BACTERIOLOGICAL STUDY OF TURAG RIVER

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DECLARATION

It is hereby declared that,

1. The thesis submitted, titled 'Bacteriological Study Of Turag River'' is our original work while completing our degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all the main sources of help.

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DEDICATION

Over the course of one year, we've worked through the ups and downs to complete this colossal task like we've never experienced it before. So this dedication goes to Almighty Allah for giving us the strength to fight through those ups and down and also guiding us on the right path. Also, our parents who have supported us in this, and our respected supervisor's for giving us this chance to complete this colossal task and believing in us and guiding us throughout the journey.

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ABSTRACT

The main purpose of this thesis is to identify the bacterium present in the Turag River and get an overview about its Bacterological identifications.

Water is the most fundamental and crucial environmental component controlling the health and well-being of all living things. Bangladesh is a riverine country & it is proud of its over 700 rivers, which have one of the greatest networks in the world. Due to microbial pollution, heavy metals, and industrial wastes disposed of by industries made the river toxic at a level that has a very adverse effect on public health .

Among these rivers is the Turag comes from the upper branch of the Buriganga, the principal river in Bangladesh known as the most polluted river after Buriganga. Tragically, more industrial effluents are dumped into the Turag River, which contaminates its water with microorganisms highly.

Water collected from 4 sampling sites by maintaining 4ºC was transported to Lab where the samples spread to different selective and non-selective media. After colony selection, gram staining, and Biochemical tests were done for identification. Antibiotic susceptibility test was done to whether the organisms are Resistant, Sensitive, or Intermediate to Multi-drugs. For the confirmation of the identity of the organisms, the DNA bands were analyzed.

The results of our study accumulate the purpose perfectly and serve as the foundational data for the bacteriology which emphasizes the presence of fecal origin bacterium named *E. coli(19%), Klebsiella pneumoneae (21%), Pseudomonas aerogenesa (16%), vibrio spp (19%), and Staphylococcus(24%)* they are mostly harmful, disease-causing bacterium.

KEYWORDS: Turag River, Bacterological identification, *Staphylococcus spp*, *Vibrio spp*, Antibiotic Sensitivity, River water .

CHAPTER-01: INTRODUCTION

1.1: SIGNIFICANCE OF WATER:

Water is the most fundamental necessity for life (Falkenmark, 2020). It is the most crucial environmental component controlling the health and well-being of all living things (Rosenstock, 2003). Our biosphere is composed of Water, Land, and Soil but water is vital because almost 70% of the earth's surface is filled with water and the rest 30% is covered by Lands, soil, etc. (Olueh, 2017;) but inside 70%, 97% of the water on Earth is stored in the seas and oceans, which are generally salty and useless. Only 3% of water is classified as freshwater, and of that, 2% is stored as ice and glaciers, which are likewise inaccessible, while the remaining 1% is held in lakes, canals, and underground, which is the only source of water used for consumption (Idowu, 2011). However, due to growing human populations and activities that have a significant detrimental impact on the chemical and microbiological characteristics of existing water supplies, access to drinkable water has long been a problem.

Bangladesh is a riverine country & it is proud of its rivers, which have one of the greatest networks in the world and have a total of over 700 rivers, including tributaries.The majority of Bangladesh's rivers begin in northern India, cut across the country, and empty into the Bay of Bengal in the south. However, there is a lack of clean water throughout Bangladesh(Banu., 2013) , due to microbial pollution, heavy metals, and industrial wastes disposed by industries made the river toxic at a level that it has a very adverse effect on public health (Hasan., 2019)

Furthermore, Over 215,000 people in Bangladesh died due to pollution; in addition, 30,000 died due to water pollution (daily star). Unfortunately, the main resource of water in Bangladesh is Rivers that are used not only domestically but also industrially because of that rivers are constantly polluted. The effect of water pollution are adverse hampering public health.

1.2: BACKGROUND:

Bangladesh is a vastly populated, low-lying, principally riverine nation with a 720 km (447 mi) long coastline along the northern littoral of the Bay of Bengal. The significance of rivers in our country is phenomenal. Due to that, rivers are connected with our daily life they flow through our communities. Also, river water is used for irrigation in agriculture, industrial use for their local household works, drinking water, transportation, producing electricity through hydroelectric dams, and leisure activities like swimming and boating(wikipedia).

Bangladesh is surrounded by 800 rivers (wikipedia) among them Turag River is one of them. Actually, the Turag River is the upper branch of Bangladesh's main river, the Buriganga, originating from the Bangshi River. An essential lifeline for the Dhaka metropolis, the Turag River flows through Gazipur from the north to reach the Buriganga at the south (Wikipedia). Turag is well known for BISSHA IJTEMA; the second-largest Islamic gathering after the Hajj(Banglapedia). It is thought to draw between two and four million Muslims annually as well as visitors from more than sixty nations (Wikipedia) also River bank has seen the growth of numerous industries over the past ten years, and this trend is continuing (Doe, 1997). Tongi region, located on the Bank of the Turag River, is largely known and developed as an industrial zone (Poit, 2017).

However, it’s very unfortunate that Turag is well known as the most polluted waterways because of a larger amount of industrial effluents released into the river In the Tongi area, there are many tanneries and chemical factories, as well as enterprises that produce metal, clothing, jute, textiles, spinning, pharmaceuticals, and food. Water quality diminishes due to heavy metal contamination, which affects water's qualities including pH, EC, and TDS as well as natural processes and communities of natural resources for habitats and fisheries resources. Heavy metal exposure has been associated with several problems in aquatic organisms as well as humans(Debnath, 2018) because the chemicals used during the cultivation of fish hampers the food chain also ecosystem this whole process is known as Biomagnification; the accumulation of certain chemicals in living organisms to a concentration higher than that occurring in the inorganic, non-living environmentislam. This biomagnification hampers our health vastly(Adriaens,2007). Not only industrial issues but also the arrangement of vast amounts of people during Bissha Ijtema also pollutes the water surface because river water works as the main source of refreshment for them as a result the amount of fecal coliform in the water increases.

Moreover, the Turag River is the main source of fish supply but as there are so many pollutants present in the water for that reason, microbial contamination must be vast. To assess the level of microbial contamination, and identify those bacteria to check if there is any health concern present by using that water is the main purpose of the thesis.

1.3: POTENTIAL FOR WATERBORNE ILLNESSES IN BANGLADESH:

Diseases that can be caught from drinking contaminated water are referred to as "water-borne diseases." Diseases in humans and other animals are typically caused by bacteria, viruses, and even parasites. These infectious pathogens adapt to their environments and spread using a wide range of mechanisms. There are three main ways that diseases can spread: through the air, water, or from person to person. The respiratory tract's pathogens use air as their entry vehicle, while the digestive tract's pathogens use water. *Aeromonas spp., Enterobacter spp., Enterococcus spp*., *Escherichia coli, and fecal coliforms, Klebsiella species, Campylobacter species, Clostridium species, Listeria species, Burkholderia pseudomallei, Helicobacter pylori, Pseudomonas species, Salmonella species, Shigella species, Staphylococcus species, and Vibrio cholera* have been identified as the major water-borne bacterial pathogens. Bangladesh is a country with about 130 million people living in an area of 148,393 square kilometres, making it one of the countries with the highest population density in the world, and waterborne diseases are a great concern in our country.

Superficial infections like skin diseases and sexually transmitted diseases like AIDS are spread primarily through close personal contact. However, these mechanisms do not rule each other out. Many pathogens, including viruses, gain access to the body through the digestive tract, but they quickly leave the gut and travel to other organs. Paralysis caused by the poliovirus is one such example; the virus, which spreads through contaminated food and water, attacks the body's nervous system.

1.4: ANALYSIS OF THE TURAG RIVER AND INDUSTRIES

The current study was carried out in Bangladesh's Turag River, which runs through the upper tributary of the Buriganga Dhaka area. The river was formed by the Bangshi River, an important tributary of the Dhaleshwari River, and flows through Gazipur before joining the Buriganga near Mirpur in Dhaka District. Turag is 75 kilometers long, however, only roughly

18.4 kilometers are inside the study area, beginning from Amin Bazar Bridge (23°47' N, 90°20'E) and ending with Kamar Para Bridge.

The Turag River is surrounded by a heavy industrial area in Tongi. The range of DO levels for those industries is 2.32 to 6.28. In the current situation, the maximum value for DO—29.6 mg/L—has decreased to 1.85 to 4.2 mg/L,(water-borne disease, Banglapedia,2012) in the confluence point of the Turag River, which means a higher BOD value. Due to the majority of the factories being located close to the river's bank, the biological oxygen demand (BOD) is considerable. They release organic waste, such as sewage treatment plant effluent, stormwater runoff, home wastewater (including food and human waste), industrial wastewater (from the tannery, textile, and food processing sectors), agricultural slurry, and silage liquor. Industrial waste, untreated sewage, wastewater, oil spills, silt, encroachment, and other pollutants contaminate the Turag River. Solid and liquid pollutants both enter the body of water. It is clear from all measured indices, especially DO and BOD, that Turag water contamination has reached a catastrophic degree and is only getting worse. And the industries pose a significant environmental risk.

1.5: LITERATURE ASSESSMENT:

Rivers have been acknowledged as the backbone of human civilization since the beginning. The significance of rivers regarding agriculture, transport, and municipal sewage is phenomenal. Notwithstanding the availability of a huge quantity of water, only 3% of the water in the universe is fresh water. Among fresh water, only about 5% of them, or 0.15% of the total world water, is readily available for beneficial use (Rani, 2010). The significance of water is a marvel in terms of supporting surface, ground, marine, and coastal waters that support all living things (Rani, 2010).

Aquatic environments are currently contaminated physically, chemically, and biologically due to bathing and recreational activities. There are designated bodies of water that are only used for bathing and are frequently inspected. According to (Liu,2022), "the recreational use of such water-bodies against the official recommendations may lead to their ecological degradation and is an epidemiological threat." According to (Afzal , 2021), 90% of deaths in children under the age of five are attributed to water contamination in underdeveloped nations, where this epidemiological danger is most common. The importance of water to public health is

enormous. Basically, the fecal-oral pathway is used to spread a wide range of infectious diseases, causing the deaths of roughly 5 million children each year and the illness of 1/6th of the world's population (Shittu, 2010). Not to mention that, with 22 million fatalities per year (Cho, 2020), waterborne illnesses are the main cause of mortality and morbidity worldwide. When compared to developing nations like India, Bangladesh, and Pakistan, where a sizable portion of the population relies on untreated surface water for drinking, bathing, recreation, etc. (Baten & Titumir, 2015b), developed countries have experienced some success in reducing the quantity and impact of the worst infections like cholera and typhoid.

1.6: AIM AND OBJECTIVES

- This study aimed to identify the antibiotic-resistant bacteria in the Turag River.
- Also, the isolates' antimicrobial susceptibility patterns and, for further bacteriological confirmation, PCR were included in this study.
- The authors believed that because of the high levels of pollution from industries, there would be an abundance of antimicrobial-resistant bacteria found in the waters.

1.7: RESEARCH NOVELTY: As Bangladesh is a developing country, most of the industries are situated on the bank of the river. As a result of industrial effluents river water gets contaminated very easily. However, not only industrial effluents but also, people who are living at the bank, usage of river water for their daily life also contaminates the water For that reason the level of contamination especially various microorganisms present in the water. Most of the article focuses only *Vibrio cholera* in the river but there must be other bacteria present that can cause severe health problems and they can be multidrug resistant. In order to, find out those multidrug-resistant organisms to prevent health problems we used Turag River water for the identification of those species. Species like *E.coli, Klebsiella spp, Staphylococcus spp ,* *Pseudomonas spp* , *Vibrio genus* by taking resistance strain and PCR for further analysis represents that multidrug resistance strains present on the water and causing severe health effects.

CHAPTER-02: MATERIALS & METHODS:

2.1 :SAMPLING SITES

The research was carried out in Bangladesh's Turag River (23°47' N, 90°20' E) in Dhaka Upazila, Dhaka district. The study area is approximately 10 km2. In July, water samples were collected. The river was sampled at four locations (Point 1 Amin Bazar Bridge, Point 2 Birulia Landing Station, Point 3 Rustampur, and Point 4 Ashulia Landing Station) in consideration of water pollution and human consumption.

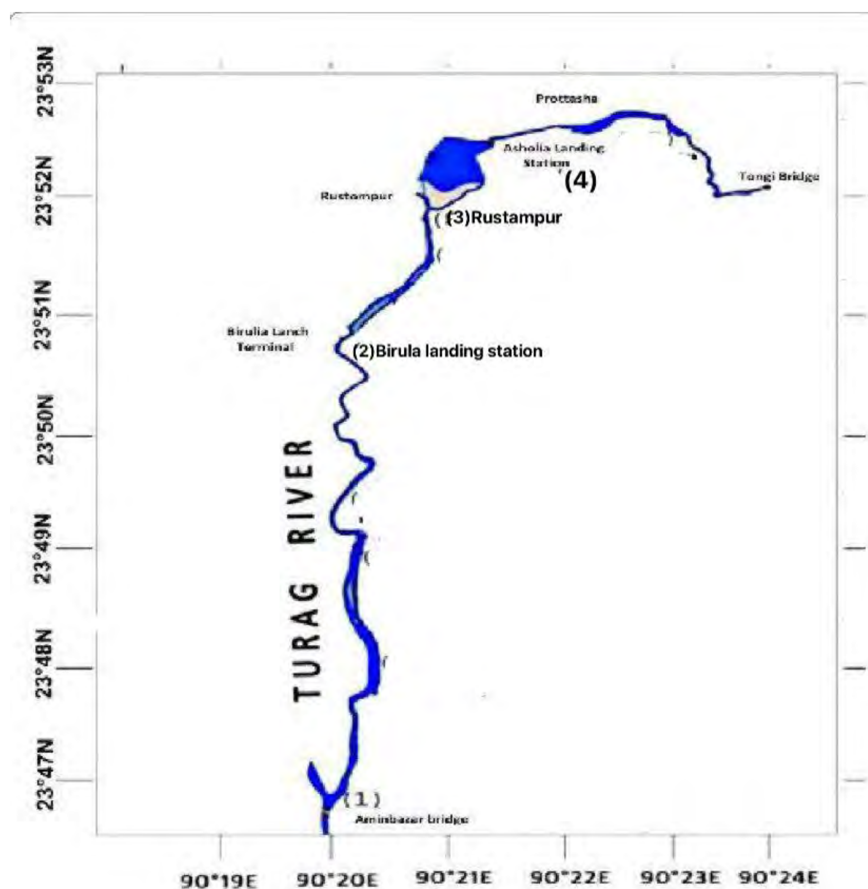

FIGURE 1: SAMPLING SITES OF TURAG RIVER

Adapted from (Bhouiyan & Baki, 2016)

2.2: SAMPLE COLLECTION

Each sampling site yielded a total of 16 water samples, collected in two separate sets. The raw river water samples were collected from each of the aforementioned sampling locations in sterile collection bottles that had been autoclaved previously. Each container had a 500-ml capacity. Aseptically, the container's caps were removed. After removal, the bottles were

lowered to depths of approximately 2 meters at the sampling sites. To reduce the risk of contamination, the bottles were brought back to the surface and sealed as soon as they were brought to the surface. In each sampling location, the same method of sample collection was utilized.

2.3: TRANSPORTATION OF SAMPLES: The samples were then transported to the laboratory at refrigerator temperature and assessed within 12 hours of collection. To maintain the cold chain, all of the containers were kept in an icebox at 4 ºC. Typically, samples must be processed within six hours of collection. However, the lab was approximately 22.9 kilometres away from the location. The cold chain contributed to the maintenance of stable conditions, as most microorganisms cannot grow at such low temperatures. In addition, the containers were airtight to prevent contamination and sealed to prevent spillage. It took approximately two hours to travel from sample collection sites to the laboratory, as our location was not very far. The samples were analyzed within five hours of being collected.

2.4: PREPARING THE SAMPLE:

The collected samples were processed in three steps. Those were: codename generation, serial dilution, and membrane filtration.

2.5: SERIAL DILUTION

For the initial step of primary processing, 100 μl of each sample was extracted without dilution. The authors have referred to those undiluted samples as "raw" in this paper. This step was completed using the spread plate method. Xylose Lysine Deoxycholate Agar, Thiosulfate Citrate Bile Salts Agar, Mannitol Salt Agar, and Cetrimide Agar were used to test the 50l raw samples. *Salmonella spp., Shigella spp., Vibrio spp., Staphylococcus spp., and Pseudomonas spp*. were isolated and identified using these specific media.

50 μl of each sample was collected again, and serial dilution was used. These samples were diluted to a factor of 10^5 times. The diluted samples were spread onto MacConkey agar and Nutrient agar using the spread plate method. The general growth of microorganisms was observed using nutrient agar, whereas E. coli was identified and counted using MacConkey. All samples, both diluted and raw, were incubated at 37°C for 24 hours.

2.6: MEMBRANE FILTRATION

E. coli and other total and fecal coliform bacteria were isolated using the membrane filtration method in the second stage of primary processing. The samples were examined for total heterotrophic count, total coliform presence, and fecal coliform. This procedure makes use of MFC agar.

100 ml of each water sample was filtered through sterile white filter paper labeled with a grid, measuring 47 mm in diameter and 0.45 mm in pore size. The membrane filtration unit was set up in accordance with. Membrane filtering In the second stage of primary processing, *E. coli* and other total and fecal coliform bacteria were isolated using the membrane filtration technique. The samples were examined for the presence of fecal coliform, total coliform, and total heterotrophic count. This procedure made use of MFC agar.

For the first step of primary processing, 50 µl of each sample was taken without dilution. For this paper, the authors have referred to those undiluted samples as "raw." For this specific stage, the spread plate approach was used. On top of Xylose Lysine Deoxycholate Agar, Thiosulfate Citrate Bile Salts Agar, Mannitol Salt Agar, and Cetrimide Agar were placed in the 50 µl raw samples. For the isolation and identification of *Salmonella spp., Shigella spp., Vibrio spp., Staphylococcus spp., and Pseudomonas spp.,* respectively, these specific mediums were chosen.

2.7: COLONIES SELECTION

Colonies with distinctive morphologies were chosen from each sample after bacterial enumeration and counting. These colonies were then analyzed thoroughly. In total 45 colonies in total were chosen. We then came up with codenames for our samples.

2.8 MEDIA:

Culture media are used as the initial stage in all microbiological studies for a fundamental assessment. Various types of media were employed for this experiment. As media, the thiosulfate-citrate-bile salts sucrose agar, xylose-lysine-deoxycholate agar, Mannitol salt agar, HiCrome UTI agar, nutrition agar, and cetrimide agar were used.

Thiosulfate-citrate-bile salts-sucrose agar (TCBS agar) :

In order to separate different *Vibrio* species, microbiology labs utilise a selective agar medium called TCBS agar, also referred to as thiosulfate-citrate-bile salts-sucrose agar. The TCBS Agar is differential and selective at the same time. It is selective for specific *Vibrio* species because of the inclusion of dyes and sugar.

TCBS agar can be used to isolate many *Vibrio* species, as well as *V. cholerae and V. parahaemolyticus.* The sodium thiosulfate and sodium citrate concentrations in TCBS agar are high enough to inhibit the growth of Enterobacteriaceae. Gram-positive bacteria are inhibited by ox gall, a naturally occurring substance made up of a combination of bile salts and sodium cholate, a pure bile salt. Sodium thiosulfate, another sulfur source, works in conjunction with ferric citrate to quickly detect the production of hydrogen sulfide. The alkaline pH of the medium encourages *V. cholerae* recovery while inhibiting the growth of other microbes. As pH change indicators, thymol blue and bromothymol blue are employed.

XYLOSE-LYSINE DEOXYCHOLATE AGAR (XLD AGAR) :

When isolating *Salmonella* and *Shigella* species, XLD media is employed as a selective growth medium. Utilizing XLD Agar, gram-negative enteric pathogens can be identified. It also contains yeast extract, which is a source of vitamins and minerals. As a selective agent, sodium deoxycholate is utilized in this instance to inhibit Gram-positive bacteria.

Mannitol Salt Agar (MSA AGAR) :

The selective and diverse growing medium is known as mannitol salt agar (MSA). It contains a sizeable amount of salt (NaCl), which is harmful to the majority of bacteria, in the range of 7.5–10%. Utilizing Mannitol Salt Agar, *Staphylococcus aureus* strains from clinical and non-clinical material are extracted and identified.

Macconkey Agar Medium:

MacConkey agar is a specialized and distinct microorganism culture medium. It is intended to distinguish and selectively separate Gram-negative and positive bacteria based on lactose fermentation and enteric bacilli, which are usually found in the digestive system. It includes bile salts, which avoid the growth of the vast majority of Gram-positive bacteria, except Enterococcus and some species of Staphylococcus, like *Staphylococcus aureus,* as well as crystal violet dye, which inhibits the growth of some Gram-positive bacteria, and neutral red dye, used as a stain for lactose- and peptone-fermenting microbes.

Hi Chrome UTI:

Hi Chrome UTI in samples like water that may contain many *Proteus* species as well as potentially pathogenic gram-positive organisms, agar is a chromogenic differential medium for recognizing, distinguishing, and determining the conformation of enteric bacteria. In chromogenic UTI media, two distinct chromogenic substrates are broken down by enzymes generated by *Enterococcus species, Escherichia coli,* and *coliforms.* Furthermore, it contains tryptophan, which, given the activity of tryptophan deaminase (TDA), supports the presence of *Proteus spp.*

Nutrient Agar:

Microorganisms that promote the growth of a range of non-fastidious organisms are grown in nutrient agar. Because nutrient agar lacks an indicator, a selecting agent, differential ingredients, and enriching chemicals, it can be employed for better pigmentation expression, biochemical tests, and even serotyping.

Cetrimide Agar:

It is recommended to utilize cetrimide agar, a selective solid medium agar, for *Pseudomonas spp.* selective isolation and presumed qualitative identification methods. The selective ingredient, cetrimide, acts as a detergent to prevent the majority of bacteria. Here, cetyltrimethylammonium bromide, a quaternary ammonium, is used as a cationic detergent.

2.9 MICROBIAL IDENTIFICATION

Biochemical Tests:

Selected isolates were subcultured onto nutrient agar for pure culture isolation so that microbial identification could be performed by biochemical testing. The specific biochemical tests selected for this particular study were Gram staining Citrate Utilization. Triple Sugar Iron (TSI) test, Oxidase, Catalase Methyl Red (MR), Voges-Proskauer (VP), and Motility-Indole-Urease (MIU) test.

Gram Staining:

Gram staining is a technique used for morphological identification. Based on cell wall composition, Gram staining is used to distinguish between Gram-positive and Gram-negative bacteria. The hypothesis for Gram staining involves the ability of bacterial cell walls to retain crystal violet staining during treatment with ethanol solvents. In gram-positive organisms, after ingestion of the crystal violet dye, 95% ethanol is first used to dehydrate and shrink the thick peptidoglycan cell walls, closing the pores and preventing the dye from exiting the cells. Therefore, crystal violet-iodine complexes bound to the thick peptidoglycan layer of gram-positive bacteria are not removed and come out blue or purple. In gram-negative organisms, the thin layer of peptidoglycan causes the lipid layer to dissolve and the primary pigment to be lost. A basic fuchsin dye such as safranin is used to give the decolorized Gram-negative bacteria a pink color for easy identification.

Citrate Utilization Test:

The citrate utilization of the isolates was observed in the growth on the slant of Simmons citrate agar. After incubation, citrate-positive cultures were determined by the presence of growth on the surface of the slant and staining in dark Prussian blue, while Citrate negative was determined by the absence of growth and green staining of the medium. This test determines whether bacteria can use citrate as their sole carbon source. A single colony was extracted from each isolate using a sterile needle.

The solidified medium was then inserted by the needle, and the slant surface is recorded when the needle is withdrawn. Results were observed after the vials were incubated for 24 hours at 37°C.

TSI/ Triple Sugar Iron Test

This experiment aims to determine the fermentation of glucose, lactose, or sucrose and H_2S production. It contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulphate, and the pH indicator phenol red. It is used to distinguish enteric based on their ability to decrease sulphur and fermentable carbohydrates. For the test, the mixture was mixed with various other ingredients, such as sodium thiolate, ferrous ammonium sulphate, 1% lactose, 1% sucrose, and 1% glucose. These ingredients allowed solidification at a slanted angle, and the test tube was created. Phenol Red is an indicator that turns yellow at an acidic pH due to fermentation. The glucose concentration was 0.1% and the lactose and sucrose concentrations were both 1%. If an organism only ferments glucose, fermentation takes place in the butt, causing the butt to turn yellow. At the slant, aerobic oxidation takes place, and it remains red. If sucrose or lactose is fermented, the amount of acid produced during butt fermentation is enough to lower the pH of both the butt and the slant, causing both of them to turn yellow. Gas can also be detected as bubbles trapped in the agar or separating from or pushing up the agar. The generation of H2S was detected by the formation of ferrous sulphide (FeS), an insoluble black precipitate in which ferrous ammonium sulphate reacts with H2S gas.

Oxidase

The oxidase test is used to indicate if an organism has the cytochrome c oxidase enzyme, which is an enzyme of the bacterial electron transport chain that catalyzes the oxidation of cytochrome c. Again, for the electron transport chain (ETC), the cytochrome-c oxidase enzyme plays a vital role. It indicates the existence of the cytochrome-c oxidase enzyme when oxidizing TMPPEH (tetramethylp- 34 phenylenediamine dihydrochloride, the redox dye).

The blue-purple colour can be noticed after the oxidizing, which indicates an oxidase-positive result. The organism does not contain the cytochrome-c oxidase enzyme, which remains colourless, which indicates an oxidase negative result. Oxidase-positive bacteria are considered aerobes, and oxidase-negative bacteria are considered anaerobes, aerobes, or facultative.

Catalase

The catalase test is used to determine the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) could be a harmful by-product. It is utilized as a reagent; 3% hydrogen peroxide is utilized. Hydrogen peroxide (H_2O_2) is changed into oxygen and water through the action of an enzyme called catalase. It is evident that the enzyme is displayed if a little inoculum of a bacterial isolate is included with hydrogen peroxide and the rapid elaboration of oxygen bubbles takes place. The non-appearance of catalase is evident from a lack of drowsy bubble generation.

When a small inoculum of a bacterial isolate is added to hydrogen peroxide and the rapid elaboration of oxygen bubbles takes place, it is clear that the enzyme is present. The absence of catalase is clear from a lack of bubble production. The culture shouldn't be older than twenty-four hours. By doing this, microbes guard themselves against hydrogen peroxide's dangerous impacts. As a byproduct of aerobic carbohydrate metabolism, accumulation occurs.

Facultative anaerobic and strict aerobic bacteria can be classified as catalase positive. Therefore, oxygen is the final electron acceptor in all these bacteria. To perform the catalase test, a small amount of the isolated bacteria was mixed in a 3% hydrogen peroxide solution. As a result, the production or absence of bubbles was observed.

MR TEST

Methyl red (MR) tests determine an organism's capacity to create and sustain stable acid end products from the fermentation of glucose. Via the "mixed acid pathway," these bacteria convert glucose into pyruvic acid, which they then use to produce stable acid. The acid causes the pH to drop to 4.5 or below. Methyl red serves as the pH indicator in this test and can identify high levels of acidic end products. The indicator methyl red changes colour from yellow to cherry red as a result of the pH change, signaling a successful MR test.

This test was conducted using fresh 24-hour bacterial cultures. The MR broth was taken in one sterile tube. Then it was marked with a marker. An inoculating loop and ascetic approach were used to transfer a loop full of bacterial culture into the MR broth. It was confirmed that the loop was shaken and rubbed against the tube's side to drain any extra broth. After that, this tube was kept in the incubator at 37°C for 24 to 48 hours. Inoculated MR broth was removed from the incubator after incubation. The tube received five drops of methyl red indicator, which was

then mixed by rolling it between the palms of the hands. The yellow colour ring represented negative results for the MR test, whereas the red colour ring referred to positive results.

VP TEST

The VP test finds certain organisms that convert organic acids produced by the metabolism of glucose into neutral or nonacidic end products, such as acetyl methyl carbinol. The Barritt's reagent solution, which contains 40% KOH and 5% alpha-naphthol, reacts with the acetoin the bacteria in the solution create. Within 30 minutes of the addition of the reagent, this reaction will yield diacetyl and a deep rose or pink colour in the medium, which is a positive VP test. On the other hand, yellow or a lack of a rose or pink tint, indicates a poor outcome. Fresh 24-hour bacterial cultures were used for this test. The VP broth was taken out of one sterile tube. It was then marked with a marker. An inoculating loop and ascetic approach were used to transfer a loop full of bacterial culture into the VP broth. It was confirmed that the loop was shaken and rubbed against the tube's side to drain any extra broth. Following that, this tube was kept in the incubator at 37°C for 24 to 48 hours. Following incubation, the tube containing the bacteria-ridden VP broth was removed from the incubator, and 10–14 drops of Barritt's reagent (Barrit reagent A- 6 drops and Reagent B-2 drops) were added. The tube was gently shaken for several minutes, and a wait time of 15 to 20 minutes was required for a colour change. A positive outcome is when rose or pink colours form, while a negative outcome is when yellow colours or no rose or pink colours occur.

Nitrate Reduction Test

The nitrate reduction test measures the amount of an enzyme called nitrate reductase or nitrite reductase, which reduces nitrate (NO_3). Because anaerobic metabolism requires an electron acceptor other than air oxygen, many gram-negative bacteria employ nitrate as the final electron acceptor. (O_2). The capacity of different bacterial species to convert nitrate to nitrite or nitrogenous gases allows for the differentiation of these species. The nitrate in the broth is converted to nitrite, which can then be further converted to nitric oxide, nitrous oxide, or nitrogen by organisms that can produce the nitrate reductase enzyme. The nitrate reduction test relies on the detection of nitrite, which can combine with sulfanilic acid to form a complex (nitrite-sulfamic acid), which then reacts with a -naphthylamine to produce a red precipitate (prontosil), which is a water-soluble azo dye. The red colour will only be produced if nitrate is

present in the medium. The absence of red colour in the medium after adding sulfanilic acid and the test for nitrate reduction is negative naphthylamine merely indicates that nitrite is not present

A heavy inoculum of the test organism was incubated in a nitrate-containing broth for 24 hours at 37°C before the outcome was discovered. After 24 hours of incubation, five drops of nitrate reagent A were applied, followed by five drops of nitrate reagent B. The colour of the broth changing to red denotes a positive result and the presence of an organism that may produce nitric oxide, nitrous oxide, or nitrogen. A modest amount of zinc powder would be added if no red colour was seen. After that, if it becomes red, the organism cannot reduce nitric oxide, nitrous oxide, or nitrogen. and the test for nitrate reduction is negative.

MIU/ Motility Indole Urease Test:

For MIU, three tests are carried out in a single tube to distinguish the organisms based on their motility, urease production, and indole synthesis. The purpose of this test is to track the migration of microorganisms. The ability of an organism to move independently is referred to as motility. Bacteria that cannot move are referred to as non-motile. Motile organisms exhibit growth extending away from the stab inoculation line. Non-motile organisms exhibit growth just along the stab line. The growth of non-motile organisms, on the other hand, takes place parallel to the stab line. Ammonia and carbon dioxide are released when urea is hydrolyzed by urease, and they combine in solution to produce ammonium carbonate. A positive urease result is produced when the pH increases and the phenol's color changes from red-yellow to pink-red at an alkaline pH. The enzyme tryptophanase in indole-positive organisms converts the tryptophan in casein enzymatic hydrolysate to indole. A quinoidal red-violet chemical is created when the resultant indole combines with the p-dimethyl amino benzaldehyde in Kovac's reagent.

The MIU media was made in accordance with the HiMedia-specific specifications. Additionally, a separate 40% urea solution was made based on the required volume. Then, bacterial colonies were taken and inoculated with a single stab using a sterilized needle. It was then incubated for 24 hours at 37 °C. By adding reagents, results were seen and noted after the incubation period. When hazy growth was seen around the stab line or throughout the media, it signified the presence of motile organisms, representing positive results. The clear transparency of the medium and the absence of any hazy development indicate a failure of the

motility test. While waiting for the result of the **indole test**, five drops of Kovac's reagent were put on. A positive outcome is indicated by the development of a pink to cherry red colour ring. On the other hand, if the media continues to be yellow, the outcome will be bad. If the medium for the **urease test** changes from orange-yellow to deep pink after 24 hours of incubation, then the test is positive. Negative results will occur if there is no colour change. produced by adhering to Media's instructions.

ANTIBIOTIC SUSCEPTIBILITY TEST:

Antibiotics are drugs that are used to treat bacterial infections. They are classified into several categories. Each kind is only effective against a specific type of bacteria. An antibiotic sensitivity test can assist you in determining which antibiotic will be most effective in treating your infection.

The test may also aid in the discovery of a treatment for antibiotic-resistant illnesses. When regular antibiotics become less effective or useless against some bacteria, this is referred to as "antibiotic resistance." Antibiotic resistance has the potential to transform previously manageable diseases into serious, even life-threatening illnesses.

For this test, the bacterial isolates were enriched in saline for 2 hours at 37°C in an incubator before being swabbed on Mueller Hinton Agar (MHA). Then use a sterile swab stick to create a mat of bacteria on the agar plate. The antibiotic disc was then taken out of the cartridge and put on the agar plate with sterile forceps. In an incubator, the plate was incubated again overnight at 37°C. The zone of inhibition (clear zone) was measured using a ruler the following day between 18 - 20 hours and compared to the antibiotic disc zone diameter interpretation. A clear zone of a specified size indicates bacterial susceptibility to the specific antibiotic, and a lack of a clear zone suggests antibiotic resistance.

Eight antibiotics are used, azithromycin, levofloxacin, meropenem, tetracycline, cefixime, amikacin, & chloramphenicol for both gram positive and gram negative bacteria also used vancomycin only for gram positive bacteria.

CHAPTER: 3

MOLECULAR ANALYSIS

3.1 DNA EXTRACTION: The selected isolates' genomic DNA was extracted, and it was done by the boiling method. At first, the isolates were cultured in Luria Broth overnight at 37 °C in a shaker incubator.

After that, 700 microliters were transferred into an Eppendorf and centrifuged at 13000 rpm for 10 minutes. Then, the supernatants were discarded, and the pellet was suspended in 200 microliters of TE buffer and subjected to heat at 100 degrees C for 15 minutes in a boiling manner in a water bath, followed by cold shock for 5 minutes. After the cold shock, the pellet was centrifuged at 14000 rpm for 5 minutes at 15 °C, where, after centrifugation, cell debris precipitated at the bottom. Now supernatant was collected.

3.2 POLYMERASE CHAIN REACTION: PCR is a common laboratory technique where a specific DNA fragment can be amplified quickly. In PCR, a section of the genome to be amplified is chosen using short synthetic DNA fragments called primers. Multiple rounds of DNA synthesis are then used to amplify that segment. PCR has a vast application in terms of identifying specific genus and organisms of bacterial, viral, and fungal pathogens; Genotypes are more specific and more easily quantified and standardized between different organisms than traditional phenotype markers (Garibyan & Avashia, 2013).

Our main purpose was to specifically identify the genus of organisms where mostly fecal origin pathogenic bacteria were found, such as *E coli, Klebsiella, Staphylococcus, Pseudomonas,* and so on. By following proper conditions, PCR was done and for confirmation of that particular organism, gel electrophoresis was done

For each bacterial sample, a total of 13 microliters of final solution were prepared, and with proper conditions and an adequate cycle, PCR was done except *Vibrio genus;* for *vibrio.spp* total 15 microliters final solution made . After PCR, the gel electrophoresis method was used, and the results were observed under UV trans luminance.

3.3 Supplementary Table:

TABLE 1 : PCR SUPPLEMENTARY TABLE

NAME OF THE BACTERIA	Primer Designa-tion	PRIMER SEQUENCE	Pro-duct size	PCR Condition
E.coli Spp	Eco –F Eco-R	5’GACCTCGGTTTAGTTCACAGA-3’ 5’-CACACGCTGACGCTGACCA-3’	585 bp	I.denaturation at 95◦ C for 5min, 35 cycles of denaturation at 94◦C for 45s, annealing at 45◦C for 45 s & extension for 1 min followed by a final extension at 72◦C for 5 min
Klebsiella pneumoneae	KP Pf-F KP Pf-R	5’-ATTTGAAGAGGTTGCAAACGAT-3’ 5-TTCACTCTGAAGTTTTCTTGTGTTC-3’	130 bp	Cycling conditions were 10min at 94 ◦C followed by 35 cycles 30 sat 94◦C. 20s at 57◦Cand 20 s at 72◦C, followed by a 10 min hold at 72◦C
Vibrio spp	VG-F VG-R	GTC ARA TTG AAA ARC ART TYG GTA AAG G ACY TTR ATR CGN GTT TCR TTR CC	689 bp	I.denaturation at 94◦ C for 10 min, 30 cycles of denaturation at 94◦C for 30s, annealing at 60◦C for 1 min extension for 72 ◦C 30 s followed by a final extension at 72◦C for 10 min

Pseudomonas aerogenesa	PA-SS-F PA-SS-R	5’-GGGGGATCTTCGGACCTCA-3’ 5’-TCCTTAGAGTGCCCACCCG-3’	956 bp	Initial denaturation at 95◦ C for 2 min, 25 cycles of denaturation at 94◦C for 20s, annealing at 58◦C for 20 s extension for 72 ◦C 40 s followed by a final extension at 72◦C for 1 min
Staphylococcus spp.	Ts TaG F Ts TaG R	5′-GGCCGTGTTGAACGTGGTCAAATCA-3′ 5′-TIACCATTTCAGTACCTTCTGGTAA-3′	370 bp	3 min at 96◦C & then 30 cycles of 1s at 95◦C for the denaturation step and 30 s at 55◦C for the annealing-elongation step.

3.4 GEL ELECTROPHORESIS:

Gel electrophoresis is a technique for classifying biological components (DNA, RNA, or Proteins) according to size. These molecules are separated by putting them in a gel with tiny pores and applying an electric field to the gel as a whole. The speed of the molecules depends on their size and electric charge. Gel electrophoresis visualizes, identifies, and separates molecules that have undergone processing by an earlier technique, such as PCR, enzymatic digestion, or an experimental condition. To identify or distinguish between components, nucleic acid or protein mixtures gathered from an earlier experiment or procedure are frequently processed via gel electrophoresis(Mutch., 2018)

After dissolving 1 g of agarose into 100 ml TBE/ TAE buffer, the agarose gel was processed and comb channels were made into it. This process works by Negatively charged molecules traveling away from the negative pole of the electric current during the gel electrophoresis process, and smaller molecules move more quickly than bigger molecules. As a result, the pool of molecules flowing through the gel is separated by size. Similar to a sieve, the gel separates particles according to size. The electrophoresis uses the particles' innate electric charge to push them through the sieve. At the beginning of the gel, the samples are loaded into the channels. Because DNA is made up of the same 4 nucleotides and carries a tiny negative charge regardless of size, every DNA molecule has the same charge (-1). Each DNA molecule will therefore be pulled through the gel with the same amount of force. (Mutch., 2018)

CHAPTER-04:

4.1: Results

In this study, 42 isolates were collected from different points of the Turag River and their presumptive identification was done by biochemical tests which were subsequently confirmed by PCR .

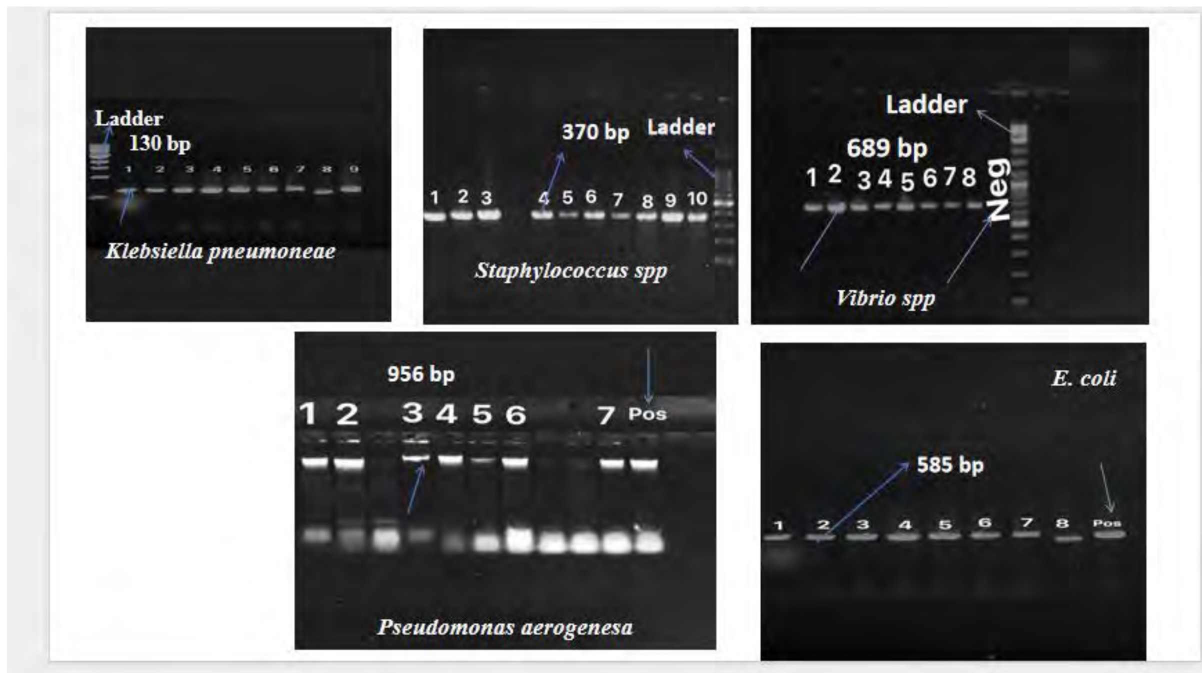

FIGURE 2 : AGAROSE GEL ELECTROPHOROSIS RESULT OF DIFFERENT ORGANISM TO CONFIRM THEIR IDENTITY.

Picture (A): Agarose gel electrophoresis of PCR assay of *Klebsiella pneumoneae* isolates. L represents DNA marker and positive samples from (1-10) at 130 bp

Picture (B): Agarose gel electrophoresis of PCR assay of *Staphylococcus spp*. isolates. positive samples from (1-7 & 9-11) at 370 base pair

Picture (C): Agarose gel electrophoresis of PCR assay of *Vibrio spp.* isolates. negative control set behind the DNA marker and positive samples from (1-8) at 689 bp.

Picture (D): Agarose gel electrophoresis of PCR assay of *Pseudomonas aerogenesa.* isolates. Here L lane represents the DNA marker ladder and positive samples from (1-7 and 9,10) and a negative control at 956 base pair.

Picture (E): Agarose gel electrophoresis of PCR assay of *E. coli* isolates positive samples from (1-9) at 585 bp.

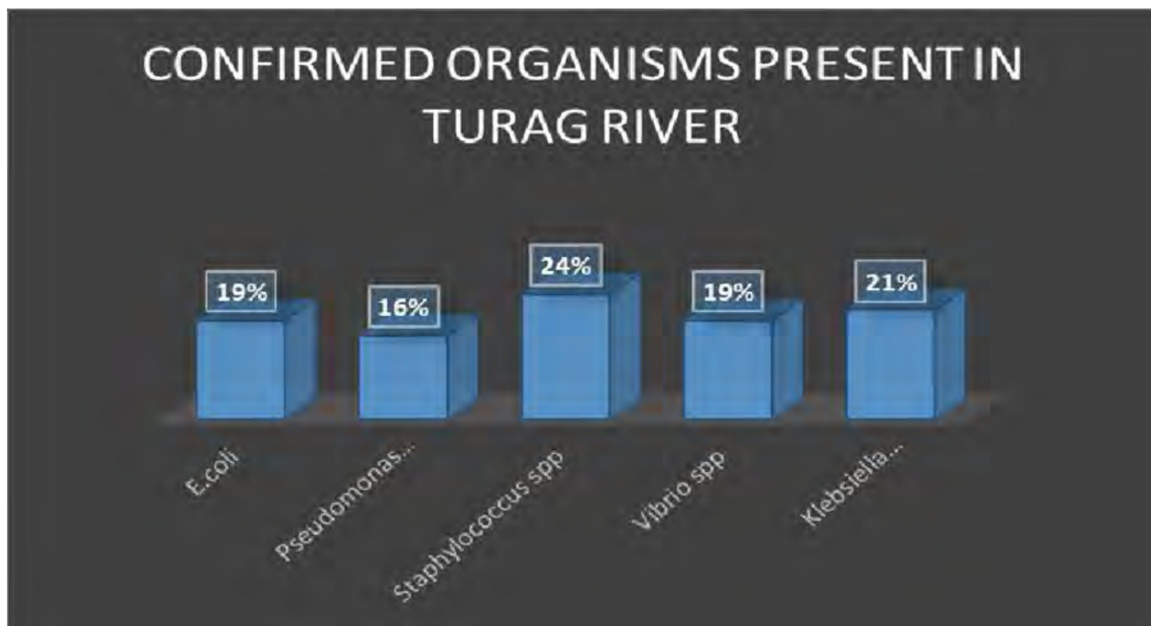

FIGURE 3 : ORGANISM IDENTIFICATION THROUGH PCR.

Figure 3. represents organisms that are surely present in the Turag River; that have been identified by PCR. The organisms were spread out in different ways at each of the four sampling sites shown in the Chart. Out of the 45 isolates found in the water samples ***E. coli*** contaminated around **19%,** while, ***Klebsiella pneumoneae 21%*** and dominating organisms

are ***Staphylococcus spp*** around **24%** whereas ***Pseudomonas aerogenesa 16%* and *Vibrio spp*** were about **19%.**

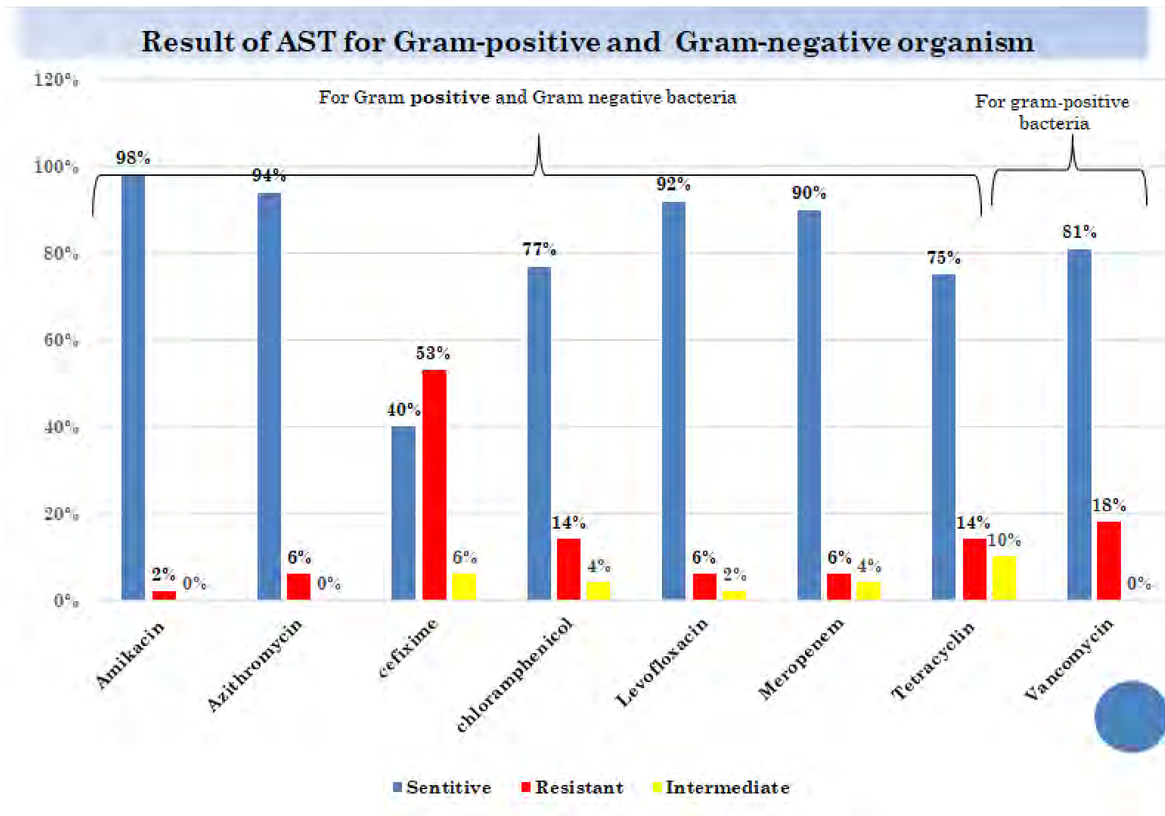

FIGURE 4 : ANTIBIOTIC SUSCEPBILITY TEST FOR DIFFERENT ORGANISM FROM DIFFERENT SITES OF TURAG RIVER SAMPLE (BOTH GRAM-POSITIVE & GRAM-NEGATIVE)

Figure 4 represents Antibiotic Susceptibility tests for different organisms (both Gram-positive and Negative) from different sites of Turag River. According to this chart, most of the organisms were Sensitive to Antibiotics like **Amikacin, Azithromycin, Chloramphenicol Levofloxacin, Meropenem , Tetracycline & Vancomycin**. & Organisms spread out in different ways after collecting from four sampling sites, and it was observed that **higher percentage** of organisms were **resistant** to **Cefixime** .

4.2 DISCUSSION:

In this investigation presence of fecal coliform and antibiotic resistance of 45 selected bacterial isolates collected from the Turag River were studied and their identity was confirmed by PCR.

ANALYSIS OF RESULT : Figure 2. shows that organisms that were present in Turag River contaminated the river water in high amounts which has an adverse effect on public health. These organisms were found from different sites(4 sampling sites) of Turag River where *Klebsiella pneumoneae (21%)* and *Staphylococcus spp (24%)* contamination are dominating because they contain equal and largest percentages than others. *E coli* contamination is another issue and from this chart, Turag River water has 19% of *E coli* contamination and *Vibrio spp* contamination. In addition, *Pseudomonas aerogenesa (16%)*

This represents that, river water is highly contaminated and people who are living on the bank of the river are likely to be affected by various diseases. Because organisms that are surely present in river water are mostly fecal origin and pathogenic causes diseases for example, By *Klebsiella* invasive diseases could emerge as a result of colonization. In particular, *Klebsiella pneumoniae* and *Klebsiella oxytoca* have the potential to induce life-threatening illnesses such as corrosive pneumonia. On the other hand, The human microbiome includes *Staphylococcus spp.*, which can cause illness in two ways despite being ubiquitous. Boils, skin sepsis, post-operative wound infections, intestinal infections, septicemia, endocarditis, osteomyelitis, and pneumonia are some of the diseases that can result from a bacterium multiplying in the body's tissues(WHO, 2022).

As *E coli* is a gram-negative pathogenic organism in fecal origin causes disease and no organisms of fecal origin should be present in any water source intended for human consumption or sanitation purposes. The World Health Organization's standards for assessing the bacteriological quality of drinking water (Idowu, 2011) state that water samples are unfit for human consumption if they contain any enteric coliforms, notably *E.coli*. Because several types of *E coli* can cause diseases including Diarrhea, Dysentery, UTI, Septicemia, and Meningitis. *Pseudomonas spp* found in soil and water is fecal origin pathogenic bacterium that causes infections in the blood, and lungs (Gebrewahd.,2020) whereas, *Vibrio spp* can cause Vibriosis and cholera (Vibriosis (Non-Cholera) - Epidemiology, 2023).

Antibiotic Susceptibility tests for different organisms (both Gram-positive and Negative) from different sites of Turag River revealed whether the organisms are Resistant, Sensitive, or Intermediate to multi-antibiotics. Fortunately, most of the organisms were Sensitive to multi-drugs except a few antibiotics. The rate of sensitivity to antibiotics is higher than Resistance-like organisms are 98% sensitive to Amikacin and 2% Resistant with 0% intermediate. Also, 94% were sensitive to Azithromycin with a 6% Resistant to the same. In terms of Levofloxacin, the sensitivity rate was 90% where a 6% resistance rate and a 4% intermediate rate. 2 exceptions were Cefixime (53%) and Vancomycin(61%) their resistance rate is higher than the sensitive rate representing that certain organisms can grow simultaneously in presence of these antibiotics. To summarize, Figure 4 represents a Lower Resistance rate, Higher Sensitive rate but a Middle Intermediate rate As sensitive represents organisms can't grow in the presence of certain antibiotics but Resistance is the opposite where organisms can grow in the presence of certain antibiotics while Intermediate represents that certain organisms still few amount of sensitive but it will be resistance soon. So, the rate of organisms sensitive to antibiotics higher than the resistance rate is good for the water environment. An antibiotic susceptibility profile was done by Author Poonia, T. Singh, and Dechen from bacteria isolated from natural sources of water from rural areas of East Sikkim where they found Ampicillin (57.50%) the most resistant antibiotic followed by Trimethoprim (39.10%). Where in Turag River we have found orgasims Resistant mostly to Cefixime and followed by Vancomycin .

PCR is done whether the organisms are present or not and AST is done for the organisms that are multi-drug resistant or sensitive or intermediate the findings of Pcr are satisfactory but AST findings are also caused mostly are sensitivity good for the environment but due to daily usage of river water for a wide variety of purposes, including drinking, bathing, and fishing, industrial, polluting the river in a large amount and presence of these certain organisms causing diseases frequently. So the experiment result is satisfactory enough to give goosebumps to everyone that rivers should be saved for Digitalized BANGLADESH.

Moreover, every day, a large number of pollutants, ranging in size from enormous amounts of trash to minute concentrations of dangerous substances, enter the oceans of our world. Additionally, the freshwater issue has been made worse by water pollution, droughts, and fast-expanding populations, affecting both our wellness and the security of numerous ecosystems. This is an alarming issue and it should be worked on globally the research is ongoing but research should be vaster to save water on Earth or else there would be one day when there will

be no fresh water present on Earth. There are lots of projects that work for the isolation and identification of multi-drug-resistant bacteria globally. To contrast with the project of Lake water in Udaipur, Rajasthan; India, they aimed contamination with bacteria from several lake water sources in and around the city of Udaipur, where mostly cocci, bacilli, and coccobacilli were found by Gram-staining primarily. After doing PCR, there was more E. coli contamination than others which ranges from 1–10 in 100 ml of lake water (WHO), so here the result was quite unsatisfactory that lake waters are very harmful for drinking. ((Bhumbla., 2020)). In Ghana, a West-African country, where the population is vast so the pollution rate because they live below poverty and this research focused on *E coli* contamination in well water where among 200 samples, all samples were positive for *E coli* contamination was significantly higher ($p<0.01$) than that of dam water, sachet water, rainwater, and tap water. This represents that well water is highly unsafe for drinking. (Sumaila, 2015). Also, similar research done in Ujjain district Triveni deals with microflora (*Staphylococcus*) in Khan River which contains a variety of infectious microorganisms that are dangerous not only to human health but also to aquatic flora and animals. The findings showed that various bacterial species, including *Pseudomonas* (37.79%), *Salmonella* (15.74%), *Klebsiella* (11.81%%), *Enterobacter* (7.08%%), *Streptococcus* (7.34%%), *Actinomycetes* (5.24%%), and *E. coli* (5.24%), were widely dispersed across the sampling sites. Other authors found that *Pseudomonas* (37.79%)` was the major bacterial strain in the sample, followed by *Salmonella* (Malik, 2022). To compare with Turag river water, it contains fecal origin organisms where 19% were *Escherichia coli*, 21% were *Klebsiella spp*., 16 % were *Pseudomonas spp,* 24 % were *Staphylococcus spp*. and 19% were *Vibrio spp*. Compare to Turag River water, it deals with *Klebsiella* and *Staphylococcus* followed By *E coli* dominates whereas Khan River deals with *Pseudomonas* and *Salmonella.* Both the river water is dangerous for public health and very dangerous for drinking and daily use. Turag River deals with mostly fecal-origin pathogenic organisms but the khan river deals with microflora which is even more dangerous because of the pollution present on it. Followed by Lake waters where *E coli* contamination is at the higher range, drinking this water can be very dangerous for health and even cause death to people every year surprisingly, this rate increase every year because 44% of wastewater return to the earth's environment untreated More than 80% of wastewater is recycled into different ecosystems untreated or uselessly. Approximately 2 billion people globally drink polluted water. The least developed countries produce 14 billion liters of wastewater daily that contains human waste, bacteria, and pathogens that breed disease. This is dumped directly into the

environment due to a lack of treatment infrastructure, affecting the soil, air, and underground water(Filipenco, 2022).

4.3 RECOMMENDATIONS:

Pollution of the Turag River must be avoided in order to rehabilitate the Turag River. Various efforts should be launched in this regard, including the following: The appropriate implementation of current rules and regulations, as well as any necessary enforcement, is required. Slums and illegal homes must be removed from the riverbed. Industries that have grown up illegally on the river's bank must be regulated. Dredging is required to keep the river flowing. Untreated home garbage and industrial effluents are polluting the river in large quantities.

The authorities should take proper action to remove this pollution so that it does not pollute the river. Governmental organizations (including DOE, WDB, and DWASA, among others) and non-governmental organizations (NGOs) need to take more proactive measures to reduce the Turag River's pollution. According to a previous study report, industries and solid waste are responsible for 90–94% of Turag River pollution. As a result, keen observation is required for industries and the generation of solid waste. Local organizations, residents, and provincial and federal departments should all be involved in the River's ecosystem restoration. The discharge of industrial effluents near-surface water, as well as domestic waste from residential areas, should be reduced, and sewage treatment plants should be established to monitor the region's human settlements. Another suggestion is to organize educational programs to provide people in the community with accurate information about wastewater management and soil conservation methods. Although some work has already started in these places, especially in terms of wastewater management, much more effort is still required to promote the use of land management techniques there.

Chapter 5: Future Prospects

5.1 LIMITATIONS

The sample collected during the rainy season was a key limitation of our experiment. That is why the water sample was already diluted enough that organisms couldn’t grow as expected, so it had a great impact. For that reason, we had to wait longer for proper sampling.

5.2 FUTURE PROSPECTS

In order to provide an extensive overview of the water quality of the Turag River, the authors' focused exclusively on the microbiological pollution of the water. The physicochemical characteristics of the water, including its pH, salinity, dissolved oxygen (DO), BOD, COD, and pathogenicity test, can be thoroughly analyzed in the future. Future research could examine pollution in water samples by collecting water sample of seasonal variations.. Suitable steps to lower the level of pollutants in industrial wastewater and renovate sewage treatment facilities should be adopted to prevent further degradation of Turag River water quality.

CHAPTER 6 : CONCLUSION

The water quality of the Turag River was initially evaluated through this investigation. The Turag River's antibiotic-resistant bacteria and genes were primarily the focus of this study, which also examined the degree of contamination there. The results of the investigation showed that *Yersinia spp., E. coli, Klebsiella spp., Pseudomonas spp., Vibrio spp., and Staphylococcus spp.* were all major microbes responsible for the contamination.

The observed bacteriological characteristics indicate that the river water was severely polluted by industrial effluent discharge, sewage waste, agricultural and municipal run-off, and human activities. Compared to the rainfall season, the dry season has a somewhat greater pollution level. To prevent further deterioration of water quality, several mitigating actions, such as ongoing evaluation of environmental conditions, promoting public consciousness, and enforcing strict standards for river usage and upkeep, are required.

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CHAPTER 8: APPENDIX

MEDIA COMPOSITIONS

The compositions of all the media (used from HiMedia), employed in the study are given below:

Saline:

Component	Amount (g/L)
Sodium chloride	9

Nutrient Agar

Composition	Amount (g/L)
Peptone	5
Sodium Chloride	5
Beef extract	3
Agar	15
pH	7

Nutrient Broth

Component	Amount (g/L)
Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Final pH at 25ºC	7.4±0.2

Luria Bertani Broth:

Component	Amount (g/L)
Casein enzymic hydrolysate	10
Yeast extract	5
Sodium Chloride	10.0
Final pH at 25ºC	7.5±0.2

Component	Amount (g/L)
Peptone	3%
Sodium chloride	0.50%
Urea	2%
Mono Potassium Phosphate	0.20%
Phenol Red	0.00%
Agar	0.40%
pH	7

Simmons Citrate Agar:

Component	Amount (g/L)
Magnesium Sulfate	0.02%
Sodium chloride	0.50%
Sodium Citrate	0.20%
Di potassium Phosphate	0.10%
Mono potassium phosphate	0.10%
Bromothymol Blue	0.01%
Agar	2%
pH	7

XLD Agar:

Composition	Amount (g/L)
Yeast Extract	3
L-Lysine	5
Lactose	7.5
Sucrose	7.5
Xylose	3.5
Sodium chloride	5
Sodium deoxycholate	2.5
Sodium thiosulfate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.8
Agar	15
Final pH at 25°C	7.4±0.2

Indole

Component	Amount (g/L)
Peptone	10
Sodium chloride	5

MacConkey Agar:

Component	Amount (g/L)
Peptone (Pancreatic digest of gelatin)	17 gm
Proteose peptone (meat and casein)	3 gm
Lactose monohydrate	10 gm
Bile salts	1.5 gm
Sodium chloride	5 gm
Neutral red	0.03 gm
Crystal Violet	0.001 g
Agar	13.5 gm

Thiosulfate Citrate Bile Salt Agar:

Component	Amount (g/L)
Proteose peptone	10
Yeast Extract	5
Sodium Thiosulfate	10
Sodium Citrate	10
Bile	8
Sucrose	20
Sodium Chloride	10
Ferric Citrate	1
Bromothymol Blue	0.04
Thymol Blue	0.04
Agar	15
Final pH at 25ºC	8.6± 0.2

T1N1 (Trypton-NaCl) Agar:

Component	(Amount g/L)
Casein Enzymic Hydrolysate	10
Sodium Chloride	10
Agar	15
pH after sterilization at 25ºC	7.2± 0.2

Methyl Red and Voges Proskauer Media (MR-VP):

Component	Amount (g/L)
Peptone	7
Dextrose	5
Dipotassium hydrogen phosphate	5
Final pH	7

Grams of iodine (300 ml)

1 g of iodine and 2 g of potassium iodide were added to 300 ml of distilled water. After being thoroughly blended on a magnetic stirrer for a whole day, the mixture was transferred to a reagent bottle and kept at room temperature.

Safranin(100 ml)

2.5 g of safranin were dissolved in 10 ml of 95% ethanol. The solution was diluted with distilled water to a final volume of 100 ml. The finished product was kept at room temperature in a reagent bottle.

Kovac's Reagent (150 ml)

150 ml of reagent-grade isoamyl alcohol, 10 g of p-dimethylaminobenzaldehyde (DMAB), and 50 ml of concentrated HCl were combined and added to a reagent bottle. The reagent bottle was then wrapped in aluminum foil to shield it from light and kept at 4°C.

methyl red (300 ml)

300 ml of ethanol (95% alcohol) were used to thoroughly dissolve 1 g of methyl red powder in the reagent bottle. 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol were made by adding 200 ml of distilled water, and they were then kept at 4 °C.

Crystal Violet (100 ml)

2 g of crystal violet were dissolved in 29 ml of 95% ethyl alcohol. Ammonium oxalate, weighing 0.8 g, was dissolved in 80 ml of distilled water. To create the stain, the two solutions were combined and kept at room temperature in a reagent bottle.

Barrit's Reagent A (100 ml)

100 ml of 100% ethanol was mixed with 5% (wt/vol) a-naphthol and kept in a reagent bottle at 4°C.

Barrit's Reagent B (100 ml)

100 ml of distilled water were mixed with 40% (wt/vol) KOH, and the mixture was kept at 4°C in a reagent bottle.

Tris HCl 1M:

1.576g of Tris HCl were put into a McCartney bottle. To create 10 ml of 1M Tris HCl, 10 ml of distilled water was then added. The pH was then adjusted to 8. It was then kept chilled at 4 °C.

Twenty (20) ml of catalase reagent in 3% hydrogen peroxide

583 l of solution from a stock solution of 35% hydrogen peroxide was added to 19.417 ml of distilled water and kept at 4 °C in a reagent container.

TBE Buffer 1X:

5.4 g of Tris base, 2.75 g of boric acid, and 2 ml of 0.5 MEDTA were put into a Durham bottle. To create 500 ml of 1X TBE Buffer, 500 ml of distilled water was then added. The buffer's pH was then adjusted to 8. It was then autoclaved at 15 psi and 121 °C. The buffer was kept at room temperature after being autoclaved.