Bacteriological Study of Karatoa River and Their Antimicrobial Resistance

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment of the requirements for the degree of Bachelor of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted, titled “Bacteriological Analysis of the Karatoa River and Their Antimicrobial Resistance” 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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Sincerely,

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Abstract

The Karatoa River in Bangladesh is extensively contaminated with various contaminants. Water is one of the major cases of infectious illness. The current study concentrates on the bacterial identification by biochemical test and susceptibility to some antibiotics of the isolated organisms. Four samples were collected from the four different sites of the Karatoa River. The water samples were spread on Xylose Lysine Deoxycholate (XLD), Mannitol salt agar (MSA), Thiosulfate-citrate-bile salts-sucrose (TCBS), Nutrient Agar (NA), Cetrimide, MacConkey (MAC), HiCrome UTI following serial dilution within 24 hours of the collection. Also, the filtration method was used for MFC media. After those different types of Biochemical tests like the Triple Sugar Iron (TSI), Motility Indole Urea (MIU), Methyl Red -Voges-Proskauer (MR-VP), Catalase, Oxidase, Gram staining, Nitrate, Indole were used to determine the characteristics. Lastly, antibiotic susceptibility test was conducted using Amoxicillin, Amoxiclav, Ceftriaxone, Cefepime, Impenem, Azithromycin, Tetracycline, Nalidixic Acid, and Tobramycin disc. Around 62 different microorganisms were isolated from the water samples. Among these, *Planococcus* was the most predominant (15.87%), followed by *Staphylococcus spp*. (14.29%), and *Marinococcus* (11.11%). Some other organisms were also isolated in a very small amount. Moreover, the highest resistance was recorded for the antibiotic Amoxicillin (72.6%). On the other hand, 85.5% of the isolates were mostly susceptible to Impenam and Nalidixic acid. From our study, based on biochemical identification we found some highly pathogenic bacteria like *Vibrio spp, Klebsiella spp*, *Enterobacter spp* and *Staphylococcus spp.* Moreover, the presence of fecal coliform was present.

Contents

Introduction

Background

Though it may seem unthinkable, our oceans contain over 97% of all the water on Earth. About 2% of the minuscule portion that isn't in the ocean is encased in ice caps and glaciers. Clean water makes up less than 1% of the total water on Earth. In our atmosphere, a very small portion of water is present as water vapor. (NOAA, 2023) According to the research, 2 billion people (26% of the global population) do not have access to clean drinking water, and 3.6 billion (46%) do not have access to adequately managed sanitation, which was issued today at the UN 2023 Water Conference in New York by UNESCO on behalf of UN-Water. Water scarcity affects two to three billion people annually for at least one month, which puts their livelihoods—particularly those related to food safety and accessibility to electricity—in danger. According to projections, the number of urban people facing water scarcity worldwide is expected to quadruple by 2050, from 930 million in 2016 to 1.7–2.4 billion. Ecosystems are under stress due to the increasing frequency of severe and protracted droughts, which have disastrous effects on species of plants and animals alike. (UNESCO/D. Bonazzi 3 May 2023)

Bangladesh is a riverine country having as many as 800 rivers that crisscross the land to create the most complex river system in the world. Bangladesh has predominantly four major river systems - (1) the Brahmaputra-Jamuna, (2) the Ganges-Padma, (3) the Surma-Meghna, and (4) the Chittagong Region river system. However, the Brahmaputra is the 22nd longest (2,850 km) and the Ganges is the 30th longest (2,510 km) river in the world. On the eve of the annual International Day of Action for Rivers on March 14, experts and officials stated that the six major rivers that encircle the capital of Bangladesh, Dhaka, which has been regarded as the megacity's lifeblood, are being alarmingly polluted by industrial and other wastes. For many years, factories that produce medications, ready-to-wear, cement, dyes, and other goods have been dumping garbage into the Buriganga, Shitalakshya, Turag, Dhaleshwari, and Balu rivers without taking any concrete steps to stop it. Approximately 350,000 kilograms (350 metric tons) of hazardous garbage are discharged into rivers daily from approximately 7,000 companies and various residential areas in greater Dhaka and surrounding areas, based on the official data of the Bangladesh Inland Water Transport Authority (BIWTA). (Kamruzzaman et al., 14 May 2022)

Waterborne disease in Bangladesh

Any illness that is communicated or transferred by polluted water is considered a water-borne disease. Numerous illnesses that affect humans and other animals are caused by pathogenic microorganisms, including viruses and bacteria, as well as certain parasitic organisms. These

pathogenic organisms use a variety of tactics to live and proliferate in the environment. Diarrhea is the type of water-borne illness that is most prevalent. Watery diarrhea and dysentery are the two main forms. The Cholera bacterium is the source of severe watery diarrhea or cholera. Shigella is one of the several bacteria (*bacilli*) that can cause dysentery, often known as bacillary dysentery. Enteric fever, the precursor of typhoid fever, is the clinical manifestation of a group of salmonella bacteria that enter the gastrointestinal tract through water and may or may not induce diarrhea at the outset of the illness. Pathogens that are water-borne or transported through the medium of water are the primary cause of diarrhea along with other gastrointestinal illnesses. About 12% of all illnesses in Bangladesh are caused by diarrhea, and 10% are caused by other gastrointestinal disorders such as enteric fever. Together, these diseases account for roughly a quarter of all illnesses. Water therefore has a significant impact on the nation's total disease profile, with air ranking as the second most significant medium and contributing to 11% of all diseases, including pneumonia and other respiratory tract infections.

Factor Contaminating Karatoa river

The primary cause of the region's poor water quality is due to upstream sources, especially Mahasthangarh and Shibganj, which brought water into the area initially. However, because of the complex system of drainage that connects to the city's commercial center, this water experiences substantial pollution as it travels through the urban landscape, particularly in the area around Chalopara Bridge. As such, the pollution spreads widely along the whole length, from Chalopara to Bejora and beyond to Sherpur, further aggravating the watercourse's toxicity.

One more factor exacerbating this environmental problem is the careless dumping of solid trash into the Karatoa River from throughout the city. Notably, the city's major correctional facility releases a significant quantity of contaminated wastewater, which deteriorates the water condition overall. Furthermore, the existence of a busy marketplace close to Chaloparar encourages the illegal disposal of rubbish into the river.

The water of the Karatoa River is now completely unfit for any use due to the cumulative effects of these actions, which are typified by the discharge of hazardous waste into the river. In addition, the stench of the contaminated water discourages people living close to the riverbanks from using this resource. This situation emphasizes how urgent it is to take comprehensive action to address and resolve the problem of water contamination to protect the local population's health and the surroundings.

Literature review

Since many people lack access to clean, safe drinking water and pass away from bacterial illnesses in water, this review examines the significance of this issue. In addition to discussing the function of pathogenic Escherichia coli strains and emerging pathogens, it concentrates on the biology and

ecology of bacterial infections spread by water, including cholera, typhoid fever, and bacillary dysentery. Fecal indicator bacteria, which are found in both human and animal excrement and exhibit host and environmental behavior, are the foundation of microbiological water analysis. The assessment also addresses which indicators should be utilized in the present drinking water microbiological analysis and identifies the origins of bacterial fecal contamination in environmental waterways. A fundamental concern of the twenty-first century is providing safe drinking water, and microbial management of drinking water needs to be standard practice. Quantitating enterococci and testing for the presence of *Escherichia coli* should be part of regular basic microbiological analysis. Further research is required to better understand the ecology and behavior of animal and human fecal bacteria in environmental waterways, as well as to ascertain the validity of ammonia for initial screening for emergency fecal contamination outbreaks. (Cabral JP, 2010)

The Karnaphuli River in Bangladesh is extensively contaminated with various contaminants, and contaminated water is a major cause of infectious illnesses. A significant portion of the population uses the water for drinking and leisure, thus it's important to assess the health concerns it presents. A crucial factor in assessing the health concerns associated with water is its microbiological purity. Surface waters frequently pick up animal and human gut microbiota, which spreads infections throughout the ecosystem. The investigation looked for both coliform and fecal coliform in the water, and it discovered that the water samples had a total coliform count that varied from about 8.7×l03 to 8.2×l04/100 ml. Compared to the WHO guideline values and the standard values of the EQSB, the total coliform count that was detected throughout the research was much greater. The Karnaphuli River includes all five of the major bacteria that can cause water-borne illnesses, which indicates serious health hazards for the general public. The investigation recommended preventive actions to shield the river from harmful contamination and opened the door for more research on river water quality. (Sujan et al., 2017)

Water collected from the Halda River was found to contain ten different types of bacteria, including *Comamonas, Acinetobacter,* and *Bacillus*. These bacteria might have come from home wastes, local markets, Moduna Ghat Bazar, and canals that were connected to the river. Extremely salt-tolerant Bacillus strains were identified, probably from saltwater or tannery wastes: *Bacillus halotolerans strain HSB1, Bacillus safensis strain HSB2, Bacillus subtilis strain HSC1, Bacillus marisflavi strain HSD3,* and *Bacillus megaterium strain HABIBE3.* Nigerian Brycinus longipinnis fish were the source of *Comamonas jiangduensis* isolation. When commercially available antibiotics were used to assess the sensitivity of each strain, *Bacillus wiedmannii strain HSA2* demonstrated resistance to many antibiotics. (Habibur et al., 2022)

According to the research, waste disposal and the excrement of humans and warm-blooded animals have severely contaminated the Turag River's water. Because of this, it is inappropriate for drinking, recreational usage, and domestic use. The water's widespread untreated use, including

drinking, puts the public's health in danger. *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae,* and *Escherichia coli* were the four dangerous bacteria discovered in the investigation. These microorganisms suggest that the water is highly polluted with impurities, such as feces. The bacteriological state of the river and the risk of numerous water-borne illnesses for those who use it are both shown by this fecal contamination. Moreover, the river's entire aquatic environment is seriously threatened by this fecal pollution. (Tahmina et al., 2018)

The Buriganga River in Bangalore was found to have a high level of heterotrophic bacterial load during the period of rainfall compared to autumn, indicating considerable bacterial population pollution. This is probably because precipitation washes the land's surface into the river. The region around Shyambazar was most severely impacted, with fall registering the largest number of enteric bacteria. Annual fluctuations in the aquatic microflora dynamic can be attributed to water movement caused by wind, tides, or currents. It was discovered that the two most common Gram-negative bacteria in the river were *Escherichia sp.* and *Proteus morganii*. The lakes and rivers in Bangalore were home to bacterial species of the following: *Flavobacterium, Aeromonas, Pseudomonas, Enterobacter,* and *Corynebacterium.* Significant levels of microbial contamination were also demonstrated by the existence and quantity of aerobic heterotrophic bacteria as well as the presence of *Bacillus sp., Escherichia sp., Proteus morganii, Plesiomonas sp., Hafnia sp.,* and *Alcaligenes sp*. in the water. Due to the low DO content in river water, aquatic organisms may not be able to maintain optimal circumstances and may become more sensitive to pollutants.

Gaps in previous studies

Numerous well-known rivers, including the Buriganga, Turag, Halda, and Karnaphuli, have been the subject of in-depth studies on the identification of bacteria in water from rivers in Bangladesh. Nonetheless, it is significant that there has been very little if any, study done on the identification of bacteria in the Karatoa River's waters. Even though it was completely unsuitable for scientific studies due to extreme pollution.

Questions over the existence of harmful bacteria, such as but not restricted to *Cholera, Shigella, Salmonella*, and several other potentially fatal forms, have been raised by the current state of the Karatetoa River, which is marked by an abnormally high pollution level. This emphasizes how important and urgent the planned study is since it attempts to close a significant information gap about the identification of bacteria in a particular water body.

Besides its significance for comprehending the Karatoa River's microbial ecology, this study offers a rare chance to look at the resistance to antibiotics of the detected bacterial species. This information can be extremely helpful in creating plans to reduce the health hazards brought on by the existence of bacteria resistant to antibiotics in this highly contaminated water body. In the end, this study may offer crucial insights into the Karatoa River's microbiological features as well as

the consequences of its pollution for public health. making it a perfect and essential project for the long-term care of this essential ecosystem and the enhancement of the neighborhood.

Aim and objectives

In this experiment, determining the bacteriological composition and the incidence of resistance to antibiotics in the Karatoa River water was the main goal. Additionally, the goal of this study was to examine how susceptible the individual microbes were to different antibiotic treatments. The study's working premise was the belief that particular aquatic settings would harbor a wide variety of pathogenic and antibiotic-resistant organisms because of the significant contamination caused by adjacent industrial activity.

Research Novelty

There is a pressing need to address Bangladesh's river water quality crisis, as pollution in Dhaka is mostly caused by garbage from homes and industrial emissions. However, in terms of the Karatoa River, the circumstances offer a distinct and hitherto undiscovered aspect. The primary cause of pollution in this case is the shocking ignorance of the significant ecological ramifications of pollutants being released into the river. The result has been widespread contamination of the river. exacerbated even more by the direct incineration of refuse from the commercial marketplace and households into its waste receptacles.

Given this unique setting and the obvious study vacuum concerning the Karatoa River, this investigation stands out as a trailblazing undertaking that promises unmatched research originality. Our project's main goal is to close the gap by carrying out an extensive microbiological assessment. We aim to specifically extract and identify bacteria that are resistant to antibiotics, such as *Salmonella/shigella*, *Staphylococcus*, *Pseudomonas bacteria*, and *Bacillus species,* from the environment of the river.

We believe that by doing this ground-breaking study, we will significantly advance the discipline of microbiology. because it represents the first baseline evaluation of the water quality of the Karatoa River. This not only demonstrates the novel character of our work but also highlights its fundamental significance for further research endeavors and the development of crucial plans for environmental conservation in this crucial waterway

Methods and material

Location (Sampling site)

Our research was conducted along the Karatoya River, a majestic waterway spanning approximately 187 kilometers. Originating from the Baikunthapur jungles in the far northwest of the Jalpaiguri district, West Bengal, India, the river serves as the boundary between Dinajpur and Rangpur districts for a considerable stretch. It gracefully meanders through Rangpur and Bogura, forming a captivating landscape. The scope of our study encompassed an expansive area of approximately 40 square kilometers.

To gain comprehensive insights into the river's water quality, we collected water samples from four distinct locations. The first two samples were obtained from Sherpur (24°40'41.4"N 89°25'04.9" E) and Bejora (24°49'38.7"N 89°23'20.4" E) at the end of September. The remaining two samples were collected from Mohasthan Garh (24°57'33.9"N 89°21'05.2" E) and Shibganj (25°00'17.0"N 89°19'08.7" E). Notably, the water flow follows a path from Shibganj to Mohasthan Garh, Bejora, and finally Sherpur. Consequently, we observed that Bejora and Sherpur exhibited a higher presence of water pollution compared to the other locations.

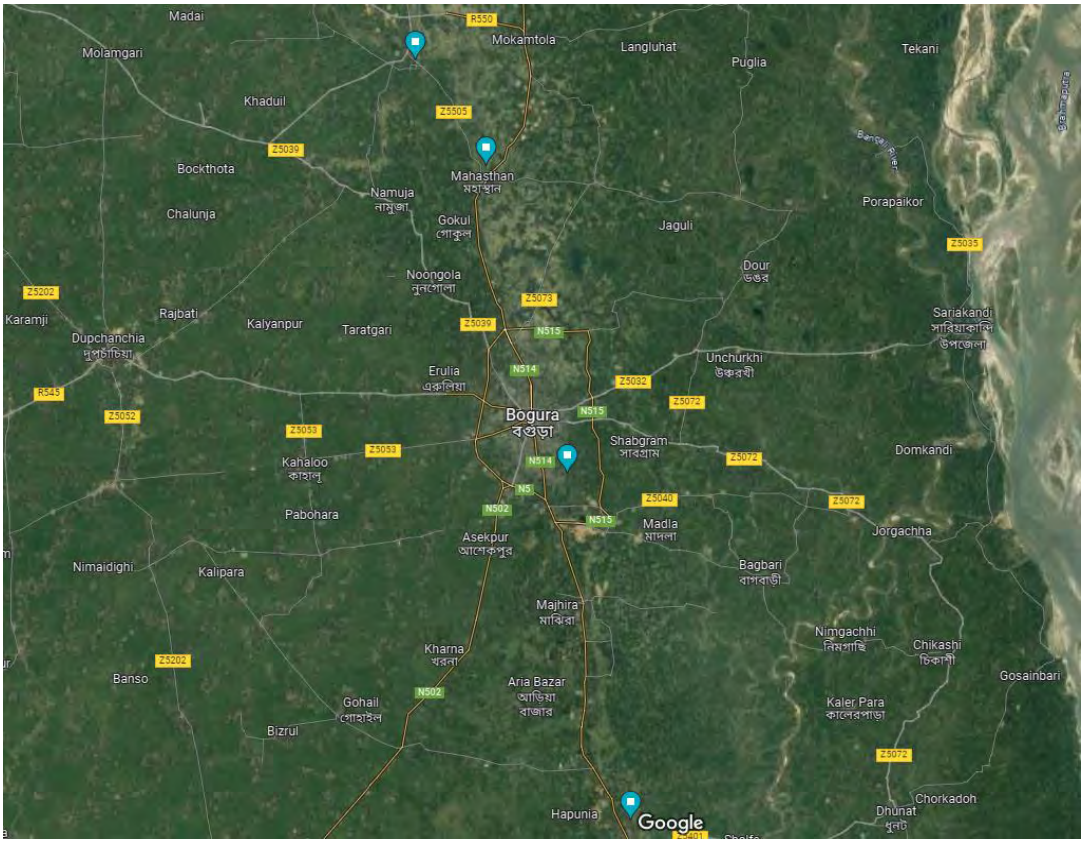

Figure 01 - Sampling site of Karatoya River

Sample Collection & Transportation

Four samples were meticulously collected from distinct locations, ensuring utmost care in the process. Before use, the sample collection bottle underwent thorough sterilization by autoclaving, rendering it completely sterile. With a generous capacity of 500 ml, each bottle was specifically designed for water collection. To procure the water, the bottle was submerged approximately 2 feet beneath the water's surface. Delicately opening the cap, the bubbles gently escaped as the bottle gradually filled with pristine water. To maintain its purity, the bottle was promptly resealed, protecting it from any potential contaminants. Following collection, the samples were promptly stored in a refrigerated environment, maintaining the minimum temperature required.
The sample must be processed within 24 hours so, within 24 hours, the samples were carefully transported to Dhaka which is around 185.4 km. For assurance of not getting contaminated, the bottles were sealed properly. The journey from sample collection to Dhaka took about 14 hours and within 17 hours the samples were processed properly using different media plates.

Sample Processing

The sample was processed using two methods-

1. Serial dilution,
2. Membrane filtration

Serial Dilution

For a serial dilution method, one must use the sample and put it in a solution. We used the NaCl solution as a solution for dilution. We used the raw sample of about 1 ml and put it in the solution of NaCl and mixed it using vortex mixture again from the 10-1 we used 1 ml of solution and diluted in 10-2 we continuously did the same process from 10-1 to 10-9.
For this paper, the authors have addressed the use of undiluted samples as ‘raw’. Then using the spread plate method, it was spread on the plate for about 50µl. The media was used - Xylose Lysine Deoxycholate (XLD), Mannitol salt agar (MSA), Thiosulfate-citrate-bile salts-sucrose (TCBS), Nutrient Agar (NA), Cetrimide, MacConkey (MAC), HiCrome UTI.
Moreover, 50µl of the sample was spread on the plate for every serial dilution. Here the (NA) plate was used for general growth. And TCBS was used to determine whether the sample contained Cholera. The plates were then put in an incubator at 37ºC for 24 hours.

Membrane Filtration

MFC media was employed for this experiment because membrane filtration is used to see fecal coliform. which is used to monitor the development of *Enterococcus faecalis, Enterobacter aerogenes, or E. coli.*
Using a raw sample, the membrane filtering procedure was employed. 100 ml of water for each sample was drawn through a unique porous membrane in this process, which was developed to catch microorganisms bigger than 0.45 m. The MFC agar plates are then covered with the filter, which is then incubated for 24 hours at 45°C. After that, the plates were tested for coliform.

Microbial Identification

Biochemical Test –
After selecting the colony, it was a subculture in NA media. It was also a subculture for pure colony and then stored in T1N1 media for long-term use. The specific biochemical test was selected for identification - The Triple Sugar Iron (TSI), Motility Indole Urea (MIU), Methyl Red - Voges-Proskauer (MR-VP), Catalase, Oxidase, Gram staining, Nitrate, Indole.

Gram Staining

Gram staining is a method for classifying bacteria into positive and negative groups by giving their cells red or violet coloring. Some bacteria maintain the stain when exposed to Crystal Violet and a mordant, whereas alcohol decolorizes other germs. Gram-positive bacteria have a strong peptidoglycan coating with little lipid content, which makes the cells dry up and constrict, stopping the stain from leaving. Because of this, the Crystal Violet-Iodine combination is unharmed and produces blue or purple hues. Gram-negative bacteria, in contrast, also take up the CV-Iodine complex, but they can be removed more easily because of their thick lipid coating and thin peptidoglycan layer. The lipids in the cell walls are dissolved by alcohol exposure, enabling the crystal violet-iodine combination to escape. When stained with safranin, Gram-negative bacteria assume a red coloration.

The material was heat-fixed by putting it over a Bunsen burner three times before being placed on a cell slide for staining. Crystal violet, the main stain, was applied and incubated for 45 seconds. To get rid of any crystal violet that was not bonded, the slide was washed with water for five seconds. For 30 seconds, Gram's iodine was applied to adhere the crystal violet onto the bacterial cell wall. If the bacteria were Gram-negative, the slide was washed with acetone or alcohol for three seconds to eliminate crystal violet. Safranin, the secondary stain, was applied, and it was incubated for 30 seconds. Through a microscope, gram-positive bacteria appeared violet or purple because they kept the main stain and did not take the secondary stain. If Gram-negative bacteria

were observed, they lost the primary stain and took up the secondary stain, resulting in a red appearance.

TSI

The Triple Sugar Iron (TSI) test is a useful microbiological assay used to assess the ability of bacteria to produce hydrogen sulfide and ferment sugar. This test uses a customized agar slant medium with the following ingredients: lactose, sucrose, glucose (all at 1% concentrations), sodium thiosulfate, and either ferrous sulfate or ferrous ammonium sulfate, a pH-sensitive dye known as phenol red, and. Lactose, sucrose, and glucose are the three fermentative sugars that are particularly added to TSI Agar to enable the monitoring of carbohydrate usage patterns. Microbes create acid during the fermentation of carbohydrates, which causes the pH to fall. An orange-to-yellow color shift in the carbohydrate medium, which is proof of carbohydrate fermentation, visually represents this acid-base process. The indication for this technique is phenol red. Additionally, alkaline byproducts are produced during oxidative decarboxylation of peptone, raising the pH. During the exam, this change from an orange-red to a deep crimson tint acts as a crucial signal.

The inclusion of sodium thiosulfate and ferrous ammonium sulfate in the medium enables the detection of hydrogen sulfide formation in addition to assessing sugar fermentation. If hydrogen sulfide is created, it will be seen by the blackening of the tube's bottom.
The tubes were tilted and TSI agar was first made before being let to cool. The bacterium was then softly poked and then streaked across the surface of the slant. And after doing so it was put into the incubator at 34°C for 24 hours. (August 10, 2022 by Sagar Aryal)

Its content in the medium is one-tenth that of lactose or sucrose to help in the detection of microorganisms that exclusively digest glucose. The slanted part of the tube quickly oxidizes the acid produced during glucose fermentation, causing the medium to either stay orange or revert to an alkaline pH. However, the reduced oxygen tension in the tube's butt keeps the acid reaction (yellow) there.

MIU

The test organisms on MIU agar have distinctive growth patterns after incubation. Non-motile organisms exhibit confined growth along the stab line, while motile organisms exhibit diffuse growth or turbidity from the stab inoculation line. The bacteria being examined produce the enzyme urease, which breaks down urea and produces ammonia and carbon dioxide as a byproduct. The test medium's pH rises as a result of the ammonia and water reaction that produces ammonium carbonate, which creates an alkaline environment. The formation of ammonia is indicated by a shift in color from yellow to pink-red in the presence of phenol red in the media.

Tryptophan, which is present in casein enzymic hydrolysate, is acted upon by the enzyme tryptophanase to create indole, skatole, and indole acetic acid in addition to urea hydrolysis. Indole is created when p-dimethyl amino benzaldehyde, a component of Kovac's reagent, interacts with indole to create a unique red-violet molecule.

The medium was originally prepared and autoclaved at 121 degrees Celsius. It was autoclaved and then allowed to cool before urea was added. To avoid contamination, the urea was carefully added using a syringe filter. The medium was chilled to the necessary minimum temperature after urea was introduced. The culture was then incubated at 34°C for 24 hours with a chosen bacterial colony pierced into its core.

Peptones in the medium supply the carbon and nitrogen required for bacterial development, whereas urea serves as a nitrogen source for species that produce the urease enzyme. Amino acids and other nitrogenous substances are provided by the hydrolysate of casein enzymes. The osmotic balance is maintained by sodium chloride, while dextrose acts as a carbohydrate that may be fermented. When the environment is alkaline, phenol red works as a pH indicator, becoming pink-red. The ability to see bacterial motion is facilitated by the low 0.2% agar concentration. By incorporating aldehyde into Kovac's reagent, indole synthesis from the casein enzymic hydrolysate, which is mediated by the tryptophanase enzyme in the test organisms, is identified. This causes a pink-red color ring to emerge in the tube. (December 22, 2022, by Medical Lab Notes)

MR-VP

The methyl red test is carried out by autoclaving the methyl red solution and pouring it into a test tube. The bacteria culture is then infused into the mixture and incubated for 24 hours at 34°C in a suitable incubator. Two or three drops of methyl red are added to the solution after 24 hours, and a quick color shift to red is watched for. (August 10, 2022 by Sagar Aryal)

On the other hand, the Voges-Proskauer (VP) test is used to identify organisms that convert glucose into acetyl methyl carbinol. Acetyl methyl carbinol, if present, undergoes deacetylation in the presence of -naphthol, strong alkali (40% KOH), and oxygen from the air. It should be noted that although -naphthol was eventually incorporated into the process, Barritt initially found that it must be introduced because it acts as a color enhancer. The peptones in the broth's diacetyl and guanidine-containing molecules then combine to produce a pinkish-crimson polymer. (August 10, 2022 by Sagar Aryal)

The medium is injected with the test microorganisms and incubated at 34°C for 24 hours to perform the VP test, which is comparable to the MR test. Six drops of 5% -naphthol is added to

the solution and carefully agitated to provide aeration before the results are interpreted. To provide aeration, two drops of 40% potassium hydroxide (KOH) are added and well mixed. It is observed to see if the color has changed to pink-red or not after waiting 30 minutes.

Catalase

The catalase test is used to determine which organisms generate the catalase enzyme, which is essential for converting hydrogen peroxide into water and oxygen gas during the detoxification process. This test helps to distinguish between bacteria that have a similar morphology, such as the catalase-negative *Enterococcus, streptococcus* and catalase-positive *Staphylococcus*. Additionally, it helps to distinguish between bacteria that are obligately anaerobic and aerobic. The semiquantitative catalase test is also used to distinguish between catalase-positive *Bacillus* species and catalase-negative aerotolerant *Clostridium* bacteria, as well as to identify *Mycobacterium tuberculosis*. The catalase test can also be used as a supplementary method to identify *Enterobacteriaceae*. (August 10, 2022 by Sagar Aryal)

For the catalase test in the project, the slide method was used. A tiny quantity of colony growth was applied to the surface of a dry, clean glass slide using a sterile wooden stick. The glass slide was then treated with a drop of hydrogen peroxide (H2O2), and the existence of oxygen bubbles was checked.

Oxidase

To ascertain if an organism has the cytochrome oxidase enzyme, the oxidase test is used. A cellular oxidase enzyme is produced by organisms whose respiratory chains include cytochrome c. The oxidation of cytochrome c is facilitated by this oxidase enzyme. Due to their oxidase positivity, these cytochrome-containing organisms change color from red to blue or purple when the oxidase reagent is used. However, organisms lacking cytochrome C in their respiratory chain fail to oxidize the reagent, keeping it colorless throughout the time limit of the test, and are categorized as oxidase-negative. These organisms also do not oxidize the reagent.

The oxidase test is carried out using Whatman's filter paper. The filter paper is initially moistened by adding a drop of distilled water to it. A colony is then chosen and put onto the wet filter paper using a toothpick. The oxidase reagent is then applied in three drops to the colony. A favorable response is indicated by the emergence of an intense deep-purple color within 5–10 seconds in terms of result interpretation. A "delayed positive" reaction is identified by color that happens between 10 and 60 seconds after the stimulus. The absence of pigment or coloration that appears more than 60 seconds after exposure, on the other hand, indicates a negative reaction.

Nitrate

The nitrate reduction test depends on the detection of nitrite, which combines with sulfanilic acid to generate the complex known as nitrite-sulfanilic acid, which produces a red molecule. This compound then combines with -naphthylamine to create prontosil, a red precipitate that is an azo dye that is water soluble. Therefore, it is vital to ascertain if the organism has reduced nitrate beyond nitrite if nitrite is not discovered. To accelerate the conversion of nitrate to nitrite, zinc powder is used. If the presence of zinc causes the formation of a red hue, the organism has either failed to decrease nitrate or is incapable of doing so. If, however, no color change takes place following the addition of zinc, it may indicate that the organism has successfully converted nitrate to another nitrogen molecule, demonstrating its effectiveness as a nitrate reducer.

An autoclaved nitrate broth is produced for the nitrate reduction test. A colony is then picked and added to the soup as an inoculant. Reagents A (sulfanilic acid) and B (-naphthylamine) are added to the infected broth after it has been incubated at 35°C for 24 hours, and any color change is then assessed. If the color does not turn red, zinc is added for more investigation. The nitrate reduction test is a useful technique for identifying and classifying organisms by evaluating whether or not they have the ability to reduce nitrate.

Indole

A biochemical test known as the indole test is used to assess a bacteria's capacity to degrade the amino acid tryptophan and create indole. By being deaminated and hydrolyzed by bacterial enzymes known as tryptophan, indole, pyruvic acid, ammonium, and energy are produced. By mixing the medium with Kovac's Reagent, which has hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol, indole may be found in the sample. Indole and Kovac's Reagent react, changing the hue from yellow to cherry red. Due to amyl alcohol's insoluble nature in water, a reddish greasy film appears on top of the soup. Microbiologists may identify and classify bacterial species according to their capacity to produce indole by monitoring the color change. In clinical and scientific contexts, this test is very important for the identification and categorization of bacteria.

The mediums were prepared and autoclaved to carry out the indole test. Kovac's reagent was added to the culture after it had been incubated for around 24 hours at 35°C to experiment. A favorable outcome was shown by a crimson ring on the media's surface, which showed that the bacteria had produced indole.

Antibiotic Susceptibility Test

Susceptibility testing is a technique used to assess how well antimicrobial medications work against bacteria or fungi that cause an illness. To generate turbidity that matches the standard (0.5 McFarland), 2-3 colonies of the bacteria are emulsified in sterile saline in this procedure. To ensure equal growth, a sterile cotton swab is dipped into the bacterial solution, the excess liquid is squeezed out, and the sample is subsequently streaked onto the Mueller-Hinton Agar (MHA) surface in three directions.

Paper disks with predetermined amounts of specific antibiotics (such as Amoxicillin, Amoxiclav, Ceftriaxone, Cefepime, Impenem, Azithromycin, Tetracycline, Nalidixic Acid and Tobramycin) are placed onto the surface of the agar using sterile tongs or a disk holder after the plates have dried for 10 to 15 minutes. After that, the plates are left to incubate for 24 hours at 37°C.

Following the incubation time, the diameter of the millimeter-sized zone of inhibition surrounding each antibiotic disk is measured. This value shows how susceptible the bacteria are to the particular antibiotic. The antibiotic is more effective in stopping bacterial growth if the zone of inhibition is greater. These findings help medics choose the best antibiotic treatments for treating infections.

Result

This table (Table 1.1) offers a detailed review of the pathogenic organisms found in four different places along the Karatoa River. In comparison to the comparatively lower contamination levels in Mahasthangarh, the data clearly shows that the highest degree of contamination is found in the city area, with the sources of pollution extending from Bejora and flowing downstream to Sherpur. This occurrence may be explained by the river's downstream flow, which carries contaminants from Bejora to Sherpur and raises the levels of pollution in these places.

Table 1.1: Organism found from different location

Location	Name of organism
Sherpur	*Caryophanon kurthia* *Centipeda* *Coprococcus* *Corynebacterium* *Marinococcus* *Megasphaera* *Micrococcus Deinobacter / Deinococcus* *Moraxella* *Peptococcus* *Planococcus* *Ruminococcus / Peptostreptococcus* *Staphylococcus*
Bejora	*Coprococcus / Peptococcus* *Enterococcus Vagococcus* *Marinococcus* *Peptococcus* *Phenylobacterium* *Planococcus* *Ruminococcus / Peptostreptococcus* *Saccharococcus (thermophilic)* *Sarcina* *Syntrophococcus*

Among **62 samples** the predominant *planococcus* was **15.87%** , by the notable ratio of some highest to lowest was *staphylococcus spp* **14.29%**, *Marinococcus* **11.11%,** *E.coli* **7.94%,** *Coprococcus,* and *Streptococcus* was both **6.35%,** *Caryophanon Kurthia* **3.17%** and some others like *Megasphaera, Micrococcus Deinobacter / Deinococcus, Moraxella, Phenylobacterium,*

Shibganj	*Shigella spp.* *Staphylococcus saprophyticus* *Staphylococcus spp.* *Streptococcus spp.* *Vibrio spp.*
Mahasthangarh	*E. coli* *Enterobacter spp.* *Klebsiella spp.* *Staphylococcus spp.* *Streptococcus spp.*

Sacharococcus, Sarcina, Shigella spp, Staphylococcus, Staphylococcus saprophyticus, Stomatococcus, Syntrophococcus, Vibrio spp, Enterobacter spp., Enterococcus Vagococcus, Klebsiella spp., Corynebacterium/ Propionibacterium/ Rothia/ Dermabacter, Centipeda are at **1.59%** which is described in the (figure 1.2) by a chart.

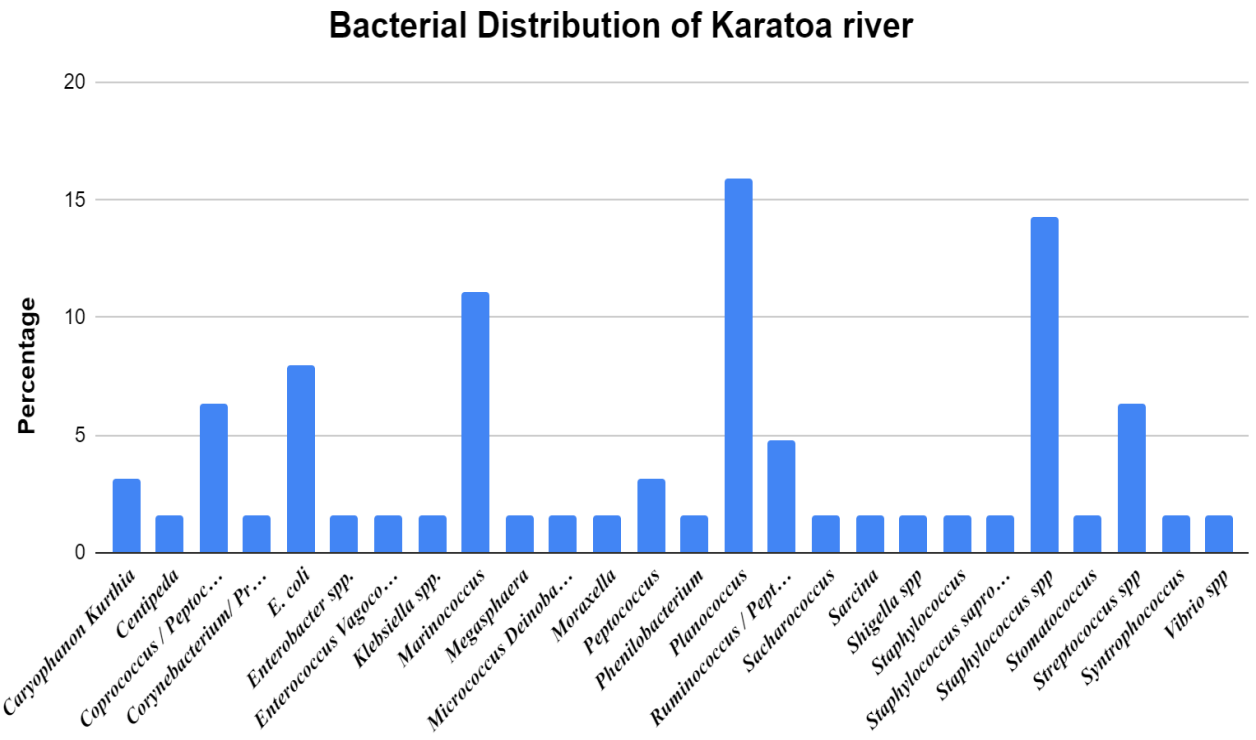

Figure 1.2 Degree of contamination

Antimicrobial resistance was detected in all kinds of organisms in the samples. According to (figure 1.3) the highest resistance level was observed with Amoxicillin at 72.6% while the lowest resistance was Impenam at 6.5%. Intermediate resistance was relatively common for Tetracycline

and Azithromycin at 29.0%. The lowest percentage of Intermediate is Nalidixic Acid at 3.2%. It means some grows invaded the zone of inhibitions. On the other hand, the highest percentage of Susceptible is Impenam and Nalidixic Acid which is 85.5%, while Amoxicillin exhibits the lowest percentage of Susceptible at 22.6%.

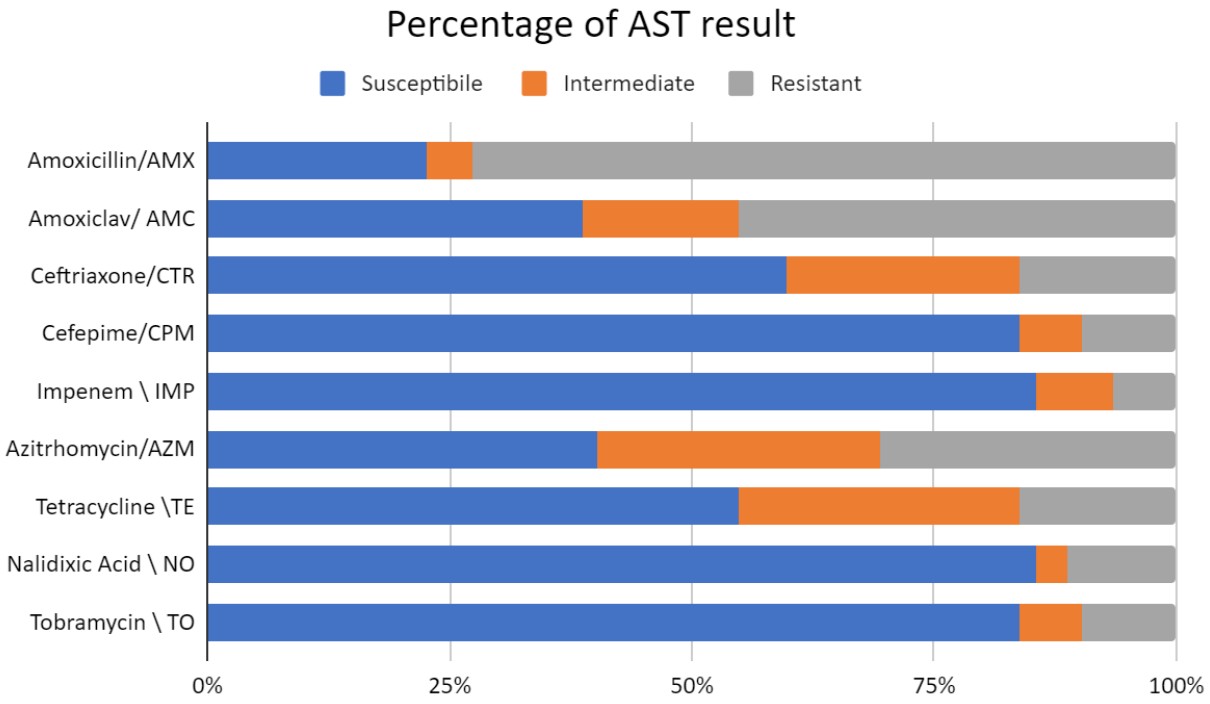

Figure 1.3: Antimicrobial susceptibility result, The highest percentage of susceptibility Impenem and Nalidixic Acid at 85.5%. Highest Percentage of Resistant at 72.6% Amoxicillin.

Discussion

The information on the existence of many pathogenic organisms in our sample is included in Table (figure 1.1). one of the four Karatoa River study locations. The fact that Sherpur and Bejora have noticeably elevated quantities of these potentially dangerous microbes is very concerning. Given that river waters are widely dispersed across densely populated metropolitan areas, there are serious public health issues raised by the higher pathogenic content in certain river sections. Most concerning is the fact that many people who live near the riverbanks suffer from socioeconomic disadvantage and rely on the waters of Bejora and Sherpur, which are highly contaminated with harmful bacteria, as their main supply of water. Unfortunately, the issue is made worse by the poor sanitary conditions in these areas, where people are frequently forced to use or eat water tainted with dangerous diseases. Furthermore, the discovery of a *Staphylococcus* water sample is particularly alarming because people's skin is home to the bacteria *Staphylococcus aureus*, sometimes known as staph. If *Staphylococcus* enters the bloodstream, it can result in fatal infections or sepsis. Methicillin-susceptible staph (MRSA) or methicillin-resistant staph (MRSSA) are the two types of *Staphylococcus* bacteria. (17) Severe and even fatal infections can also be caused by *Staphylococcus* bacteria. Serious sickness might arise from the invasion of circulation or organs by *Staphylococcus* germs. Modern antibiotics must be used for treating *Staphylococcus* strains that are immune to certain antibiotics. (S. Srakocic, el al. Oct. 2023). This emphasizes how crucial it is to act quickly and take comprehensive action to address the microbial pollution of the Karatoa River in order to protect the health and safety of both residents and visitors. It's really important to use purified water. However, some of the organisms from our research are - *Shigella, Staphylococcus, Streptococcus, Snterobacter, Klebsiella, Planococcus, Marinococcus, Centipeda, Coprococcus, Corynebacterium, Ruminococcus,* etc these are mostly pathogenic organisms mainly of fecal origin. Any water source used for drinking or cleaning purposes should not contain any organism of fecal origin. The presence of enteric coliforms especially Escherichia coli makes the water samples unsuitable for human consumption according to the guidelines set by WHO for the evaluation of the bacteriological quality of drinking water (Idowu et al., 2011) At least six different pathotypes of ***E. coli*** can cause enteric diseases, such as diarrhea or dysentery, and other pathotypes cause extra-intestinal infections, including urinary tract infections and meningitis (Kaper et al.,2004). Some more disease-causing pathogenic bacteria from the result are - A digestive tract infection called shigellosis is brought on by species of ***Shigella spp***. Shigella often infects humans and is found in their feces, but it can also contaminate water. Furthermore, the primary cause of simple urinary tract infections is the Gram-positive bacterium ***Staphylococcus saprophyticus***. In addition, it is the cause of problems such as urethritis, prostatitis, epididymitis, and acute pyelonephritis. Some Gram-positive bacteria called ***Staphylococcus spp*** species produce suppurative infections in people as well as animals. Humans may have skin infections, which are the most prevalent kind of staph infections, or bacteremia, a bloodstream infection. Sepsis, a

potentially fatal immunological reaction to infection, bone infections, endocarditis (infection of the heart's internal lining and valves), food poisoning, pneumonia, and other conditions can result from this. The potentially fatal illness known as toxic shock syndrome (TSS) is brought on by the toxins produced by specific bacteria. Most human infections linked to the naturally occurring microbiota of aquatic habitats and seafood are caused by ***Vibrio spp***. The most prevalent pathogenic species are *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus,* and *Vibrio alginolyticus*. Most cases of *Vibrio spp.* infections result from intake or exposure to tainted water. (Baker-Austin, Craig, et al Dec. 2018). There are also ***Klebsiella spp*** which cause respiratory and urinary tract infections, ***Enterobacter spp***. Causes healthcare-associated infections.

The persistence and resistance to antibiotics of bacteria in clean water have been shown by this research, which presents a serious risk to the public health of the Bogra community. We have discovered that bacteria resistant to antibiotics can live for long periods in freshwater settings while their DNA continues to function. Given the fact that the residents of Bogra depend on sources of freshwater that are closely linked to sewage and river flows from different locations, such as Bejora, Sherpur, and Mahasthangarh, this discovery is very concerning.

We have found that a significant proportion of the bacteria in the samples exhibit susceptibility to antibiotics based on our analysis of the findings of the antimicrobial susceptibility test (AST). This sensitivity suggests that even after being exposed to antibiotics, these bacteria may be able to develop and even multiply. In particular, medications that are highly efficient in fighting these bacteria include cefepime 83.9%, imipenem 85.5%, tobramycin 83.9%, and nalidixic acid 85.5%.

On the other hand, fewer bacteria showed signs of resistance to antibiotics, particularly amoxicillin 72.6% and amoxiclav 45.2%. Additionally, with azithromycin and tetracycline. An intermediate degree of resistance was shown by the bacteria. This condition raises serious concerns since it fosters the development of bacteria in the presence of antibiotics, which may result in the emergence of resistance to antimicrobial strains. This presents a significant obstacle to the control and treatment of illnesses in the area, requiring prompt action and all-encompassing approaches to stop the spread of bacteria resistant to antibiotics in Bogra.

Recommendation:

From the previous studies we can say that water from the Karatoa River is not safe at all. Different types of waterborne Bacteria are present in it, even though they are very much resistant to antibiotics. Which is very concerning as local people are using it for drinking purposes. So, the locals and the government should be aware and spread awareness among the people and take necessary steps to guard the health of people there. We should be very aware and take some steps

like drinking properly treated water. Water from private water supplies should be routinely tested twice a year for Total Coliform and other harmful bacteria. Inorganic analysis on private water supplies should preferably be done every two to three years. Do not swallow water while swimming in swimming pools, hot tubs, or interactive fountains, lakes, rivers, springs, ponds, streams, or the ocean. Do not drink untreated water from lakes, rivers, springs, ponds, streams, or shallow wells. Do not drink tap water or use ice while traveling to a high-risk destination unless the water source has been properly treated etc. Even more research, development, and investment need to go towards wastewater treatment to make the water in rivers match the WHO standards for drinking water. Various governmental and non-governmental organizations should carry out educational initiatives in rural areas which lack tap water and hence rely on surface water for drinking, on ways to purify surface water properly.

Limitations:

Our sampling site was far away from our laboratory. As we collected our samples from Bogra Zilla, Karatoa River, taking the samples and bringing them to our lab was a hustle. It is important to take the sample and work with it in a minimum time interval, as we take the sample from the river, the bacterial growth begins to change and the phases also change in time. The humidity and the pH changed over time. Though we extracted 27 samples from 4 different sites and got several organisms, if we could bring the sample earlier and do our thesis work it would be more convenient to extract more efficiently. There were greater chances to extract more organisms if the circumstances were more friendly and for the molecular lab's genetic thesis limitations, as there was a lack of some parameters, we couldn`t research more extensively. For certain reasons, we successfully extracted 27 organisms, and there were plenty of chances to get more of them.

Future prospect:

Bangladesh is a land of rivers and also a developing country and there are lots of industries and pharmacies especially tanneries established at the banks of the rivers. Since Bangladesh is home to several tanneries, there is a possibility of massive physico-chemical contamination, which is also a massive public health concern for water that is used for domestic purposes. Additionally, there are several rivers in the district as well. The data sets can be more statistically significant if future experiments are done that focus on multiple rivers. Not to mention, the seasonal variation between microorganisms can also be examined to explain the seasonal variation in waterborne diseases such as cholera and typhoid. Additional molecular work related to resistance, and further genome sequencing would be advisable.

Conclusion

This study forms the baseline study on the water quality of the Karatoa River., which is the first of its kind in the country. It focuses mostly on identifying the level of contamination and identifying antibiotic-resistant bacteria and antibiotic-resistant genes in the Karatoa River.

Our results show that from this river, we extracted many pathogenic organisms most likely *E. coli, Shigella, Staphylococcus, Streptococcus, Enterobacter, Klebsiella, Planococcus, Marinococcus, Centipeda, Coprococcus, Corynebacterium, Ruminococcus,* etc. Through the analysis, we were able to know that maximum bacteria are susceptible to antibiotics. It means they can grow after using antibiotics. Tobramycin, Nalidixic acid, Imipenem, and cefepime are most susceptible. Less number of bacteria were resistant to amoxiclav and amoxicillin. Even more, the Tetracycline and Azithromycin were intermediate. Among 27 samples, 15.8% was predominant *planococcus*, while a significant ratio of *Staphylococcus spp, Marinoco, E. coli, Coproco,* and *Streptococcus* were respectively 14.29%, 11.11%, 7.94%, and 6.35%. less than 2% were other organisms. Lastly, there is plenty of scope for further research on understanding the mechanisms of resistance and investigating the presence of certain resistance-causing genes that can be identified if we can run the research in a broader sense. So, this was all for till now from the water of Karatoa in Bogra Zilla. This research can be prolonged with higher extended versions if we want to examine the properties and also to stop the pathogenic bacteria to mitigate health issues regarding the water.

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