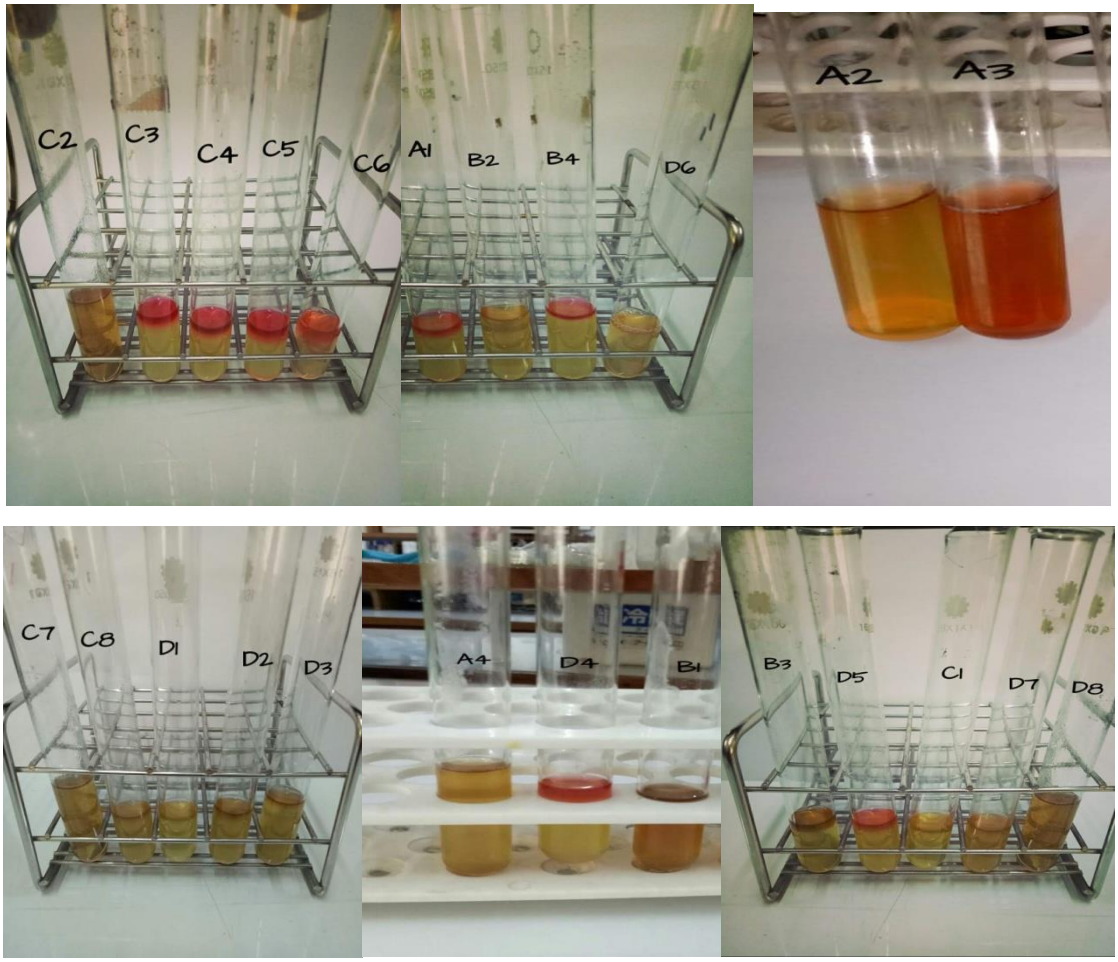


Figure 3.4: VP Test results for Twenty-four isolates. Here A1, A4, B4, C3, C5, C6, D4, D5 isolates show rose color rose color due to the production of non-acidic products that change the pH of bromothymol blue indicator present in Barritt's reagent.

3.2.2.4 Citrate Test

Citrate test was utilized to detect the isolates that were not capable of fermenting glucose and neither lactose. The citrate positive isolates produced alkaline sodium carbonate. Simmons agar medium contain bromothymol blue indicator that change the color of the medium and it becomes Prussian blue from green. Among twenty-four isolates just twelve isolates appeared as citrate positive by showing Prussian blue color.

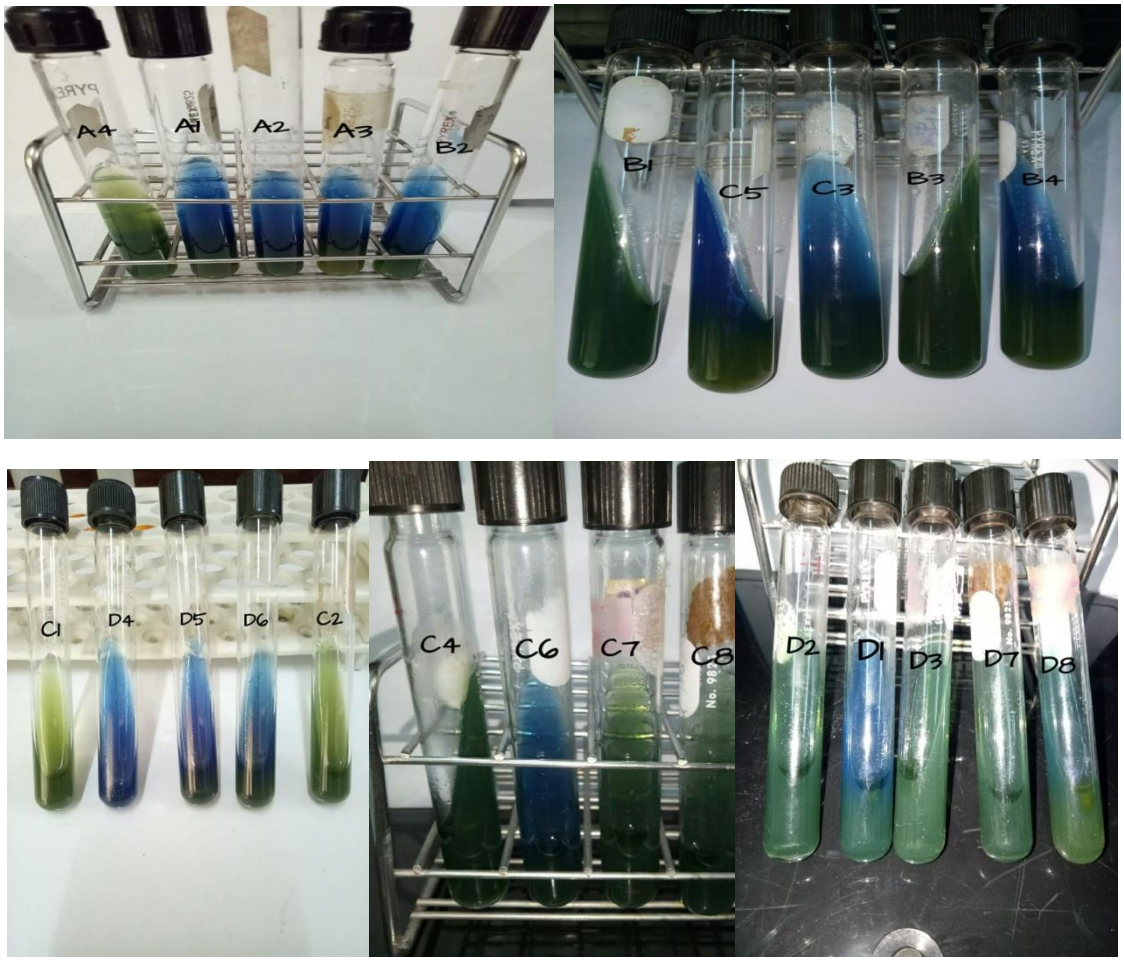


Figure 3.5: Citrate Test of twenty-four isolates. Here A1, A2, A3, B2, B4, C3, C5, C6, D1, D4, D5 and D6 isolate are positive due to the presence of Prussian blue color of the medium. These isolates produce the alkaline sodium carbonate that cause color change of bromothymol blue indicator and show blue color different from the color of the medium that was green. Remaining isolates do not show any color change of the medium.

3.2.2.5 Kligler's Iron Agar (KIA) Test

To determine the ability of the isolates whether they ferment lactose and glucose or just ferment only one the KIA test was performed and by seeing the color change and gas production of the media the isolates were identified. When the test was done it was found that amongst twenty-four isolates only two isolates showed alkaline (no lactose fermentation) slant and alkaline (no glucose fermentation) butt without any gas production and another two isolates exhibited alkaline slant (no lactose fermentation) and acid butt (glucose fermentation) without gas production and remaining all the isolates showed acid slant (lactose fermentation) and acid butt (glucose fermentation) with gas production.

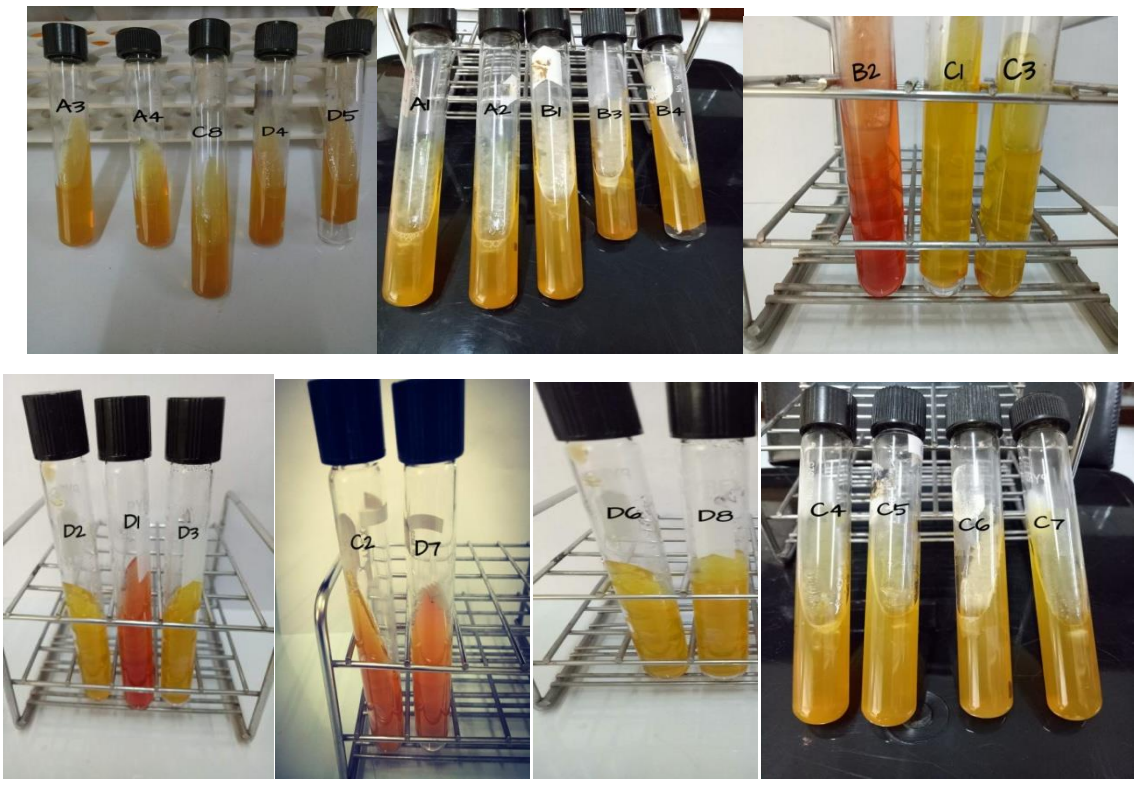


Figure 3.6: KIA Test results of twenty-four isolates. Here C2 and D7 isolate show alkaline slant (no lactose fermentation) and acid butt (glucose fermentation), B2 and D1 isolate show alkaline (no lactose fermentation) slant and alkaline (no glucose fermentation) butt and remaining all the isolates represent acid slant (lactose fermentation) and acid butt (glucose fermentation)

3.2.2.6 Motility Test

Motile isolates showed opaque and hazy appearance throughout the line of inoculation and growth of non-motile isolates were limited only to the line of inoculation. After performing the test, it was found that fifteen isolates were motile and only nine isolates were non-motile.

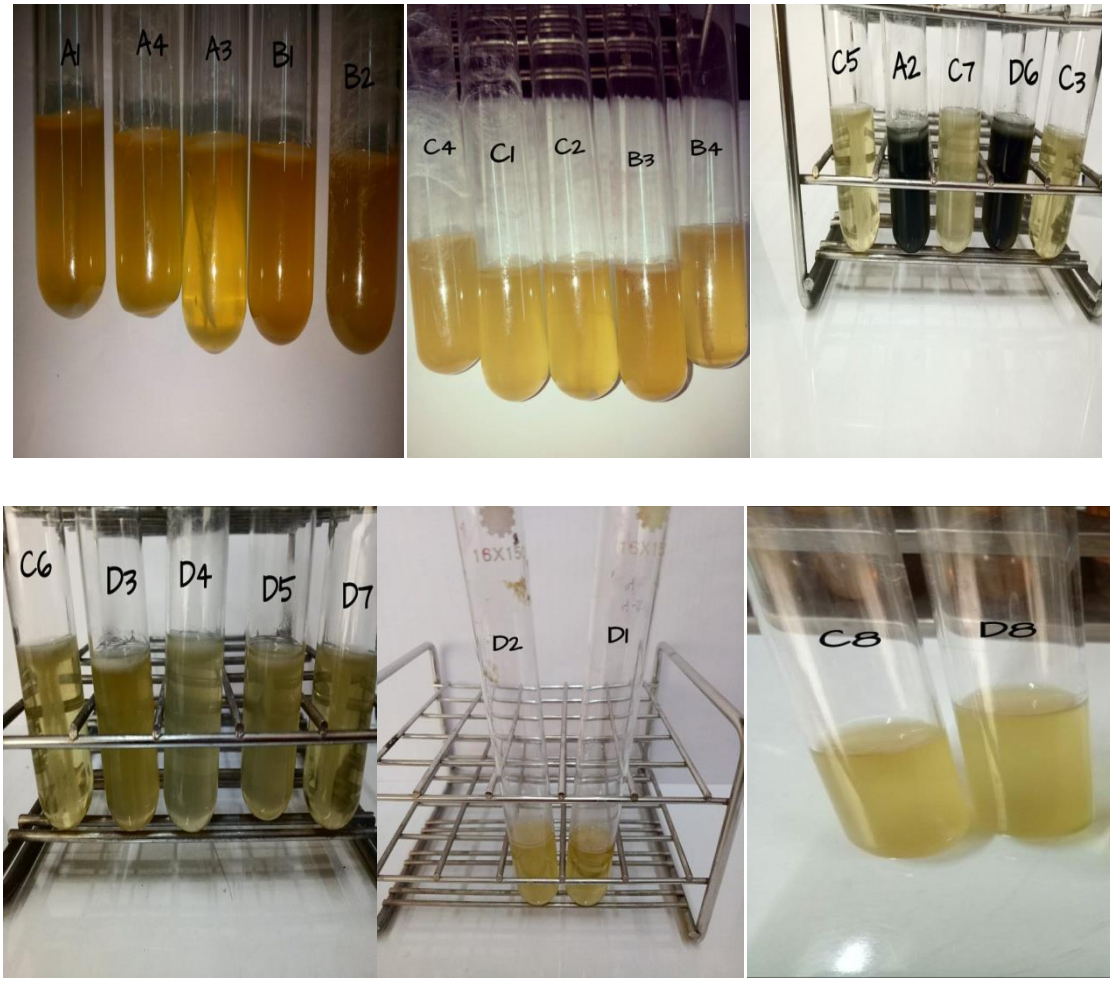


Figure 3.7: Motility Test results of twenty-four isolates. Here A1, A2, B1, B2, C1, A4, B3, C4, D1, C7, D2, C8, D3, D6 and D8 isolate are motile because their growth was throughout the line of inoculation and remaining isolate are non-motile as their growth was limited to the line of inoculation.

Summary of all the biochemical test results and name of the probable bacteria according to the biochemical test results are shown in Table 3.2.

Table 3.2: Summary of the Biochemical Test result of Twenty-four isolates.

Isolate ID	**Indole Test**	**MR Test**	**VP Test**	**Citrate Test**	**KIA Test**	**Motility Test**	**Probable Bacteria**
A1	-	-	+	+	Acid Slant, Acid butt, gas	Motile	*Enterobacter*
A2	-	+	-	+	Acid Slant, Acid butt, gas	Motile, H_2S	*Citrobacter*
A3	-	-	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*
A4	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*

Isolate ID	Indole Test	MR Test	VP Test	Citrate Test	KIA Test	Motility Test	Probable Bacteria
B1	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
B2	-	-	-	+	Alkaline slant, Alkaline butt, gas	Motile	*Pseudomonas*
B3	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
B4	-	-	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*
C1	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*

Isolate ID	Indole Test	MR Test	VP Test	Citrate Test	KIA Test	Motility Test	Probable Bacteria
C2	-	+	-	-	Alkaline slant, Acid butt, gas	Non-motile	*Shigella*
C3	-	+	+	+	Acid Slant, Acid butt, gas	Non-motile	Klebsiella
C4	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
C5	-	+	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*
C6	-	+	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*

Isolate ID	Indole Test	MR Test	VP Test	Citrate Test	KIA Test	Motility Test	Probable Bacteria
C7	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
C8	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
D1	-	-	-	+	Alkaline slant, Alkaline butt, gas	Motile	*Pseudomonas*
D2	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*
D3	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*

Isolate ID	Indole Test	MR Test	VP Test	Citrate Test	KIA Test	Motility Test	Probable Bacteria
D4	-	+	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*
D5	-	+	+	+	Acid Slant, Acid butt, gas	Non-motile	*Klebsiella*
D6	-	+	-	+	Acid Slant, Acid butt, gas	Motile H_2S	*Citrobacter*
D7	-	+	-	-	Alkaline slant, Acid butt, gas	Non-motile	*Shigella*
D8	+	+	-	-	Acid Slant, Acid butt, gas	Motile	*E. coli*

From table 3.2 it is clear that among twenty-four isolates the probable organism were *E. coli*, *Klebsiella*, *Pseudomonas*, *Shigella*, *Citrobacter* and E*nterobacter*. The prevalence of E-coli in comparison to other organisms was high in amount in lake water. On the other hand the amount of *Enterobacter* was much lower than the other organisms. The number and percentage of the probable organisms by genus are shown in Table 3.3 in below-

Table 3.2: Percentage of probable organism by genus

Probable Organism	**Number**	**Percentage**
E. coli	10	41.66%
Klebsiella	7	29.16%
Pseudomonas	2	8.33%
Shigella	2	8.33%
Citrobacter	2	8.33%
Enterobacter	1	4.16%

4. Discussion

The goal of the research was to isolate the gram-negative bacteria from water sample and characterization of them. After analyzing the result, it is found that among twenty-four isolates 41.66% was *E. coli*, 29.16% was *Klebsiella*, 8.33% was *Pseudomonas*, 8.33% was *Shigella*, 8.33% was *Citrobacter* and only 4.16% was *Enterobacter*.

There might be doubt about the presence of these bacteria in lake water but to prove the presence of these bacteria in water that cause serious health issues a variety of research-based work is accomplished. *E. coli* considered as fecal indicator to indicate the pollution and presence of other pathogenic bacteria in water. A study that was conducted in Bangladesh showed that enterotoxigenic *E. coli* that causes acute diarrheal disease is found in lake water in Dhaka, and water serves as the reservoir of transmission. Bangladesh is considered as the subtropical country and during the monsoon period it is influenced by flooding which increase the diarrheal disease. (Ahmed, Islam and Begum et al, 2013). Another research was performed to survey and characterize the *E-coli* in Lake Huron of Canada and it documented that swimming was unsafe due to the high concentration of *E. coli* found into water (Kon, Weir, Howell, 2008). The strains of *E. coli* containing the virulence gene can be found in water due to the contamination of water by human and untamed life defecation, horticultural spillover, sewage flood and tempest occasions (Titiawo, Obi and Okoh, 2015).

A significant number of the water framework detailing coli form occurrences in their conveyance systems have noticed that the dominating living being was an individual from *Klebsiella* family. The capsular sludge covering around *Klebsiella* cells give them protection from chlorinated water and disinfectants and by along these lines they multiply in water. Lake and pond water receive *Klebsiella* from fecal sources, from rural and urban runoff and by discharge of the wood and paper processing wastes. Non-Fecal *Klebsiella* are present in higher number than the fecal coli forms in the natural water (Geldreich and Rice,1987). A study was conducted in Modimola Dam situated in South

Africa to identify the contamination level of *Klebsiella* in that dam. From the middle of the dam water sample was collected and 29 (36.9%) *Klebsiella* were obtained from 504 isolates when they were tested (Siri, Sithebe and Ateba, 2011). Another study conducted by Podschun (2001) showed that *Klebsiella was* isolated with high percentage (53%) from surface water samples in Germany that had virulent factors, for example, pili, serum obstruction and siderophore.

Pseudomonas is a piece of a substantial gathering of microorganisms and it is universal in the earth. *Pseudomonas* can be seen in common waters, for example, lakes and streams in convergences of 10/100 mL to >1,000/100 mL. Normally it can be observed in 2% of tests, or less, and at concentrations up to 2.300/mL (Allen and Geldreich, 1975) or regularly at 3– 4 CFU/mL (Mena and Gerba, 2009). Presence of *Pseudomonas* in lake water was observed by various studies among which a study was performed in the Larsemann hill that contains more than 150 lakes situated in East Antarctica. In this study nine lake samples were collected and out of these nine samples *Pseudomonas* was found in one lake sample when characteristic colonies of *Pseudomonas* were grown on selective media at 20-25°C. Presence of the *Pseudomonas* in lake determines the pollution of this lake (Chauhan, Bharti and Goyal, 2015). *Pseudomonas* seems to have the capacity to stay cultivable for an extensive stretch of time contrasted and other gram-negative bacteria. As per Byrd, Xu and Colwell (1991) for a lake water types of *Pseudomonas* just 18% of the cell made due in lake water after 24 hr and no cell made due in refined water after 24 hr at 30°C. They expected that the surviving cultivable *Pseudomonas* cell would keep on decreasing after 24hr incubation in lake water.

Shigella causes Shigellosis which in endemic in Bangladesh. *Shigella* S. flexneri and S. dysenteriae type 1. are considered the most virulent in Bangladesh. According to Faruque and Khan et al (2002), surface water (lake and river) contain about 10.9% *Shigella* and it was detected by using PCR method in Bangladesh. PCR method was used to indicate the presence of *Shigella* in water by detecting the virulence gene. The

recognition of *Shigella* (15.8%) in Lake Ziway water situated in Ethiopia was in a nearby concurrence with the estimations of 10.9% from surface water (lake and stream) of Bangladesh utilizing PCR technique (Mekonnen, Asefa, Lemma et al. 2014). In like manner, 1,242 separates of enteric microbes were recognized from an assortment of drinking water wellsprings of Yaounde, of which Shigella had 0.24% event. The discoveries of this and past examinations affirm the predominance of enteric pathogens in surface crude waters and show that surface waters can transport these microscopic organisms, and there is a probability of a persistent wellspring of pollution into the aquatic bodies (Chouhan, 2015).

The survival of a smaller scale organism in a domain in which it isn't indigenous relies on its capacity to endure an outsider arrangement of natural, physical and compound conditions. Survival of bacteria in water depends on several factors. These components might be, for instance, starvation, temperature, pH, toxically manufactured substances, the animal's physiological express, its flexibility of supplement depleted circumstances, coordinated efforts with various animals, inhibitory blends conveyed by other living creatures, and the closeness of a residual disinfectant (Darcan and Kahyaoglu, 2012). After a pollution of drinking with non-nearby microorganisms the gatecrasher's factors like supplement openness, temperature, predators and sedimentation affect the survival and stop to exist cases of these interlopers. Different components, such as microbial survival in water can move with the possibility of the earth itself; e.g. zooplankton populaces can have the effect on the consistent quality of sensible bacterial cells, both by ejection through predation and by duplication as the consequence of supplement release. Microorganisms develop until the point that they deplete the supply of supplement or gather lethal side-effects of digestion; at that point they receive cautious measures, some take part in human tissue utilization or make ultra-microcells; some mix weight proteins or express starvation characteristics; some become dormant; some shape spores. To get by at low temperature, microbes can reduce their cell evaluate; decrease their capsular

polysaccharide coat thickness; change their unsaturated fat and phospholipids synthesis and lessen the halfway volume of cell water (Price and Sowers, 2004).

The presence of the organism in water cause serious health issues. These organisms can contaminate the water by many ways. Potential sources of the organisms in water include human fecal waste, invasion of household or wild creature fecal issue, spouting from discharging septic structures or sewage release, urban storm water, wastes of the hospitals and agricultural flood (Ye, Yang, Li et al, 2013). In many areas the discharge of the human fecal wastes into water is done without the prior treatment of the fecal wastes. Human fecal wastes that are not treated contain a variety of bacteria that affect the health of the community (Domingo and Edge, 2010). Fecal waste from wildlife for example bird feces also contain organism that can cause human health risk. According to Serrano the coli form level of lake water situated in South California rise with the entry of the migratory bird. During the era of urbanization, the regular land surfaces are supplanted by man-made fake scope, for example, cleared streets, parking areas and rooftops. In the midst of storms water streams over these impenetrable surfaces activating contaminants and transporting them to getting waters. Urban storm water can convey critical measures of contaminants, including human pathogens from diffuse sources to accepting waters. In regions where there is lacking, or maybe no sewage or septic treatment forms, there can be noteworthy dangers of waterborne pathogens spoiling water bodies (Younis, 2008). The hospitals that are situated near the lakes or rivers discharge all the wastes containing various organisms into the water that further cause contamination of the water.

There is a wide scope for the continuation of my study by performing Real time PCR that can be used for the precise quantification of the genes of the isolated. Real time PCR is a procedure to estimate the species of the probable organism that cannot be done by culture-based techniques. While differentiating evaluation by culture techniques versus qPCR, aftereffects of this examination uncovered that qPCR created higher values contrasted with the way of life-based technique. Many studies have been conducted to identify the different species of the organism using PCR techniques. A few investigations

have been directed to assess the productivity of techniques in distinguishing *Klebsiella* species. PCR examination has been accounted for precise recognizable proof of Klebsiella species when contrasted with other starter recognizable proof tests. To isolate the *Klebsiella* species from Modimola dam arranged in South Africa PCR methods was used. After performing the PCR it was found that the 28 isolates were under the family *of Klebsiella.* The extents of the segregates were high in amount in the upper-stream (15; 53.6%). The amount of these segregates in the middle stream and in the lower stream were (8; 28.6%) and (5; 17.8%) (Siri, Sithebe and Ateba, 2011). According to Podschun there are many species of *Klebsiella*, including K. oxytoca, K. planticola, K. pneumoniae among which K. pneumoniae are largely found in lakes, saltwater and buckish water (Barati, Ghaderpour and Chewet al, 2016). For the hereditary distinguishing proof and portrayal of Pseudomonas species by means of PCR-based techniques, different targets have been accounted for. To recognize P. aeruginosa a novel P. aeruginosa particular continuous PCR measure is produced. Due to a higher atomic development rate and less flat gene exchange, Real Time PCR examine focusing on the gyrB quality has been outlined and assessed as an exceedingly delicate and particular test when tried with P. aeruginosa strains and types of pseudomonads firmly identified with P. aeruginosa (Lee, Wetzel, Buckley et al, 2012). According to Ekwanzala, Abia et al (2017), the genus *Shigella* include four species, namely S. flexneri, S. sonnei, S. boydii and S. dysenteriae. By using qPCR, the ipaH gene sequence of the *Shigella* was dissected and *Shigella* flexneri was observed prominent in water what's more, dregs, at 79%, all things considered. In water, two strains were distinguished as Shigella sonnei what's more, Shigella dysenteriae with arrangement similitude's of 7% and 14%, separately (Ekwanzala, Abia and Keshri et al, 2017).

Therefore, in this study the results described above clearly showed that all the isolates were gram negative bacteria that are present in Dhanmondi Lake water.

5. Conclusion

The main target of this study was to isolate, identify and characterize the gram-negative bacteria from water sample. Under the applied conditions the occurrence of *E. coli, Klebsiella, Pseudomonas, Shigella, Citrobacter* and *Enterobacter* were found in the water sample that was collected from Dhanmondi Lake. Further research can be conducted to precisely know about the species of this organism by using PCR technique and it is suggested that corrective measures should be taken to control the extension of these bacteria in water.

Appendix

Unless otherwise mentioned, all the media were sterilized by autoclaving at 121ºC for 55 minutes. Distilled water was used for preparation of all the media. The media used in this study have been given below:

1. **MacConkey medium:**

Ingredients	**Amount(g/L)**
Pancreatic digest of gelatin	17.000 g
Peptone of meat	1.500 g
Peptone of casein	1.500 g
Lactose monohydrate	10.000 g
Lactose	10.000 g
NaCl	5.000 g
Crystal Violet	0.001 g
Neutral Red	0.030 g
Bile Salts	1.500 g
Agar	13.500 g
pH	7.1

2. Nutrient Broth

Ingredients	Amount(g/L)
Beef Extract	1.000 g
Yeast Extract	1.000 g
Peptone	5.000 g
Sodium chloride (NaCl)	5.000 g

3. Methyl Red VogesProskauer (MR-VP) Broth

Ingredients	Amount(g/L)
Buffered peptone	7.000 g
Dextrose	5.000 g
Dipotassium phosphate	5.000 g
pH	6.9

4.Simmons citrate agar

Ingredients	Amount(g/L)
Sodium Chloride (NaCl)	5.000 g
Sodium Citrate	2.000 g
Ammonium Dihydrogen Phosphate	1.000 g
Dipotassium Phosphate	1.000 g
Magnesium Sulfate	0.200 g
Bromothymol Blue	0.080 g
Agar	15.000 g
pH	6.9

5. Kligler's agar medium

Ingredients	Amount(g/L)
Peptone	15 g
Beef extract	3 g
Yeast extract	3 g

Proteose Peptone	5 g
Lactose	10 g
Dextrose	1 g
Ferrous sulphate	0.200 g
Sodium chloride	5 g
Sodium thiosulphate	0.300 g
Phenol red	0.024 g
Agar	15.000 g
pH (at 25ºC)	7.4

6. SIM agar medium

Ingredients	**Amount(g/L)**
Beef extract	3.000 g
Peptone	30.000 g
Peptonized iron	0.200 g
Sodium thiosulphate	0.025 g

Agar	3.000 g
pH	7.5

7. Kovac's Reagent

Ingredients	**Amount**
P-Dimethylaminobenzaldehyde	5.000 g
Amyl alcohol	75.000 ml
Hydrochloric acid (conc.)	25.000 ml

8. Methyl Red Indicator

Ingredients	**Amount**
Methyl Red	0.200 g
Ethyl alcohol	60.000 ml
Distilled water	40.000 ml

9.Barritt Reagent A

Ingredient	Amount
α-Naphthol	5.000 g
Absolute ethanol	100.00 ml

10. Barritt Reagent B

Ingredient	Amount
Potassium hydroxide	40.000 g
Distilled water	100.00 ml

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