MICROBIAL PREVALENCE OF KAPTAI LAKE FROM TWO DIFFERENT SPOTS

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Declaration

It is hereby declared that the work presented in this thesis titled "MICROBIAL PREVALENCE OF KAPTAI LAKE FROM TWO DIFFERENT SPOTS" has been completed by - Sharmila Alam, Amreen Nahin Neha and Pretom Saha as a prerequisite submission for the undergraduate thesis under the course BTE 450: Biotech Project in the Biotechnology program of the Department of Mathematics and Natural Sciences of BRAC University, Dhaka. The thesis submitted is our own work of review paper while completing a degree at Brac University. It does not contain any materials that have been accepted, submitted, or published for any other degree or diploma at a university or other institution. The thesis does not contain material previously published or written by a third party, and in places where it is, these are appropriately cited through full and accurate referencing. All the primary sources of help have been rightfully acknowledged.

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MICROBIAL PREVALENCE OF KAPTAI LAKE FROM TWO DIFFERENT SPOTS

ABSTRACT

Kaptai Lake one of the largest artificial freshwater lakes in South-east Asia which is situated in Rangamati district of Bangladesh. The people of Rangamati are greatly dependent on Kaptai Lake for their food source, agriculture, etc. So, the water sample of Kaptai Lake is tested in this study to check the microbial load of the water. The samples were collected from two different spots include Banarupa and Fisharighat. A total of 6 samples were collected in different time interval from those spots. The experiment was performed with a combination of spread, pour and streak plating on different selective media includes MacConkey, SS, TCBS and EMB.

Characterization and Gram Staining were performed to identify the species. Got different types of coliforms and fecal contaminators- *Escherichia, Vibrio, Shigella, Salmonella, Klebsiella, Enterobacter*. Moreover, Antibiotic Susceptibility test was performed to check the resistance pattern. Around 14 different antibiotics from different class were used. In the study, approximately 68 microorganisms were isolated from the water samples of Kaptai Lake, most of the microorganisms were gram negative (92%) and rest of them are Gram Positive (8%). Most of the organisms showed sensitivity to most of antibiotics that used. Comparing to other researches, susceptibility rate is higher than the water samples of Dhaka city. So, we found the low resistance rate. A small amount of organism showed resistance to those antibiotics.

Keywords- Kaptai lake, Rangamati, Bangladesh, Water,Banarupa, Fisharighat Pathogens, Antimicrobial resistance, Antibiotic susceptibility, Isolation, Identification, Samples, Sensitive, Intermediate, Resistance, Gram positive, Gram negative.

1. INTRODUCTION

1.1 BACKGROUND

Since water is necessary for life, Kaptai Lake (KL) one of the largest artificial freshwater lakes in South-east Asia, was chosen. It is situated in Bangladesh's Rangamati district, which is also the principal residence of the indigenous ethnic minority. The people who live on the islands within the lake use the lake water for drinking and other domestic purposes without first cleaning it. Throughout the world, freshwater usage has significantly increased during the past ten years (Doria, 2006). The increase has also been observed in nations where tap water is used for drinking.

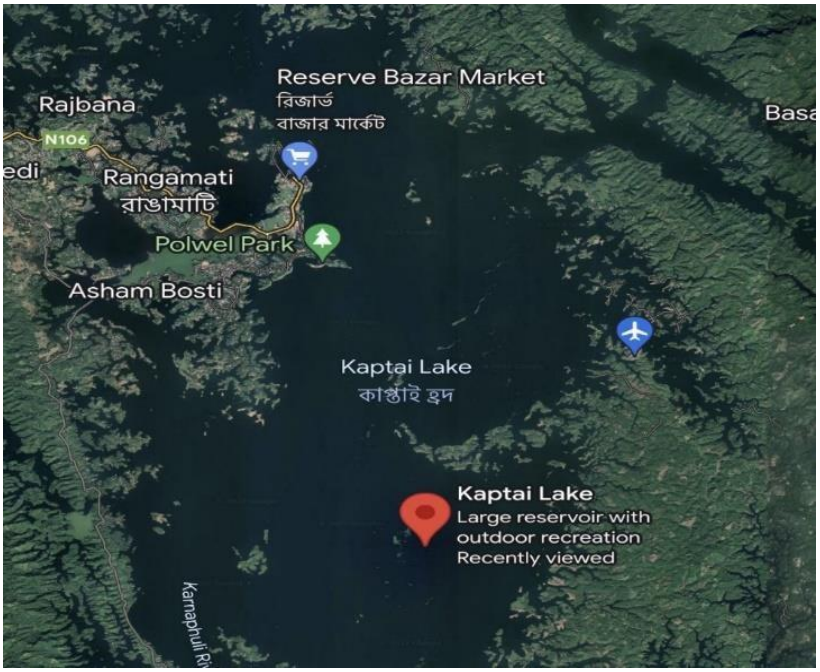


Figure (1): KAPTAI LAKE's position in google map

Safety and health advantages are the main considerations in the criteria. Everyone must have availability to enough that is safe and convenient. Enhancing access to clean drinking water can have a positive impact on health. To ensure the safest possible drinking water quality, every precaution should be taken. Safe drinking water is necessary for maintaining environmental and public health, which means that it must be devoid of dangerous germs.

The largest man-made freshwater body in Bangladesh is Kaptai Lake. Although designed largely to produce hydroelectric power, it also helps with navigation, flood control, agriculture, and the production of a significant number of freshwater fish. The reservoir was built by damming the river Karnafuli in the Chittagong Hill Tracts, close to Kaptai Town. The lake has developed into a valuable resource for some other purposes in addition to producing hydroelectricity. It has produced a lengthy, varied watercourse (*Kaptai Lake*,2018). Today, traveling to many locations that once required a day or longer by speedboat or launch just takes an hour. Resources in forests

that were previously out of reach have become far more accessible. Today, the entire lake is a popular tourist destination. The lake has made a substantial contribution to the growth of the agricultural and fishing industries. The lakeshores are irrigated automatically and are extremely fruitful because the lake's water level is kept at various levels throughout the year. On a leasehold basis, on approximately 6,075 acres of land, people engage in controlled farming under agreements with the local government (*Kaptai Lake*,2018). The World Health Report (2002) states that more than 3.4 million people die each year from water-related illnesses, making these the leading cause of death worldwide. In Bangladesh, water-related diseases continue to be a major cause of mortality and morbidity despite efforts to promote the use of safe water sources, and it has been hypothesized that consuming polluted water is a key method of pathogen transmission. Even though disinfection is used in water supply systems, inadequate management could cause the disinfection system to fail, posing major health risks and post-contamination risks. The amount of drinking water that is thought to be contaminated with different organic and inorganic substances is 1%. Fecal waste from poultry and cattle farms, pesticides, herbicides, numerous industrial wastes, minerals (including dangerous metals like lead, copper, etc. and biological agents like bacteria, virus, fungus, algae, etc. are among the organic materials that cause water contamination. River water included Enterobacteriaceae and contained a significant number of enteric types of organisms (Mieres and Bastardo,1975). Most water sources have chemical pollutants that are man-made. Industrial waste is one of the main pollutants in metropolitan areas that contributes to the chemical contamination of the water sources.

The goal of the current study was to test the bacteriological, physicochemical, and metal features of the lake water and compare them to WHO, USEPA, and Bangladesh EQS criteria in order to assess the lake water quality in relation to health problems. But the coliform was discovered. *Salmonella, Pseudomonas, Vibrio*, and *Enterococcus spp*. were all detected in all the water samples. Our research's conclusions show that Kaptai Lake's water lacks the bacteriological and metal qualities that make it appropriate for drinking and may even be harmful to users' long-term health.

In the current investigation, pathogenic bacteria such as *Escherichia coli, Enterobacter, Staphylococcus aureus, Pseudomonas,* and *Vibrio* species (*V. cholerae, V. parahaemolyticus, V. mimicus,* and *valginolyticus)* were identified from mineral water in the city of Dhaka. The

prevention of waterborne infection in this area will be aided by the detection of pathogenic bacteria in water. According to estimates from the Globe Health Organization (2018), poor sanitation, contaminated water, or a lack of access to water are to blame for up to 80% of all sickness and disease in the world. According to estimates, approximately 1.5 billion people lack access to clean drinking water, and at least 5 million people every year pass away from a water-related illness. Most people in Bangladesh lack basic literacy skills and are unaware of health risks. This is a result of the populace's low literacy rate. Most people reside in rural areas with few initiatives to inform them of the risks to their health. Therefore, these people's health is put at risk due to their religious and cultural beliefs. On the other hand, city dwellers are very aware of the dangers to their health. They are aware of the advantages of taking preventative measures to stay healthy. Bacterial concentrations in water that are excessive and much over regulatory thresholds can result from the release of germs together with resuspended sediments. The current models for microbiological water quality that support management choices neglect this bacterial input from sediment. The presence of significant populations of fecal coliforms and *E. coli* in freshwater, both in the surface and ground water, has long been recognized. But it wasn't until recently that the relative significance of sediments as bacterial habitats and as a source of water-borne fecal-coliforms and *E. coli* was realized. Many publications have demonstrated that in many instances elevated E. coli concentrations in water can be caused by the resuspension of sediment rather than runoff from nearby lands. The threats that pathogenic members of the group represent to humans has drawn a lot of interest to the persistence of enteric bacteria in aquatic habitats. (Lim, Yoon, and Hovde ,2010)

Therefore, the goal of the study was to identify the kaptai bacteria and conduct AMR (Antimicrobial Resistance) testing to determine the resistance to the potency of antibiotics against the illness. It is the capacity of germs to survive or develop when exposed to medications intended to stop them or kill them. Antimicrobials, as these medications are also known, are prescribed to treat infectious disorders brought on by bacteria, fungi, viruses, and protozoan parasites. Thus, the analysis can be beneficial in many medical or dietary issues.

1.2- LITERATURE REVIEW

Water resources with a large potential are abundant in Bangladesh and are spread out across the nation. According to sources the total area of the inland water body is 46, 99, 394 hectares, of

which 39, 10, 053 hectares are open water and 7, 89, 341 hectares are closed water. 9,95,805 metric tons of the total inland production and 19, 56,925 metric tons of it originate from open water bodies. Additionally, 1, 76,120 mt of shrimp were produced in closed water fisheries. The availability of 19.30 kg of fish per person annually, compared to the minimal requirement of roughly 21.90 kg, is reflected in this production level. There are 266 indigenous fish species, 13 foreign fish species, and 24 prawn species that can be found in Bangladesh's inland waterways. 475 different fish species may be found in the marine water bodies, which are located 200 nautical miles off the shore. At least 36 species of marine shrimp, 24 of which are freshwater species, may be found in Bangladesh. Bangladesh is home to 266 freshwater, 475 marine, 24 freshwater, 36 marine, and 12 exotic kinds of fish. Kaptai Lake was initially created with the intention of creating hydroelectric power at the time Kaptai Dam was built in 1962. 54,000 acres of farmland were flooded by the Karnafuli Hydropower Station's dam. During that time, over 100,000 locals from about 18,000 households, largely from indigenous communities, were uprooted, forcing over 35,000 Chakmas and Hajongs to flee to the neighboring country of India. One of the main fishing resources in Bangladesh is the Kaptai Lake (Lat. 2220' - 2318'N Long. 9200' - 9226'E) (Kabir, M. T. H., Rahman, M. A., Chowdhury, P., Haque, S. M., & Miah, M. I. ,2020). It developed because of the construction of a dam across the Karnaphuli River in the Kaptai section of the Rangamati district to generate hydroelectric power, which later transformed it into an appropriate habitat for several fish species with significant commercial value. The lake, which has a surface size of over 68,800 hectares, makes a significant annual contribution to the nation's total fish production. The lake's average water area is 58,300 ha, and its full surface area is 68,800 ha. The lake's maximum and minimum depths are 35 meters and 9 meters, respectively. According to a study, the Kaptai Lake is home to 74 species of freshwater fish and two types of prawns. Therefore, it goes without saying that Kaptai Lake's physiological and microbiological status must be in a state where it won't cause any environmental problems or health problems for living things. Fishing at Kaptai Lake is primarily focused on the types of fishing equipment utilized, the species composition, and the marketing channel with current management techniques. One of the busy areas we targeted for the study was Banarupa and Fisharighat. Boats keep coming in from the hills delivering a variety of goods and produce to the Shamata Ghat of the Banarupa Bazaar in Rangamati. In this floating market by the Kaptai Lake, it doesn't take long for the produce to sell out. Another busy location from Rangamati that links to Kaptai Lake is Fisharighat.

Nevertheless, to succeed, we must move forward with pathogen isolation and AMR. A bacterial infectious agent is often subjected to AST after being cultured and species identified. However, AST takes time since it requires the organism to grow both in the absence and presence of the appropriate antibiotics. Antimicrobial resistance rates have risen since the beta-lactam antibiotic penicillin was discovered. Methods for determining a bacteria's susceptibility to antibiotics have evolved and varied over time. In the 1920s, Alexander Fleming created the initial susceptibility testing technique (Wheat, P. F ,2001).

In our study, it was essential to carry out AST bacterial isolation and characterization. Robert Koch created methods to cultivate and extract bacteria cells in the late 1800s. These bacteria cells were then recognized and defined by biochemical staining, microscopic examination of their appearance, and the use of enrichment cultures. Gram staining and biochemical tests were carried out to identify the isolates of bacteria. It was discovered that 79% of the isolates were gram positive and 26% were gram negative. Some bacteria had a circular shape upon microscopic examination, whereas others had a rod shape.

1.3- Objective

The presence of bacterial pollutants from various water sources in and around Kaptai Lake was the main goal of the current investigation. By monitoring numerous microbiological pathogens, lake water from BANARUPA and FISHARIGHAT was researched together with the risk factors connected to it. And run an antimicrobial susceptibility test to evaluate which medications are effective against bacteria that are resistant to antibiotics. Many diseases today have become resistant to the treatment provided by antimicrobial drugs. Antibiotics may no longer work as intended due to the development of resistant bacteria because of improper use, which poses a serious threat, for instance, to the treatment of cancer. Major surgeries and cancer chemotherapy would fail if there were no good antibiotics available to fight off germs like *Escherichia coli, Enterobacter,* and *Vibrio, Enterococcus, Shigella*, and *Salmonella.*

1.4- Pathogens of lake water (KAPTAI)

The sources of coastal water contamination are discharges of both treated and untreated sewage from coastline outfalls and non-point releases are the origins of coastal water contamination. Pathogens are released into coastal waterways by non-point sources, such as runoff from naturally

vegetated areas. Most of the waterborne infections detected in recreational waters are caused by feces. Some of the sources of these wastes in surface waters include swimmers who are ill and have diarrhea and/or vomiting, livestock, waterfowl, and animal waste (Bacteriological and physicochemical characteristics of Kaptai Lake ,2016). There are numerous connections between Kaptai Lake and animals, plants, or chemicals. Major bacterial infections can spread through contact with tainted water. Infected humans and other animals as well as animal or human wastes are the most frequent sources of contamination. Both a reservoir for harmful bacteria and a means of disease transmission can be found in fresh water (Benenson, 1995). Common bacteria, water-borne human pathogens like *Shigella* species, *Salmonella* species, *E. coli, Enterobacter* species, *Vibrio cholerae*, and *Vibrio* other species, as well as opportunistic pathogens like *Enterococcus* and *Pseudomonas* species, *Klebsiella pneumoniae*, can all be found naturally in lake water. (Bacteriological and physicochemical characteristics of Kaptai Lake ,2016)

Escherichia coli

Gram-negative, rod-shaped, facultatively anaerobic is how *Escherichia coli* (E. coli) is described. Theodor Escherich published the first description of this bacterium in 1885. Most E. coli strains are a typical part of the flora in the gastrointestinal tracts of both humans and animals. Some *E. coli* strains, however, have acquired virulence traits via plasmids, transposons, bacteriophages, and/or pathogenicity islands, resulting in their becoming pathogenic. Based on serogroups, pathogenicity mechanisms, clinical signs, or virulence variables, this pathogenic *E. coli* can be grouped. A pathogenic strain of *E. coli* that produces Shiga toxins (Stxs), causes hemorrhagic colitis (HC), and has the potentially fatal side effects of hemolytic uremic syndrome (HUS) is known as enterohemorrhagic *E. coli* (EHEC). Many EHEC serotypes, including O26:H11, O91:H21, O111:H8, O157: NM, and O157:H7, have a strong association with human illnesses. E. coli O157:H7 is capable of surviving and persisting in a wide range of conditions, including food, water, soil, and animal reservoirs. The 5.5 Mb chromosome size of *E. coli* O157:H7. The 4. 1 Mb backbone sequence of this genome is the same in all strains of *E. coli*. All the rest are unique to E. coli O157:H7. Furthermore, a genome comparison of pathogenic *E. coli* O157:H7 and nonpathogenic E. coli K12 reveals that 0.53 Mb of DNA is absent in *E. coli* O157:H7, indicating that genomic reduction may also have contributed to the evolution of *E. coli* O157:H7. A large portion of the 1.4 Mb of *E. coli* O157:H7-specific DNA sequences are horizontally transmitted foreign sequences. Horizontally transmitted foreign DNAs like prophage and prophage-like

elements make up the majority of the 1.4 Mb of *E. coli* O157:H7-specific DNA sequences. (Lim, , Yoon, and Hovde ,2010)

Vibrio

A family of widespread, rod-shaped, Gram-negative bacteria called Vibrio spp. is a natural component of freshwater, estuary, and marine environments1. Several biological and genetic traits are shared by Vibrio species. Their two chromosomes, which have been sculpted by horizontal gene transfer and recombination, each contain half of their genomes (HGT; that is, the acquisition of genetic material by transfer from other organisms). According to *Nature News*, the origin of these pathogens may vary in terms of their genomic makeup, but they all come from aquatic and marine environments. They prefer warm, brackish (slightly salty) water, and the abundance of these pathogens in the natural world often reflects the temperature of the surrounding environment. (Baker-Austin, *et al.*, 2018)

Twelve of the more than 100 *Vibrio* species that have been reported cause illnesses in people. *Vibrio* is a genus of ubiquitous bacteria that can be found in a wide range of aquatic and marine settings. Cholera is a severe diarrheal illness that can soon be fatal if left untreated and is mainly spread through contaminated water and direct human contact. *Vibrio cholerae* can cause this illness. Non-cholera Vibrio spp., such as *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio vulnificus*, cause vibriosis, diseases that are typically contracted by meeting polluted seawater or undercooked or raw seafood. Except for *V. vulnificus*, an opportunistic pathogen with a high mortality that causes wound infections that can quickly progress to septicemia, non-cholera bacteria can produce a variety of clinical symptoms, most frequently mild, self-limiting gastroenteritis. For example, rehydration therapy for *V. cholerae* infections and debridement of infected tissues for *V. vulnificus* associated wound infections, with antibiotic therapy for severe cholera and systemic infections, determine the appropriate course of treatment for *Vibrio spp*. infections. (Baker-Austin, *et al.*, 2018)

Shigella

Shigellosis is brought on by the Gram-negative bacteria *Shigella. Shigella* research has helped scientists learn more about how the host reacts to bacterial infection and how bacteria have evolved to successfully bypass the host's defenses over time. In this review, we give a summary of recent developments in our knowledge of key aspects of *Shigella* infection, including invasion into host

cells, metabolic alterations in the bacterium and the infected cell, cell-to-cell spread mechanisms, autophagy and membrane trafficking, inflammatory signaling, and cell death. Recent research gives a clearer understanding of the intricate interactions between host cells and bacterial infections in general as well as new insight into the mechanisms behind *Shigella* pathogenesis.

The tribe Escherichia of the Enterobacteriaceae family includes organisms of the genus Shigella. (Hale,1996) Four distinct species make up the genus Shigella: Serogroup A of *S. dysenteriae* has 12 serotypes, while Serogroup A of *S. flexneri* has 6 serotypes, Serogroup C of *S. boydii* has 18 serotypes, and Serogroup D of *S sonnei* has 12 serotypes (serogroup D, consisting of a single serotype). Serogoups A, B, and C share many physiological characteristics, whereas *S. sonnei* can be distinguished from the other serogroups by positive -D-galactosidase and ornithine decarboxylase biochemical reactions. Slide agglutination with commercially available, absorbed rabbit antisera is typically used in the clinical laboratory to identify *shigellae* by species. A self-limiting complication of *S. flexneri* infection, reactive arthritis, can affect as many as 2% of those who exhibit the HLA-B27 histocompatibility antigen. A rare consequence in kids with *S dysenteriae* serotype 1 infection is hemolytic-uremic syndrome, which is defined by a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (Hale & Keusch, 1996).

Salmonella

In the family Enterobacteriaceae, the genus *Salmonella* contains a variety of rod-shaped, gram-negative, facultatively anaerobic bacteria. The gastrointestinal tracts of people and other animals serve as their primary home. Animals can harbor some species without showing any signs of sickness, while others can lead to any of a wide variety of mild to serious infections known as salmonellosis. *Salmonella* infections in humans are typically brought on by consuming infected food or drink. (Britannica. Editors of Encyclopaedia, 2022) Typhoid fever is brought on by *Salmonella typhi*, while *S. paratyphi, S. schottmuelleri*, and *S. hirschfeldii*—all of which are thought to be variations of *S. enteritidis*—cause paratyphoid fever.

Klebsiella

A variety of healthcare-associated diseases, such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis, can be brought on by *Klebsiella*, a kind of Gram-negative bacteria. Antibiotic resistance in *Klebsiella* bacteria is on the rise, most recently to the antibiotic class known as carbapenems. Human intestines typically include *Klebsiella* bacteria (where they do not cause disease). They can be detected in human feces (*Klebsiella pneumoniae* in healthcare settings, 2010). Additionally, according to the Centers for Disease Control and Prevention, patients who are ill and getting treatment for various diseases frequently contract Klebsiella infections in healthcare settings. The most vulnerable individuals to *Klebsiella* infections are those whose care necessitates the use of equipment like ventilators (breathing machines) or intravenous (vein) catheters, as well as those undergoing prolonged courses of specific antibiotics. *Klebsiella* infections typically do not occur in healthy individuals. *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella terrigena*, and *Klebsiella planticola* are the four known species. Neonatal infections of the bloodstream, urinary tract, central nervous system, lungs, skin, and soft tissues are brought on by *K. pneumoniae*, the most prevalent human pathogen, and *K. oxytoca*.

Enterococcus

In the nosocomial context, enterococci, which are Gram-positive facultative anaerobic cocci in short and medium chains, cause infections that are challenging to treat. They occasionally cause intra-abdominal infections and meningitis, but they frequently cause UTI, bacteremia, and infective endocarditis. (G.M.S.C.D.B.I.Y.S, 2022)

According to the National Center for Biotechnology Information, *E. faecalis* is thought to be the primary cause of 85% to 90% of enterococci infections, which are predominantly nosocomial (hospital-acquired). Poor hand hygiene, growth in medical equipment, and tainted food or drink are some common sources of *E. faecalis* infections.

1.5- Importance of AMR & AST in this study

AMR (Antimicrobial resistance)

Particularly in environments of intense animal production, the enormous volume of antibiotics used in food-producing animals contributes to the growth of antimicrobial-resistant bacteria. WHO estimates that in some nations, the overall amount of antibiotics given to animals is four times more than the total amount given to people. These are just a few instances of the ways in which AMR is currently influencing our daily life. AMR happens when bacteria, viruses, fungi, parasites, and other microorganisms evolve over time and cease to respond to antibiotics, making infections more difficult to cure and raising the risk of disease spread, life-threatening illness, and death. In turn, this makes the medications less effective and causes infections to linger in the body, raising the chance of infection spreading. Superbugs are a term used to describe bacteria that become resistant to antibiotics. Specific bacteria that are resistant to numerous types of antibiotics are referred to as "superbugs." (Antimicrobial resistance, 2022) Other names for superbugs include:

1. **multi-resistant organisms (MROs)**
2. **multi-drug resistant organisms (MDRs).**

A major hazard to human health, antimicrobial resistance (AMR) has huge worldwide economic and security ramifications. A global action plan to combat AMR was unanimously accepted by WHO Member States in 2015. (GAP-AMR). GAP-mission AMR's statement reads, "To ensure, for as long as feasible, continuity of successful treatment and prevention of infectious illnesses with effective and safe medicines that are quality-assured, used responsibly, and accessible to all who need them. “In order to inform policy and infection prevention and control strategies, surveillance is a crucial tool. Data is essential because it provides the foundation for figuring out how AMR is spreading and for guiding and monitoring the success of regional, global, and international activities. The Global Antimicrobial Resistance and Use Surveillance System (GLASS), the first international collaborative effort to standardize AMR surveillance, was introduced by WHO on October 22, 2015. GLASS was established to support the second goal of the GAP-AMR initiative, "strengthen knowledge through surveillance and research," and to continue filling knowledge gaps with the aim of informing strategies at all levels (Molecular Methods for Antimicrobial Resistance (AMR) Diagnostics to Enhance the Global Antimicrobial Resistance Surveillance System. Geneva, 2019). This goal was endorsed by the Sixty-eighth World

Health Assembly in resolution WHA68.7. Antimicrobial medications include antibiotics. They function by eliminating bacteria, reducing their growth, or preventing infection. The native immune system of the body uses antibiotics to combat bacterial infections.

AST (Antibiotic Sensitivity Test)

Finding the appropriate course of action for a bacterial infection involves using an antibiotic sensitivity test. It can also be used to determine which treatment will be most effective for a particular fungus infection. Result descriptions typically take one of the following forms:

• Susceptible- The drug under test halted or eliminated the bacterium or fungus that was infecting you. The drug might be an effective therapy option.

• Intermediate- higher dose of the drug might still be effective.

• Resistant- the medication did not prevent the infection-causing bacteria or fungus from growing or killing it. It wouldn't make a good therapy option.

Infections that are resistant to antibiotics may benefit from the test in finding a cure. When common drugs lose their effectiveness or are rendered useless against bacteria, antibiotic resistanceoccurs. Antibiotic resistance can transform diseases that were once easily curable into serious, potentially fatal conditions (van Belkum et al., 2018). Here, drinking water samples taken from two separate sources of Kaptai Lake were analyzed for bacteria and for antibiotic sensitivity patterns. The antibiotics that were mostly considered in our investigation to determine thesensitivity were some of those that were often utilized in this region to treat infectious illnesses. If there is any antimicrobial resistance, the test may be useful in identifying an antibiotic-resistant infection's treatment options.

2. Materials and Methods

2.1 Study place

This research work was carried out at the Microbiology, Biotechnology, and Molecular Biology Laboratory of the Department of Mathematics and Natural Sciences of BRAC University.

2.2 Flow Diagram of the Study Design-

Collection of mineral water sample

⇩

Sample purification through filtration

⇩

Culture in LB shaker

⇩

Performing serial dilution

⇩

Spread plate method

⇩

Estimation of colony forming unit (CFU)

⇩

Selection of isolates

⇩

Making stocks of the isolates to work further

⇩

Characterization through Gram staining

⇩

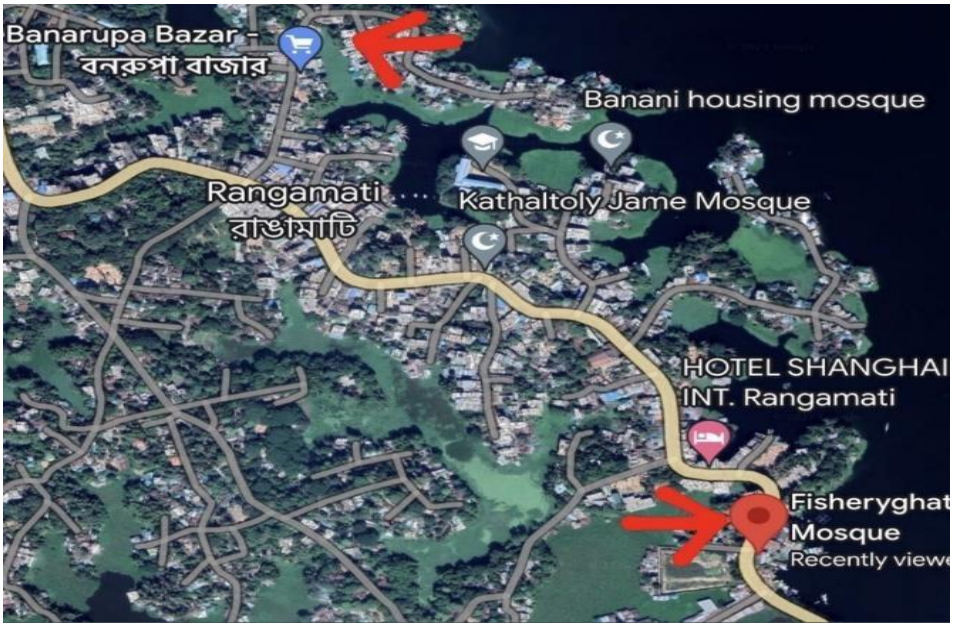
Microscopic observation

⇩

Antibiotic Susceptibility Test (AST)

2.3 Collection of Samples

Two water samples of different places of Kaptai Lake were collected from June 2022 to October 2022. The samples were collected from "**Banarupa**" and "**Fisharighat**". Samples were labeled in the field, transported to the laboratory, and processed in the Laboratory of Microbiology, BRAC University within 3 hours of collection.



Figure(2):Banarupaghat and Fisharighat position in google map

2.4 Isolation of bacteria water sample

After collecting the samples, filtration of the samples was done through 0.4mm Whatman filter paper. Then, 5 ml of each filtrated sample were added to 50 ml of LB in two different autoclaved falcon tube for growth enrichment. After that, these tubes were in shaker for 3 hours. Serial dilution was done till 10^9 Four different selective agar media MacConkey, TCBS, SS and EMB agar media were used for the isolation of Escherichia coli, Enterobacter, and Vibrio species respectively by spread plate method.

a) *E. coli:* spread 300 µl sample of 10^1, 10^3, 10^5, 10^7 and 10^9 dilution in MacConkey plate.

b) *V. cholera:* spread 300 µl sample of 10^1, 10^3, 10^5, 10^7 and 10^9 dilution in TCBS plate.

All the plates were then incubated at 37 °C for 24 hours. After incubation, all the colonies were counted, and several colonies were streaked for the isolation of single colonies. Then again, all the streaked plates were incubated at 37 °C for 24 hours. Lastly, single colonies were stocked in semi-solid agar containing vials named LA.

2.5 Microscopic observation of isolates

For evaluation of microscopic character, a pure colony of each isolate was picked, and Gram staining was performed according to Hacker's modified method (Doetsch, 1981). The isolates' size, shape, arrangement, and Gram reaction properties were carefully observed. If the organism appeared purple, then it was noted as gram negative organism and if the organism appeared pink then it was noted as gram positive bacteria.

2.6 Possible Identification

After evaluating the microscopic result, we have identified the organisms as per the "HiMedia Publication". From this publication we have been able to identify the organisms of TCBS, SS, EMB and Mac media. The color of the colony indicates the organism according to this publication. The chart of the identification way is given below-

Agar name	Organism name	Color of the colony
ss	*Escherichia coli*	Pink with bile precipitate
ss	*Enterobacter aerogenes*	Cream Pink
ss	*Enterococcus faecalis*	Colorless
ss	*Salmonella typhii*	Colorless with black center
ss	*Salmonella typhimurium*	Colorless with black center
ss	*Salmonella enteridis*	Colorless with black center

ss	*Salmonella flexneri*	Colorless with black center
MacConkey	*Escherichia coli*	Pink to red with bile precipitate
MacConkey	*Klebsiella aerogenes*	Pale pink to red
MacConkey	*Enterococcus faecalis*	Pale pink to red
MacConkey	*Salmonella paratyphii*	colorless
MacConkey	*Shigella flexneri*	colorless
MacConkey	*Salmonella paratyphi*	colorless
MacConkey	*Salmonella enteridis*	colorless
MacConkey	*Salmonella typhi*	Pink to red
TCBS	*Vibrio cholera*	Yellow
TCBS	*Vibrio fluvialis*	Yellow
TCBS	*Vibrio parahaemolyticus*	Bluish Green
TCBS	*Vibrio vulnificus*	Greenish Yellow
EMB	*Klebsiella aerogenes*	Pink without sheen
EMB	*Escherichia coli*	Purple with black center and green metallic sheen
EMB	*Klebsiella pneumoniae*	Pink, mucoid
EMB	*Salmonella typhimurium*	colorless

2.7 Antimicrobial Susceptibility

Susceptibility and resistance of different antibiotics were measured in vitro by employing the Kirby-Bauer method (Bauer et al., 1996). This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that resulted from the diffusion of the agent into the medium surrounding the disc. For performing this test, each colony was taken and missed in 10ml of saline (0.5-1.0 McFarland Standard). Then, using an autoclaved cotton swab, lawn the MHA plate in such a way that it covers the entire surface. After that, place antibiotic discs and gently press them on the plate. Then, place the Petri dishes bottom up in the incubator. For the best results, the plates were observed within 14-16 hours. The incubation period didn't exceed 24 hours. The antibiotics discs used were:

1. Ampicillin 2mcg.

2. Aztreonam 30 mcg.

3. cefepime 30 mcg.

4. Chloramphenicol 30 mcg.

5. Co- Trimoxazole 25 mcg.

6. Doxycycline Hydrochloride 30 mcg.

7. Erythromycin 15 mcg.

8. Gentamicin 10 mcg.

9. Imipenem 10 mcg.

10. Kanamycin 30 mcg.

11. Levofloxacin 5 mcg.

12. Linezolid 30 mcg.

13. Oxacillin 1 mcg.

14. Vancomycin 5 mcg.

Sterile glass spreader was used to spread the culture homogenously on the medium. Antibiotic discs were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of sterile forceps. After incubation, the plates were examined and the diameters of the zone of complete inhibition were observed. Here is the table of antibiotics and diameter of zone inhibition:

Antibiotic Names	Disc Concentration	Diameter of zone of inhibition		
		Resistant <or=nm	Intermediate (nm)	Susceptible =or>nm
1. Ampicillin	2 mcg	10	8-9	8
2. Aztreonam	30 mcg	21	18-20	17

3. cefepime	30 mcg	25	19-24	18
4. Chloramphenicol	30 mcg	18	13-17	12
5. Co-Trimoxazole	25 mcg	16	11-15	10
6. Doxycycline Hydrochloride	30 mcg	14	11-13	10
7. Erythromycin	15 mcg	23	14-22	13
8. Gentamicin	10 mcg	15	13-14	12
9. Imipenem	10 mcg	23	20-22	19
10. Kanamycin	30 mcg	18	14-17	13
11. Levofloxacin	5 mcg	21	17-20	16
12. Linezolid	30 mcg	23	21-22	20
13. Oxacillin	1 mcg	18	-	17
14. Vancomycin	5 mcg	12	-	12

Result

We have collected our samples from two different areas (Banarupa and Fisharighat) of Kaptai Lake. As we have collected the samples from two different areas, we created code names for the samples. For example,

Sample area: Banarupa (B) and Fisharighat (F)

Media: First two letters of the media name-

- TCBS- TC
- EMB- EM
- MAC- MA
- SS- SS

Isolate number: 1,2,3 …

For example: BEM1

Isolates from Banarupa Samples:

The following tables contain gram staining results of all samples of Banarupa-

Sample 01

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BTC2	Yellow		(-)	Rod/Comma	*Vibrio*
2.	BSS1	Colorless with black center	(+)		Cocci(chain)	*Shigella*
3.	BSS3	Pink with bile precipitate		(-)	Comma	*Escherichia*
4.	BSS4	Colorless with black center		(+)	Rod	*Salmonella*
5.	BSS5	Cream pink	(+)		Spiral	*Enterobacter*
6.	BMA6	Pink to red with bile precipitate/ pale pink to red	(+)		Comma	*Enterococcus*

Sample 02

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BSS1	Cream pink		(-)	Rod/Comma	*Enterobacter*
2.	BSS4	Colorless with black center		(-)	Rod	*Salmonella*
3.	BEM2	Purple with black center and green metallic sheen		(-)	Rod	*Escherichia*
4.	BTC4	Bluish green		(-)	Rod	*Vibrio*
5.	BMA4	Pink to red with bile precipitate		(-)	Rod	*Escherichia*

Sample 03

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BSS1	Cream pink		(-)	Rod	*Enterobacter*
2.	BMA1	Pale pink to red	(+)		Cocci	*Enterococcus*
3.	BMA2	Pale pink to red		(-)	Rod/comma	*Klebsiella*
4.	BEM2	Pink, mucoid		(-)	Rod	*Klebsiella*
5.	BEM5	Purple with black center and green metallic sheen		(-)	Long rod	*Escherichia*

Sample 04

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BSS2	Pink with bile precipitate		(-)	Rod	*Escherichia*
2.	BSS5	Colorless with black center		(-)	Rod	*Salmonella*
3.	BMA1	Pale pink to red		(-)	Rod	*Klebsiella*
4.	BMA3	Colorless	(+)		Cocci	*Shigella*
5.	BEM5	Pink, mucoid		(-)	Rod	*Klebsiella*
6.	BTC2	Greenish yellow		(-)	Rod/comma	*Vibrio*
7.	BTC3	Bluish green		(-)	Rod	*Vibrio*

Sample 05

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BTC2	Bluish green		(-)	Rod	*Vibrio*
2.	BSS7	Colorless, may have black center		(-)	Rod	*Salmonella*
3.	BMA1	Pale pink to red		(-)	Rod	*Klebsiella*
4.	BMA2	Pink to red with bile precipitate		(-)	Cocci	*Escherichia*
5.	BMA3	Pink to red		(-)	Rod	*Salmonella*
6.	BEM3	Pink, mucoid		(-)	Cocci	*Klebsiella*

Sample 06

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	BTC2	Bluish green		(-)	Rod	*Vibrio*
2.	BMA1	Pink to red		(-)	Rod	*Salmonella*
3.	BMA2	Pink to red		(-)	Rod	*Shigella*
4.	BEM2	Pink, mucoid		(-)	Cocci	*Klebsiella*

Antibiogram Result (BANARUPA)

Here, we will be indicating,

Sensitive as **S**

Intermediate as **I**

Resistant as **R**

The following tables contain antibiogram results of all samples of Banarupa.

Sample 01

Antimicrobial Agent	Sample Susceptibility Result										
	BTC2 (Vibrio)		BSS1 (Shigella)		BSS3 (Escherichia)		BSS4 (Salmonella)		BSS6 (Enterobacter)		BMA6 (Enterococcus)
	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	R	0	R	22	S	0	S	0	R	0	R
Aztreonam	S	40	S	38	S	32	S	36	S	38	S
Cefepime	S	38	S	38	S	28	S	36	S	36	S
Chloramphenicol	S	26	S	24	S	26	S	26	S	32	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	S	30	S	30	S	32	S	34	S	28	S
Doxycycline Hydrochloride	S	24	S	0	R	22	S	22	I	20	S
Erythromycin	R	0	R	0	R	0	R	0	R	0	R
Imipenem	S	32	S	30	S	28	S	30	S	24	S
Gentamycin	S	26	S	30	S	24	S	24	S	24	S
Kanamycin	S	18	S	22	S	18	S	22	S	18	S
Levofloxacin	S	31	S	36	S	30	S	30	S	38	S
Linezolid	R	0	R	0	R	0	R	0	R	0	R
Oxacillin	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	R	0	R	0	R	0	R	0	R	0	R

Sample 02

Antimicrobial Agent	Sample Susceptibility Result									
	BSS1 (Enterobacter)		BSS4 (Salmonella)		BTC4(Vibrio)		BEM2 (Escherichia)		BMA4 (Escherichia)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	17	S	20	S	0	R	O	R	15	I
Aztreonam	41	S	41	S	42	S	33	S	37	S
Cefepime	34	S	37	S	34	S	36	S	36	S
Chloramphenicol	25	S	20	S	30	S	27	S	30	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	27	S	30	S	32	S	30	S	33	S
Doxycycline Hydrochloride	0	R	0	R	0	R	14	S	21	S
Erythromycin	0	R	0	R	0	R	0	R	21	R
Imipenem	32	S	30	S	26	S	28	S	39	S
Gentamycin	25	S	28	S	28	S	22	S	24	S
Kanamycin	19	S	21	S	22	S	12	R	18	S
Levofloxacin	42	S	34	S	30	S	36	S	38	S
Linezolid	0	R	11	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	0	R	0	R

Sample 03

Antimicrobial Agent	Sample Susceptibility Result									
	BSS1 (Enterobacter)		BMA1 (Enterococcus)		BMA2 (Klebsiella)		BEM2 (Klebsiella)		BEM5 (Escherichia)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	0	R	0	R	0	R	O	R	21	S
Aztreonam	37	S	19	R	16	R	20	I	38	S
Cefepime	34	S	30	S	28	S	29	S	36	S
Chloramphenicol	28	S	13	I	0	R	21	S	30	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	38	S	29	S	28	S	30	S	0	R
Doxycycline Hydrochloride	25	S	40	S	37	S	41	S	24	S
Erythromycin	10	R	21	I	20	I	19	I	20	I
Imipenem	30	S	40	S	39	S	39	S	32	S
Gentamycin	22	S	30	S	32	S	30	S	27	S
Kanamycin	20	S	22	S	22	S	26	S	20	S
Levofloxacin	31	S	32	S	36	S	37	S	33	S
Linezolid	0	R	11	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	0	R	18	S

Sample 04

Antimicrobial Agent	Sample Susceptibility Result													
	BSS2 (Escherichia)		BSS5 (Salmonella)		BMA1 (Klebsiella)		BMA3 (Shigella)		BEM5 (Klebsiella)		BTC2 (Vibrio)		BTC4 (Vibrio)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	11	S	13	S	0	R	14	S	0	R	0	R	0	R
Aztreonam	32	S	32	S	22	S	28	S	36	S	42	S	40	S
Cefepime	36	S	34	S	28	S	32	S	36	S	32	S	40	S
Chloramphenicol	28	S	29	S	19	S	30	S	28	S	26	S	31	S
Co-Trimoxazole (Trimethoprim)	32	S	36	S	30	S	36	S	31	S	34	S	37	S
Doxycycline Hydrochloride	26	S	16	S	32	S	36	S	24	S	21	S	28	S
Erythromycin	9	R	14	I	24	S	32	S	10	R	0	R	0	R
Imipenem	28	S	30	S	32	S	32	S	28	S	28	S	28	S
Gentamycin	29	S	21	S	27	S	34	S	24	S	24	S	30	S
Kanamycin	20	S	23	S	26	S	28	S	17	I	22	S	21	S
Levofloxacin	30	S	35	S	35	S	40	S	31	S	34	S	35	S
Linezolid	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	17	S	16	S	0	R	0	R	0	R

Sample 05

Antimicrobial Agent	Sample Susceptibility Result											
	BSS7 (Salmonella)		BMA1 (Klebsiella)		BMA2 (Escherichia)		BMA3 (Salmonella)		BEM3 (Klebsiella)		BTC2 (Vibrio)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	11	R	0	R	0	S	14	I	0	R	0	R
Aztreonam	34	S	34	S	36	S	39	S	22	S	42	S
Cefepime	35	S	33	S	33	S	32	S	26	S	32	S
Chloramphenicol	29	S	30	S	32	S	31	S	18	S	24	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	34	S	30	S	30	S	28	S	30	S	29	S
Doxycycline Hydrochloride	19	S	19	S	20	S	22	S	30	S	0	R
Erythromycin	11	R	0	R	14	I	13	R	22	I	0	R
Imipenem	30	S	28	S	26	S	20	I	22	I	21	I
Gentamycin	20	S	20	S	20	S	20	S	34	S	21	S
Kanamycin	20	S	21	S	21	S	22	S	29	S	27	S
Levofloxacin	30	S	30	S	29	S	34	S	27	S	32	S
Linezolid	0	R	0	R	0	R	0	R	36	S	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	9	R	0	R	0	R	10	R	0	R	0	S

Sample 06

Antimicrobial Agent	Sample Susceptibility Result							
	BMA1 (Salmonella)		BTC2(Vibrio)		BEM2 (Klebsiella)		BMA2 (Shigella)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	0	R	0	R	0	R	0	R
Aztreonam	39	S	40	S	38	S	40	S
Cefepime	35	S	32	S	37	S	42	S
Chloramphenicol	32	S	30	S	28	S	40	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	33	S	32	S	35	S	40	S
Doxycycline Hydrochloride	21	S	0	R	20	S	38	S
Erythromycin	0	R	0	R	0	R	0	R
Imipenem	31	S	22	S	33	S	38	S
Gentamycin	24	S	27	S	21	S	22	S
Kanamycin	24	S	21	S	20	S	25	S
Levofloxacin	35	S	28	S	30	S	38	S
Linezolid	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	0	R

Isolates from Fisharighat Samples:

The following tables contain antibiogram results of all samples of Fisharighat-

Sample 01

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC1	Yellow		(-)	Rod/Comma	*Vibrio*
2.	FTC2	Greenish yellow		(-)	Rod	*Vibrio*
3.	FTC4	Bluish green		(-)	Rod	*Vibrio*
4.	FSS2	Pink with bile precipitate		(-)	Rod	*Escherichia*
5.	FSS3	Cream pink		(-)	Rod	*Enterobacter*
6.	FEM2	Pink, mucoid		(-)	Rod	*Klebsiella*
7.	FMA2	Pink to red		(-)	Rod	*Klebsiella*

Sample 02

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC1	Yellow		(-)	Rod	*Vibrio*
2.	FSS3	Cream pink		(-)	Rod	*Enterobacter*
3.	FSS5	Pink with bile precipitate		(-)	Rod	*Escherichia*
4.	FMA4	Pale pink to red	(+)		Long rod	*Enterococcus*
5.	FEM2	Pink, mucoid		(-)	cocci	*Klebsiella*
6.	FEM8	Pink, without sheen		(-)	Rod	*Klebsiella*

Sample 03

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC2	Yellow		(-)	Rod/Comma	*Vibrio*
2.	FSS1	Cream pink		(-)	Rod	*Enterobacter*
3.	FSS5	Black (colorless side)		(-)	Rod	*Salmonella*
4.	FMA1	Pink to red with bile precipitate/ pale pink to red		(-)	Rod	*Enterococcus*

Sample 04

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC2	Yellow		(-)	Rod	*Vibrio*
2.	FSS1	Cream pink		(-)	Rod	*Enterobacter*
3.	FMA1	Colorless		(-)	Rod/Cocci	*Shigella*
4.	FMA2	Colorless		(-)	Rod	*Shigella*
5.	FMA5	Pink to red		(-)	Rod	*Salmonella*
6.	FEM1	Pink, without sheen		(-)	rod	*Klebsiella*
7.	FEM2	Purple with black center and green metallic sheen		(-)	Long rod	*Escherichia*

Sample 05

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC1	Bluish green		(-)	Rod/Comma	*Vibrio*
2.	FSS1	Pink with bile precipitate		(-)	Rod	*Escherichia*
3.	FSS2	Cream Pink		(-)	Rod	*Enterobacter*
4.	FEM2	Pink without sheen		(-)	Long Rod	*Klebsiella*

Sample 06

Serial number	Sample Name	Characteristics	Gram Staining			
			Result		Shape	Species
1.	FTC1	Bluish green		(-)	Rod	*Vibrio*
2.	FTC2	Yellow		(-)	Rod	*Vibrio*
3.	FSS2	Cream pink		(-)	Rod	*Enterobacter*
4.	FSS9	Colorless		(-)	Rod/Spiral	*Enterococcus*
5.	FSS10	Pink with bile precipitate		(-)	Rod	*Escherichia*
6.	FMA3	Colorless		(-)	rod	*Salmonella*
7.	FEM1	Pink, mucoid		(-)	Rod	*Klebsiella*

Antibiogram Result (FISHARIGHAT)

Here we will be indicating-

Sensitive as **S**

Intermediate as **I**

Resistant as **R**

The following tables contain antibiogram results of all samples of Fisharighat.

Sample 01

Antimicrobial Agent	Sample Susceptibility Result							
	FTC2(Vibrio)		FSS1 (Enterobacter)		FSS5 (Salmonella)		FMA1 (Enterococcus)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	0	R	16	S	0	R	0	R
Aztreonam	40	S	40	S	17	I	30	S
Cefepime	36	S	32	S	36	S	34	S
Chloramphenicol	32	S	24	S	14	R	28	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	30	S	26	S	16	S	30	S
Doxycycline Hydrochloride	30	S	0	R	11	I	11	I
Erythromycin	20	I	0	R	0	R	0	R
Imipenem	26	S	30	S	14	R	28	S
Gentamycin	24	S	24	S	13	I	24	S
Kanamycin	18	S	20	S	20	S	30	S
Levofloxacin	32	S	40	S	13	R		
Linezolid	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	0	R

Sample 02

Antimicrobial Agent	Sample Susceptibility Result													
	FMA2(Klebsiella)		FTC1(Vibrio)		FTC2 (Vibrio)		FTC4(Vibrio)		FSS2(Escherichia)		FSS3(Enterobacter)		FEM2(Klebsiella)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size	Ctriteria	Zone size(mm)	Criteria
Ampicillin	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Aztreonam	21	S	40	S	42	S	15	R	21	S	40	S	15	R
Cefepime	28	S	41	S	33	S	23	I	28	S	39	S	23	I
Chloramphenicol	18	S	30	S	32	S	21	S	0	R	28	S	21	S
Co-Trimoxazole (Trimethoprim)	30	S	29	S	30	S	27	S	27	S	33	S	27	S
Doxycycline Hydrochloride	37	S	16	S	29	S	30	S	38	S	26	S	30	S
Erythromycin	21	NO DATA	0	R	19	I	0	R	24	R	10	NO DATA	0	R
Imipenem	30	S	25	S	25	S	24	S	30	S	27	S	24	S
Gentamycin	27	S	28	S	23	S	25	S	28	S	26	S	25	S
Kanamycin	28	S	21	S	17	I	19	S	19	S	20	S	19	S
Levofloxacin	33	S	41	S	31	S	28	S	34	S	35	S	28	S
Linezolid	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	18	S	0	R	0	R	0	R	0	R	0	R	0	R

Sample 03

Antimicrobial Agent	Sample Susceptibility Result											
	FTC1 (Vibrio)		FSS3 (Enterobacter)		FSS5 (Escherichia)		FMA4 (Enterococcus)		FEM2 (Klebsiella)		FEM8 (Klebsiella)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size	Criteria	Zone size(mm)	Criteria	Zone size	Criteria
Ampicillin	17	S	0	R	0	R	0	R	0	R	0	R
Aztreonam	28	S	40	S	22	S	18	I	22	S	18	I
Cefepime	30	S	40	S	30	S	30	S	30	S	30	S
Chloramphenicol	34	S	31	S	22	S	18	S	22	S	18	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	0	R	37	S	35	S	31	S	35	S	31	S
Doxycycline Hydrochloride	29	S	28	S	40	S	38	S	40	S	38	S
Erythromycin	22	I	0	R	24	S	20	I	24	S	20	I
Imipenem	25	S	28	S	40	S	37	S	40	S	37	S
Gentamycin	25	S	30	S	35	S	30	S	35	S	30	S
Kanamycin	0	R	21	S	26	S	22	S	26	S	22	S
Levofloxacin	26	S	35	S	36	S	37	S	36	S	37	S
Linezolid	22	S	0	R	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	15	S	0	R	15	S

Sample 04

Antimicrobial Agent	Sample Susceptibility Result													
	FTC2 (Vibrio)		FSS1 (Enterobacter)		FMA2 (Shigella)		FMA5 (Salmonella)		FMA1 (Shigella)		FEM1 (Klebsiella)		FEM2 (Escherichia)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size	Zone size(mm)	Criteria	Zone size(mm)	Zone size	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	0	R	0	R	0	0	R	0	15	S	0	R	0	R
Aztreonam	40	S	31	S	40	37	S	0	29	S	37	S	0	R
Cefepime	36	S	30	S	34	34	S	0	32	S	34	S	0	R
Chloramphenicol	0	R	28	S	32	31	S	0	38	S	31	S	0	R
Co-Trimoxazole (Trimethoprim)	30	S	29	S	34	30	S	0	32	S	30	S	0	R
Doxycycline Hydrochloride	10	R	18	S	23	21	S	0	28	S	21	S	0	R
Erythromycin	0	R	11	R	15	19	I	0	28	I	19	I	0	R
Imipenem	28	S	28	S	28	30	S	0	37	S	30	S	0	R
Gentamycin	27	S	27	S	21	19	S	0	30	S	19	S	0	R
Kanamycin	24	S	24	S	22	20	S	0	29	S	20	S	0	R
Levofloxacin	26	S	30	S	32	31	S	0	34	S	31	S	0	R
Linezolid	0	R	0	R	0	0	R	0	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	0	R	0	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	0	R	0	20	S	0	R	0	R

Sample 05

Antimicrobial Agent	Sample Susceptibility Result													
	FTC1(Vibrio)		FTC2(Vibrio)		FSS2 (Enterobacter)		FSS9 (Enterococcus)		FSS10 (Escherichia)		FMA3 (Salmonella)		FEM1 (Klebsiella)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size	Criteria	Zone size	Ctriteria	Zone size	Criteria
Ampicillin	0	R	22	S	8	R	0	R	12	R	0	R	0	R
Aztreonam	38	S	40	S	36	S	40	S	36	S	34	S	33	S
Cefepime	32	S	33	S	33	S	34	S	34	S	34	S	34	S
Chloramphenicol	33	S	34	S	30	S	34	S	34	S	30	S	28	S
Co-Trimoxazole	26	S	30	S	26	S	30	S	30	S	34	S	0	R
Doxycycline Hydrochloride	26	S	23	S	18	S	26	S	19	S	19	S	12	I
Erythromycin	19	I	20	I	14	I	20	I	11	R	10	R	17	I
Imipenem	18	R	32	S	28	S	22	I	29	S	25	S	26	S
Gentamycin	22	S	19	S	21	S	21	S	20	S	22	S	19	S
Kanamycin	23	S	18	S	21	S	22	S	21	S	24	S	21	S
Levofloxacin	29	S	38	S	34	S	42	S	28	S	31	S	32	S
Linezolid	14	R	15	R	0	R	14	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Vancomycin	13	S	18	S	0	R	20	S	0	R	8	R	12	S

Sample 06

Antimicrobial Agent	Sample Susceptibility Result							
	FTC2(Vibrio)		FSS1 (Enterobacter)		FSS2 (Salmonella)		FEM2 (Enterococcus)	
	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria	Zone size(mm)	Criteria
Ampicillin	0	R	0	R	16	S	0	R
Aztreonam	40	S	42	S	40	S	40	S
Cefepime	36	S	45	S	38	S	35	S
Chloramphenicol	32	S	30	S	40	R	33	S
Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)	30	S	35	S	40	S	36	S
Doxycycline Hydrochloride	30	S	10	I	12	I	21	S
Erythomycin	20	I	0	R	0	R	7	R
Imipenem	26	S	40	S	30	S	32	S
Gentamycin	24	S	22	S	25	S	21	S
Kanamycin	18	S	29	S	28	S	22	S
Levofloxacin	32	S	35	S	38	S	33	S
Linezolid	0	R	0	R	0	R	0	R
Oxacillin	0	R	0	R	0	R	0	R
Vancomycin	0	R	0	R	0	R	0	R

Analysis & Discussion

Water is considered an inevitable element of life. Around 75% of the earth is surrounded by water, but only 1% of water can be used as a source of drinking water for animals and human beings. Consumption of contaminated water may cause various gastrointestinal diseases like diarrhea, dysentery, and other waterborne diseases like cholera, and typhoid in humans, poultry, and livestock. The World Health Organization has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water, or unavailability of water and at least 5 million deaths per year can be attributed to water-borne diseases (Karn et al., 2001).

Kaptai water generally requires further treatment by the consumer before consumption, so it's not microbiological safety and quality are of paramount importance. The primary objective of this research work was to assess the bacteriological profile of Kaptai Lake water. As per this study, the water quality is hazardous for the people of Rangamati as the people there depend on the water of Kaptai lake. After performing all required tests (characterization of the specific bacteria, antibiotic susceptibility), the result of the study is revealed. Bacterial colonies of different morphology and color were observed.

A total of 23 isolates of different organisms were identified based on cultural characteristics from the "BANARUPA" and "FISHARIGHAT" water samples used in this study.

Gram Staining-

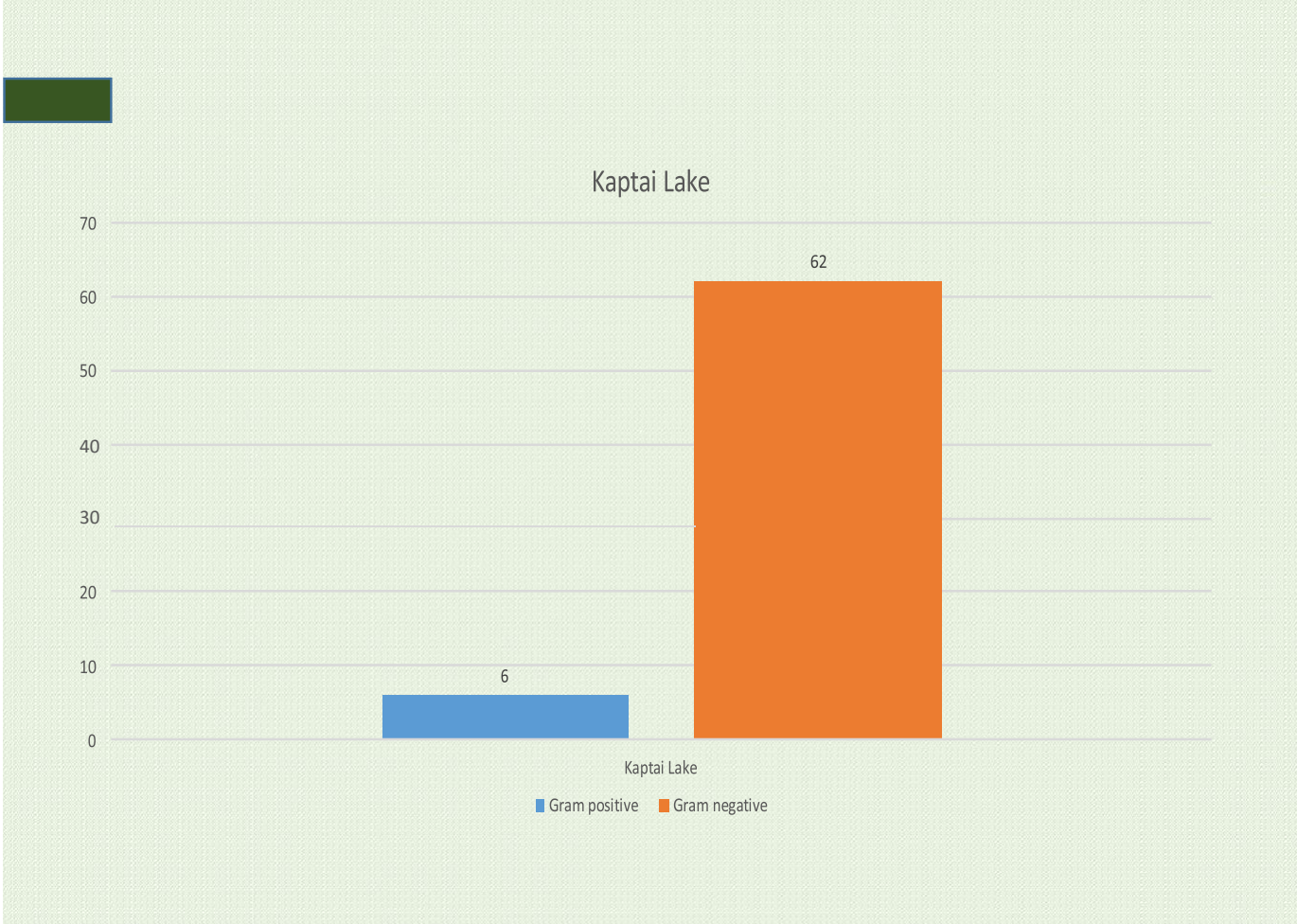
The purpose of this process is to characterize bacteria. It helps to differentiate organisms of the domain bacteria according to the cell wall structure. Gram-positive cells have a thick peptidoglycan layer and stain blue to purple. Gram-negative cells have a thin peptidoglycan layer and stain pink to red.

The Gram stain, the most widely used staining procedure in bacteriology, is a complex and differential staining procedure. Through a series of staining and decolorization steps, organisms in

the Domain of Bacteria are differentiated according to cell wall composition. Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of the cell wall). These stain purples. Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of the wall), and high lipid content. These stain pinks. This staining procedure is not used for Archaea or Eukaryotes as both lack peptidoglycan. The performance of the Gram Stain on any sample requires four basic steps that include applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram's iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstaining with safranin.

If we want to compare the results of both places, the organisms we have got are almost the same. But here one thing should be noted, during the fourth sample, there were floods in Rangamati. As a result, the water of Kaptai lake got diluted and we have got fewer vibrio species. At first, we couldn’t get the vibrio species with our procedure means, we had to reduce the amount of LB where 5 ml of sample would be diluted. After that, we got vibrio species but still that was less than the other samples vibrio species.

From the Banarupa samples, we have found approximately 33 microorganisms. Among these 33 microorganisms, 5 of them are gram positive and 28 of them are gram negative bacteria. So, approximately 15% microorganisms of Banarupa are gram positive and 85% microorganisms are gram negative. Again, from the Fisharighat samples, we have found approximately 35 microorganisms. Among these 35 microorganisms, 1 of them is gram positive and 34 of them are gram negative bacteria. So, approximately 3% microorganisms of Fisharighat are gram positive and 97% microorganisms are gram negative. However, in the whole Kaptai lake combining the Banarupa samples and Fisharighat samples, among 68 microorganisms 6 of them are gram positive and 62 of them are gram negative. So, 9% of the microorganisms are gram positive and 91% of the microorganisms are gram negative.



Figure(3): Quantity of gram positive and negative organisms

Here in the picture blue bar represents Gram positive bacteria and the orange bar represents Gram negative bacteria. Also, here the chart shows that we found 6 gram positive and 62 gram negative organism.

Although, in our research, we observed organisms through 40* and 100* microscopic views. We initially got these results based on the microscopic view. The results will be clearer with further research that will be conducted later.

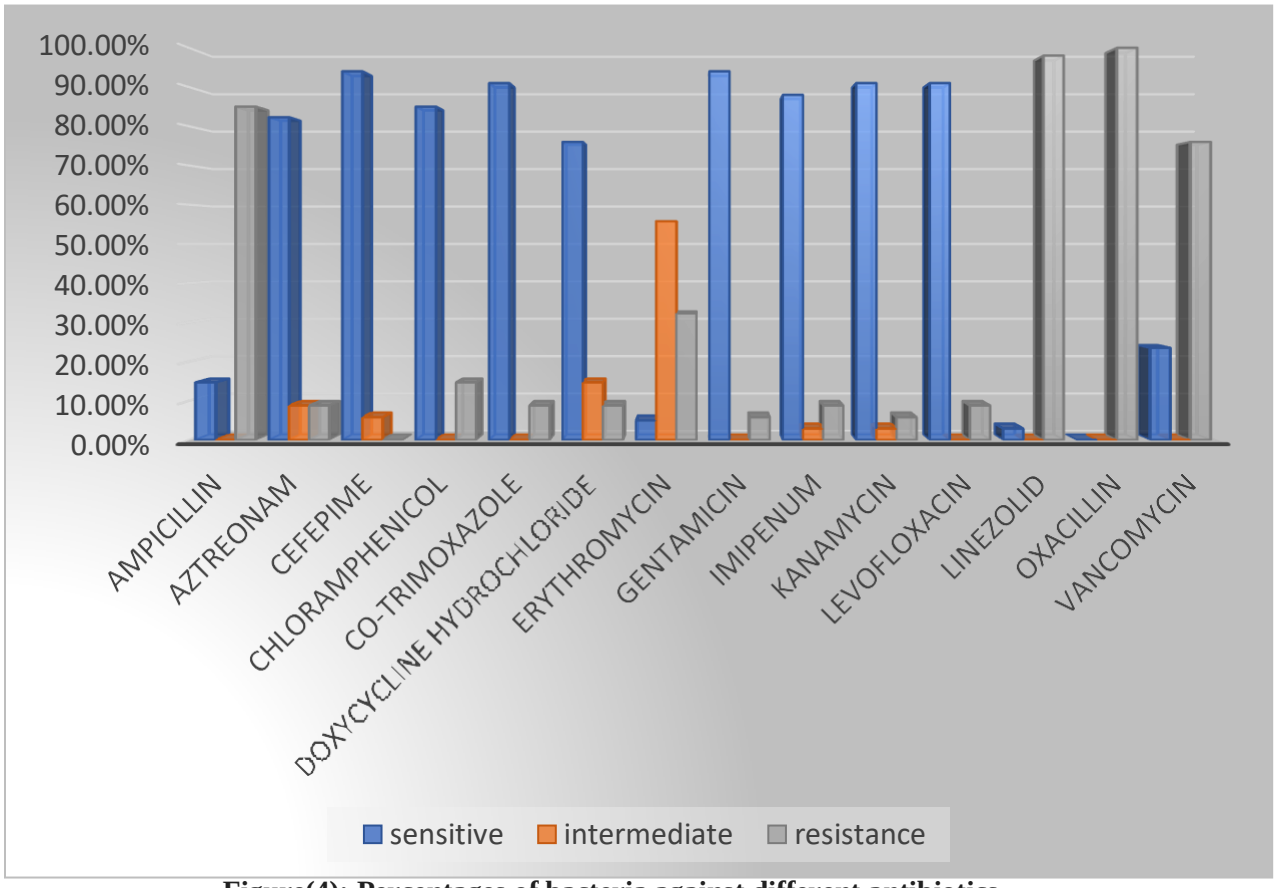
Antibiotic Susceptibility –

Microbiological profile analysis of two different places of Kaptai Lake- “Banarupa” & “Fisharighat”, Rangamati was conducted at BRAC University Biotechnology Lab. The types of bacteria available in the lakes were also assessed. We have got different types of coliforms and

fecal contaminators. Like- vibrio, Enterobacter, Salmonella etc. Antibiotic resistance of these organisms in different wharfs is different. Here is some information on our research work given so that people of Rangamati can be more cautious while taking antibiotics frequently. We have used 14 types of antibiotic discs to conduct the antibiotic susceptibility test. About 61% of total organisms showed susceptibility against antibiotic discs and about 33% of total organism showed antibiotic resistance against antibiotic discs and rest 6% showed intermediate property against the antibiotic discs.

If we briefly discuss the individual disc, there are some noticeable differences between their susceptibility. For Ampicillin 10 mcg, among the total of 68 organisms from 6 samples, organisms showed a noticeable susceptibility against antibiotics like- Aztreonam 30 mcg, Cefepime 30 mcg, Co-Trimoxazole 25 mcg, Imipenum 10 mcg, Gentamycin 10 mcg, Levofloxacin 5 mcg, Chloramphenicol 30 mcg, Kanamycin 30 mcg. Their percentage of susceptibility is respectively- 82%, 94%, 91%, 88%, 94%, 91%, 85%, 91%. Among them, susceptibility against Cefepime30 mcg, Gentamycin 10 mcg, Co-Trimoxazole 25 mcg, Levofloxacin 5 mcg, Kanamycin 30 mcg showed the highest. It means these antibiotics work well against the organisms that we have got. Another antibiotic named Erythromycin 15 mcg, showed less susceptibility than resistance. Its intermediate property is also more noticeable than other antibiotics. 32% is its resistance and 38%is its intermediate result which is the highest among all the antibiotics. The resistance percentage of some antibiotics is not negligible.

An antibiotic named oxacillin showed 100% resistance against it, which is a warning sign for the human being while using this antibiotic. Also, there are 4 more antibiotics that have a noticeable resistance percentage and respectively these are- Vancomycin 5mcg (76%) Linezolid 30 mcg (97%) Erythromycin 15mcg (32%) ampicillin 30 mcg (85%). It means, people of Kaptai Lake should be more cautious while using these antibiotics.



Figure(4): Percentages of bacteria against different antibiotics

Among total organisms, about 61% of total organisms showed susceptibility against antibiotic discs and about 33% of total organism showed antibiotic resistance against antibiotic discs and rest 6% showed intermediate property against the antibiotic discs. Here, the blue bar shows the sensitivity percentage. The grey bar shows the resistance rate and the orange bar indicates the intermediate rate of the organisms.

Conclusion

The main purpose of the experiment was to check the microbial load, antibiotic resistant pattern of the water of Kaptai Lake, as this type of analysis has not been done there yet. In our findings we have found several total coliform and fecal coliform bacteria in the water which is a clear indication of polluted and contaminated water. Got different types of coliforms and fecal contaminators- Escherichia, Vibrio, Shigella, Salmonella, Klebsiella, Enterobacter. We got detect that, susceptibility rate is higher than the water samples of Dhaka city. So, we found the low resistance rate.

Moreover, the findings from the antibiotic resistant pattern will help the doctor to treat diseases more effectively. To reduce the contamination, more awareness is needed. A proper sanitation system should be introduced in the houses near the lake. Moreover, throwing waste, for example, household waste, into the water should be avoided. Kaptai Lake plays an important role for the people of Rangamati. They are highly dependent on the lake for their food source, agriculture, flood control and power generation. Point to conclude that, people around Kaptai Lake should be more cautious while using these antibiotics which are resistance and be more cautious about using water from kaptai resource. As well as, it is their first and foremost duty to save the lake from contamination.

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