Antimicrobial Resistance *E. coli*, *Staphylococcus* and *Salmonella* Hatirjheel Lake and Buriganga River: A Threat to Water Quality and Public Health

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of
BSc in Microbiology

Mathematics and Natural Science

Brac University
March 2024

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing 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 which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

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Ethics Statement

There are no conflicts of interest in the entire research project. There was no use of a human or animal model in this experiment.

Abstract/ Executive Summary

Antimicrobial resistance (AMR) poses a significant threat to global public health, with environmental reservoirs such as lakes and rivers playing a crucial role in its dissemination. This study addresses the prevalence and patterns of AMR in *Escherichia coli*, *Staphylococcus*, and *Salmonella* isolated from Hatirjheel Lake and Buriganga River in Bangladesh.

Water samples were collected from multiple sites along each water body and subjected to microbiological analyses. Isolates of *E. coli*, *Staphylococcus*, and *Salmonella* were identified using standard biochemical and molecular methods. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer Disk Diffusion method.

Antimicrobial resistance among the isolated microorganisms from both water sources is worrying, according to our data. Multiple antibiotics, including widely used medicines like ampicillin, aztreonam, and amoxicillin, were shown to be resistant to *E. coli*. Likewise, isolates of *Staphylococcus* species demonstrated resistance to cefixime, erythromycin, ceftazidime, and linezolid. Antibiotic resistance was detected in *Salmonella* isolates against ampicillin, ciprofloxacin, cefixime, and ceftazidime. The probability of a waterborne infection spreading to humans is increased by the simultaneous presence of microorganisms resistant to multiple antibiotics in both water sources. The discovery of resistance genes such as *tsst* and *mecA* highlights the role that environmental reservoirs play in the dissemination of resistance determinants.

To reduce the emergence of antibiotic resistance in aquatic ecosystems, immediate interventions are needed, such as improved wastewater management, public awareness campaigns, and surveillance of antimicrobial usage. The significance of keeping an eye on antibiotic resistance in environmental contexts and its effects on public health and water quality are highlighted by this study. Multidisciplinary strategies combining microbiologists, environmental scientists, legislators, and public health professionals should be used to tackle antimicrobial resistance.

Keywords: antimicrobial resistance, *E. coli*, *Staphylococcus*, *Salmonella*, Hatirjheel Lake, Buriganga River, water quality, public health, multidrug resistance, environmental reservoirs

Dedication

This thesis is dedicated to our parents

Acknowledgement

First and foremost, we would like to express our thanks to Almighty Allah because He has given us the opportunity and strength to finish this research. We are also thankful for His blessings on our daily life, good health, and a healthy mind. We acknowledge my esteem to Professor A F M Yusuf Haider and Chairperson of the MNS Department.

Our regards, gratitude, indebtedness, and appreciation go to our respected supervisor Dr. Mahbubul Hasan Siddiqee, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and never-ending inspiration throughout the entire period of our research work. We would like to thank and express our deepest gratitude for guiding us in our report writing and providing time-to-time suggestions regarding the setting of experimental designs, interpretation of results, and subsequent directions for the whole work without being impatient. It would have been impossible to submit our report without his cordial help.

We would like to extend our appreciation to the respective Lab officers, also our mentor Mahmudul Hasan and Somnath Gupta.

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Chapter 1

Introduction

Water stands out as the most extraordinary substance. It serves as the medium for various activities, including bathing, fishing, swimming, drinking, and cooking—though typically not all at once. Constituting about two-thirds of our bodies, water is essential for sustaining life. The evolution of life, as we recognize it, would have been impossible without water, and existence would cease in its absence. Droughts led to famines, and floods bring about death and disease. Due to its undeniable significance, water has become the most extensively studied material on Earth. Surprisingly, despite its clear importance, both the general public and scientists working with it daily often possess a limited understanding of this vital substance. Also, water is an indispensable natural resource critical for both human survival and economic advancement. As economies expand, the issue of water scarcity eventually evolves into a global concern. The primary underlying cause of this water deficiency is the excessive utilization of water resources by humans, surpassing the available supply of fresh water (M. A. S. Islam et al., 2021) This situation underscores the inherent conflict between human exploitation of water resources and the natural availability of such resources (Rijsberman, 2006) Waterborne diseases resulting from contaminated water pose a significant challenge for developing communities. This issue stems from insufficient awareness about sanitation, inadequate environmental and water management practices, a lack of education and training, and the overwhelming burden of waste containing harmful microorganisms. This study focuses on assessing the post-contamination levels and identifying the reasons for water contamination after its treatment and collection from the direct water supply source.

Furthermore, the growing global population's heightened demand for food will continue to exert pressure on water resources, exacerbating the problem of water scarcity. Water resources also play a pivotal role in supporting food production (Mueller et al., 2012), and the prudent management of these resources is a fundamental factor in promoting food production (Rosa et al., 2018). To enhance agricultural yields, there is a need for a transition from rain-fed agriculture to irrigated agriculture, which, in turn, will amplify the demand for freshwater (Rosa et al. 2018). However, the widespread adoption of irrigation practices may intensify the strain on water resources, potentially leading to heightened water stress (Elliott et al., 2014). In the face of water scarcity due to limitations in providing sufficient fresh water, effective water

resource management is imperative to address these pressing water-related challenges (Sin et al., n.d.).

Bangladesh is a low-lying riverine country with over 230 major and minor rivers traversing its landscape (Hasan et al., 2019),(Harris et al., 2016). The capital city, Dhaka, is intricately connected through six different rivers. Dhaka is currently undergoing rapid urbanization and industrialization along the riverbanks. This industrial development has led to the concentration of contaminated areas along these industrialized river districts due to the convenience of disposal facilities (Rahman et al., 2020). The contamination of river water in these areas poses significant ecological and human health risks, rendering it unsuitable for purposes such as drinking, fishing, and agriculture. (Rakib et al., 2022) Recent findings indicate a substantial deterioration in the physicochemical characteristics of water and the overall environmental damage level in Dhaka's surrounding rivers, as reflected in water quality indicators (M. A. S. Islam et al., 2021). Among the various industries established in Dhaka district's Savar Upazila, the garment industry stands out as a major contributor, accounting for a significant portion of the country's total export income (approximately 28 billion USD annually) (Sakamoto et al., 2019). Additionally, several industrial operations have emerged in the Hazaribagh region along the Buriganga River, encompassing activities such as dyeing, textiles, batteries, and glass manufacturing. These industrial processes release substantial waste loads and various environmental pollutants into the nearby water bodies of Dhaka City. Furthermore, river water contamination is exacerbated by the discharge of agricultural runoff and urban municipal wastewater. Of particular concern are heavy metals like Cadmium, Mercury, Lead, Copper, and Zinc, which are recognized as significant marine pollutants due to their toxicity, presence in food chains, and their ability to persist in the environment over extended periods (Aprile & De Bellis, 2020).

Globally, approximately 24% of the overall disease burden and 23% of total deaths are ascribed to environmental factors. (WHO,2006) In many low- and middle-income countries, inadequate sanitation and improper management of fecal sludge pose a significant threat to public health by introducing fecal contamination into the environment (Detrimental Effects of Heavy Metals in Soil, Plants, and Aquatic Ecosystems and in Humans, 2018), (America Bolivia Santa Cruz, n.d.). Dhaka, the capital of Bangladesh, stands out as one of the world's most densely populated cities. (UN, 2014) Within Dhaka's neighborhoods, environmental contamination resulting from fecal matter is a prevalent issue, driven by various factors that encompass substandard sanitation and sewage systems, rapid and unplanned urbanization, recurrent instances of

flooding (Yeasmin et al., 2017), (Croes et al., 2009) and ineffective management of solid waste (Hossain et al., 2018), (Yeasmin et al., 2017). Extensive research has been conducted in urban Bangladesh, including Dhaka, to investigate the direct ingestion of fecal contamination through polluted drinking water. This research, conducted at both household and community levels, has utilized fecal indicator bacteria as a measurement tool (Sakamoto et al., 2019), (Al et al., n.d.), (Sirajul Islam et al., 2007). Furthermore, adverse health outcomes such as diarrhea, environmental enteric dysfunction, and stunting have been associated with various exposure pathways in urban Bangladesh, including contaminated soil (Harris et al., 2016), market produce (Harris et al., 2018), and street food (M. A. Islam et al., 2010), (Wang et al., 2017). The management of medical waste is a pressing issue in Bangladesh, and the unprocessed release of such waste into the environment through hospital drainage systems presents a significant risk to public health (M. A. S. Islam et al., 2021). Limited research has been carried out in Dhaka city to evaluate the possible consequences of this situation (Rahman et al., 2020), (Yeasmin et al., 2017).

Hatirjheel plays a crucial role in western Dhaka City by providing storage for stormwater runoff and serving as a drainage pathway for an area spanning more than 23 square kilometers. (Atauzzaman & Ali, 2022). This area primarily consists of densely populated residential and commercial zones. Currently, stormwater runoff from this extensive region, which includes high-density urban developments, is channeled into Hatirjheel through 11 storm sewer outfalls. Additionally, some runoff enters Hatirjheel via Gulshan and Banani lakes. The restoration efforts undertaken in Hatirjheel involved several key activities, including the excavation of low-lying areas to remove accumulated sludge, the construction of 11 Combined Sewer Overflow (CSO) structures positioned at the outfall locations of storm sewers (referred to as CSO-1 through CSO-11), the installation of diversion sewers along Hatirjheel's periphery (connected to the CSO structures), and the implementation of a flow control system at Hatirjheel's downstream end (Al-Mizan et al., 2020), (Atauzzaman & Ali, 2022). Hatirjheel's average bottom level is established at 0.0 meters according to the Public Works Department (PWD) datum.

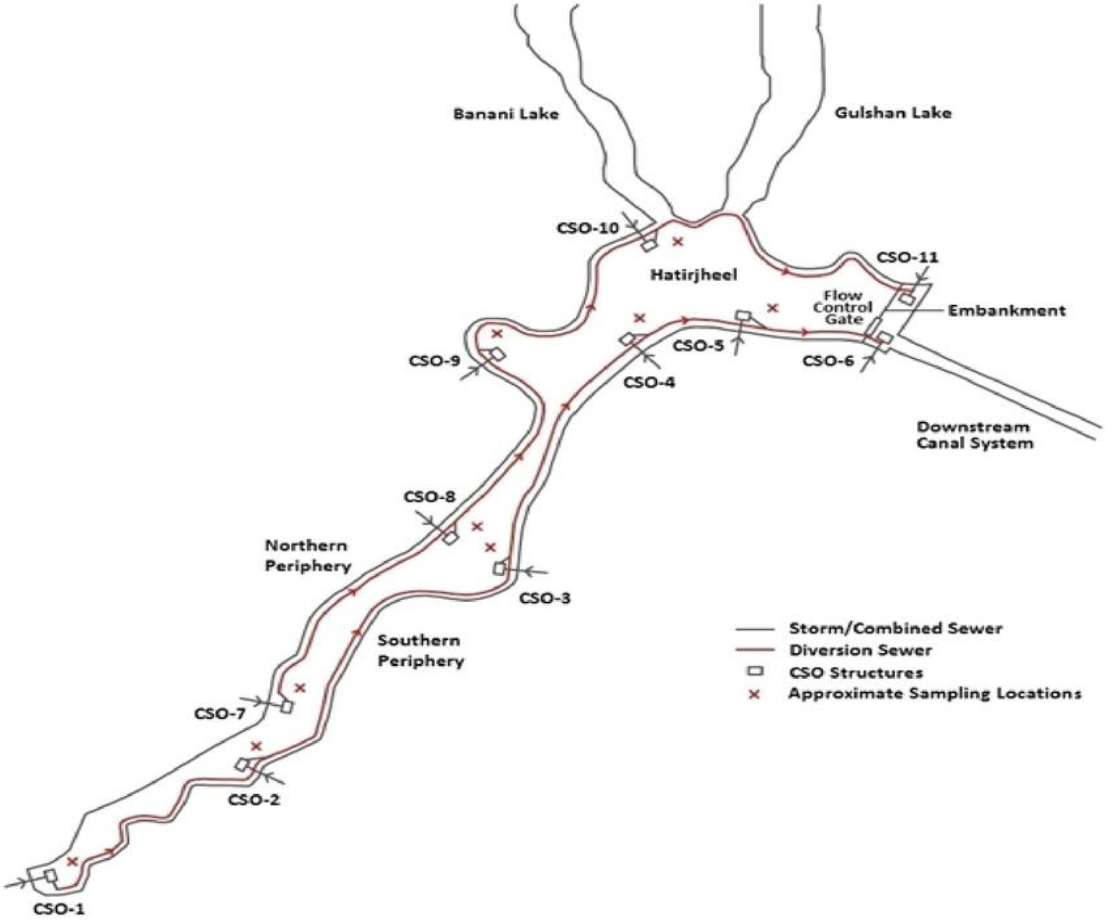

Figure 1 Schematic representation of Hatirjheel Lake showing the CSO structures, diversion sewers, and the approximate sampling location

The flow control gate located at the eastern end of Hatirjheel allows it to function as a storm retention pond. The water level within Hatirjheel can be regulated, typically maintained at around +3.5 meters (PWD datum), and can be adjusted within the range of +2.5 meters to +5.00 meters by raising or lowering the flow control gates. During the monsoon season, the canal system downstream of Hatirjheel connects to Dhaka City's peripheral river system. In response to increased upstream flows and precipitation, the water level in this canal-river system may rise. To prevent water from flowing back into Hatirjheel during such conditions, the flow control gates are raised. Consequently, Hatirjheel acts as a retention pond, storing excess water from CSO structures and surface runoff. Depending on weather conditions, the flow control gates may remain raised for varying durations, ranging from weeks to a few months, particularly during prolonged periods of flooding in the monsoon season each year (Atauzzaman & Ali, 2022).

The **Buriganga River** system is located in the southeastern part of Bangladesh's North Central Zone, in close proximity to the Padma (Ganges) and Upper Meghna rivers. It is actually a

tributary of the Dhaleswari River, which is the second largest river in the North Central Region, ranking just after the Old Brahmaputra River. Contrary to previous beliefs, the Buriganga River is not isolated from a hydrological standpoint.In the vicinity of Dhaka, the Buriganga River boasts an average width exceeding 500 meters and extends for nearly 27 kilometers.(Uddin MG et al., 2016)The primary source of water for the Buriganga River is the Turag River, which gathers rainfall runoff from the surrounding area and receives overflow from the left bank of the Jamuna River. The Turag River, originating in the nearby Gazipur district, spans approximately 63 kilometers in length (Hossain et al., 2018) which is connected to the industrial waste water system.

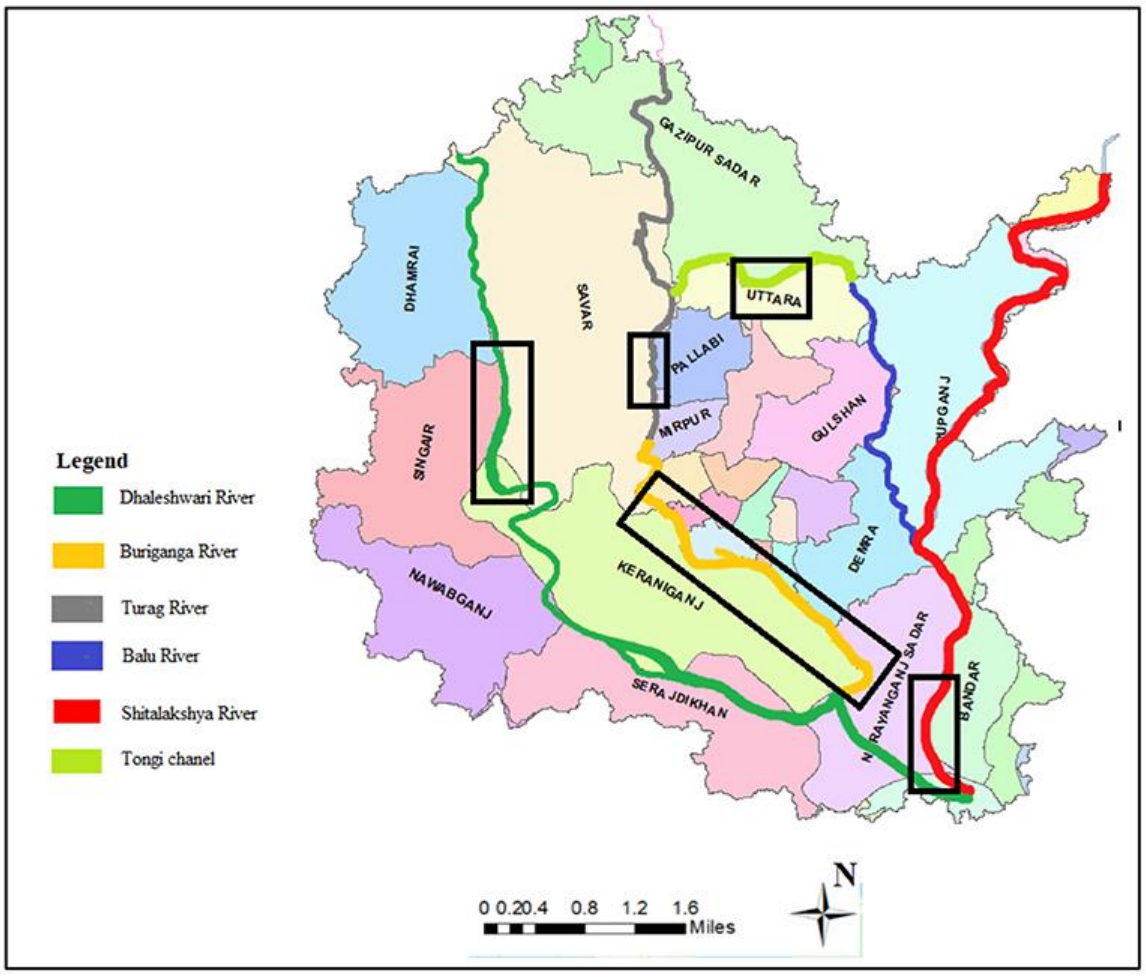

Figure 2 GIS map showing peripheral rivers around Dhaka watershed

Leather production involves various chemical substances such as chromium sulfate, tannins, bactericides, and ammonia salt (Al-Mizan et al., 2020). These chemicals, including heavy metals like Cadmium (Cd), Lead (Pb), and Zinc (Zn), can enter ecosystems and contribute to non-biodegradable contaminants. Consequently, these heavy metals persist within the ecological system, posing risks to humans and other animals (Ayangbenro & Babalola, 2017).

They enter water bodies through anthropogenic sources, primarily via untreated or partially treated waste discharge, and can also infiltrate agricultural land through fertilizers and pesticides (Martin, 2000). The accumulation of metals in sediments and water facilitates their entry into the food chain through water and vegetation (Bhuyan et al., 2017). In aquatic environments, heavy metals can impede the generation of reactive oxygen species (ROS), adversely affecting fish and other aquatic organisms (Rakib et al., 2022). The enduring presence of these heavy metals in the ecosystem poses challenges due to their non-degradability (Rowe-Magnus et al., 2003). Furthermore, their distribution and accumulation in aquatic ecosystems raise significant concerns due to their toxic and pervasive nature, leading to bioaccumulation in living organisms, particularly at higher trophic levels (Atauzzaman & Ali, 2022). Consequently, there is a heightened risk of river water contamination in Bangladesh's capital, potentially resulting in severe consequences for riverine ecology and nearby residents due to health issues arising from direct consumption, ingestion, and dermal exposure (Rahman et al., 2020).

Environmental pollution poses significant microbiological health risks worldwide. In Bangladesh, the majority of major water bodies, especially those in urban or semi-urban areas, are grappling with severe pollution. Addressing this issue is crucial, as safeguarding surface water in and around cities can alleviate the demand for groundwater among urban residents through the proper utilization of lakes and ponds.This research focused on assessing the microbial condition of Hatirjheel water in Dhaka City. Microbial analysis aimed to identify the presence of bacteria and other pathogens. The study findings unveiled potential health hazards associated with Hatirjheel water, suggesting it may not be safe for human consumption. Moreover, the analysis indicated the vulnerability of this water source to waterborne diseases such as diarrhea, dysentery, typhoid fever, shigellosis, salmonellosis, parasitic worm infections, hemolytic uremic syndrome, etc. (Ribeiro, 2021). The compromised water quality points to contamination, primarily from domestic and industrial waste, as well as other anthropogenic activities affecting the lake. Controlling pollution is imperative for ensuring the proper use of this water for various household purposes.

The assessment of water microbial quality relies on the identification of specific microorganisms known as **indicator microorganisms.** (WHO, 2006) This approach is adopted due to the challenges associated with counting all microorganisms, particularly pathogens, in water samples. Key characteristics considered for these microorganisms include being part of

the normal flora of warm-blooded animals' gastrointestinal tracts, non-pathogenic nature, higher abundance compared to pathogenic microbes, absence in uncontaminated samples with pathogens, resistance to adverse environmental conditions and disinfectants similar to pathogenic microorganisms, inability to reproduce naturally in the environment, and traceability and detection through simple and cost-effective methods. Coliforms were initially chosen as the first group of microorganisms to serve as indicators. These bacteria, which are bacilliform, non-spore-forming Gram-negative organisms, exhibit facultative aerobic and anaerobic characteristics and ferment lactose with gas production at 35°C. Coliform bacteria such as Escherichia coli, Citrobacter, Enterobacter, and Klebsiella are found in the digestive tracts of humans, warm-blooded animals, plants, soil, and water. Fecal or thermotolerant coliforms, a subset of coliforms, can produce blue colonies at 44.5°C in 24 hours in EC broth gas or M-FC medium. *Salmonella*, Shigella, and Escherichia coli are prominent bacterial species within the Enterobacteriaceae family, playing a significant role in water-related disease outbreaks. *Salmonella* pathogenesis primarily stems from fecal contamination originating from humans, livestock, and wild animals entering water supplies, as well as contaminating various foods like milk and meat. Control measures involve safeguarding raw water supplies from animal and human wastewater and implementing proper treatment and monitoring of drinking water during distribution (Selvam et al., 2022).

Staphylococcus aureus is a type of gram-positive bacterium that commonly inhabits various regions of a healthy adult's body, increasing the risk of various infections in both community and hospital settings.This bacterium, commonly referred to as "Staph," has the potential to induce food poisoning. It is widely distributed in the environment, present in soil, water, air, and on various everyday objects and surfaces. Staph can thrive in both humans and animals. *Staphylococcus* aureus is detected in food items and can generate toxins, specifically enterotoxins, which may resist destruction through cooking, though the bacterium itself can be eliminated by heat. These toxins can lead to symptoms such as nausea, stomach cramps, vomiting, and diarrhea. In severe cases, they may result in dehydration, headache, muscle cramps, and temporary alterations in blood pressure and heart rate. The illness is typically intense but typically resolves within a few hours to a day. The toxins exhibit rapid action, manifesting symptoms within 1 to 7 hours after consuming contaminated food (Ayangbenro & Babalola, 2017). Staphylococcal species, characterized as Gram-positive, non-motile, catalase-positive, small, spherical bacteria (cocci), are observed under microscopic examination in pairs,

short chains, or clustered in grape-like formations. Staphylococci are pervasive and challenging to eliminate from the environment. The genus *Staphylococcus* comprises 32 potentially foodborne species and subspecies due to environmental, human, and animal contamination. Several *staphylococcal* species, encompassing both coagulase-negative and coagulase-positive strains, possess the capability to produce highly heat-stable enterotoxins, leading to gastroenteritis in humans. *Staphylococcus aureus* is the primary agent associated with staphylococcal food poisoning and is a versatile human pathogen, causing conditions such as toxic shock syndrome, pneumonia, postoperative wound infection, and nosocomial bacteremia. *S. aureus* releases various extracellular products, many of which act as virulence factors. *Staphylococcal enterotoxins* can function as superantigens, stimulating an elevated percentage of T-cells. S. aureus is among the most resilient non-spore-forming human pathogens, capable of surviving for extended periods in a dry state. *Staphylococci* are mesophilic, with S. aureus exhibiting a growth range from 7°C to 47.8°C, with an optimum temperature for growth at 35°C. The growth pH range is between 4.5 and 9.3, with an optimum between 7.0 and 7.5. *Staphylococci* display atypical growth characteristics, thriving at low water activity levels, even as low as 0.83 under ideal conditions. The optimum growth of *S. aureus* occurs at a water activity level greater than 0.99 (Fey & Olson, 2010). In general, S. aureus strains demonstrate high tolerance to salts and sugars. *S. aureus* is recognized as a significant causative agent of infections acquired in hospitals and communities, which complicates disease management efforts (Sin et al., n.d.). This bacterium has the ability to attach to surfaces of medical devices and host tissues, leading to the formation of biofilms (Naimi et al., n.d.). The capacity to form biofilms contributes to the chronic nature of infections caused by S. aureus, as it helps the bacterium evade the host's immune system and resist antimicrobial drugs (Sin et al., n.d.). Infections resulting from biofilm-producing organisms are often chronic and predominantly occur in hospital settings, with S. aureus being a major contributor to biofilm-associated infections (Azeredo et al., 2017). Studies have shown that these infections have led to increased global healthcare costs, including higher hospital bills and prolonged hospital stays (Tong et al., 2015).The process of biofilm formation involves several steps, including initial attachment to surfaces, the accumulation of bacterial populations, the maturation of complex biofilm layers, and eventual dispersal in response to environmental changes (Croes et al., 2009), (Zmantar et al., 2010). S. aureus can also form biofilms on host surfaces like heart valves, bones, cartilage, and medical devices such as catheters and orthopedic devices (Archer et al., 2011). The bacterium adheres firmly to these surfaces either by directly interacting with the device's polymer surface or by binding to human matrix proteins that have covered the device.

Consequently, *S. aureus* is considered a significant pathogen involved in foreign-body infections (Azeredo et al., 2017), (Kiedrowski et al., 2011). Moreover, *S. aureus* has the capability to form biofilms on inanimate objects such as pipes or any foreign materials (Fey & Olson, 2010). In addition to biofilm formation, *S. aureus* employs a wide range of virulence factors to evade the host's defense mechanisms. These factors include extracellular toxins like hemolysin and leukotoxins, enzymes such as coagulases and proteases, and surface proteins like clumping factor and adhesins. The combination of these virulence factors and the ability to form biofilms presents challenges in treating S. aureus infections associated with biofilms (Paharik & Horswill, 2016). Clinical manifestations of *S. aureus* infections can vary from mild superficial skin infections to severe, life-threatening systemic conditions that involve the production of toxins (Sin et al., n.d.). Infections like *S. aureus* bacteremia (SAB) is widely reported, and cases of pneumonia, infective endocarditis resulting from S. aureus infections, and drug-resistant forms like Methicillin-Resistant *Staphylococcus aureus* (MRSA) have been observed in both community and hospital settings (Archer et al., 2011).

Escherichia coli, commonly known as *E. coli*, is a highly adaptable bacterium with a widespread presence in various water sources, including streams, rivers, wells, and other aquatic environments. This Gram-negative, rod-shaped bacterium is typically found in the lower intestine of warm-blooded organisms, as well as in water, soil, and vegetation. Belonging to the family Enterobacteriaceae, informally referred to as "coliforms," *E. coli* shares its bacterial lineage with notable pathogens like *Salmonella, Yersinia pestis, Klebsiella, Shigella,* and other related enteric bacteria. While many strains of *E. coli* are harmless and contribute to the normal flora of the gut, certain serotypes have the potential to cause severe food poisoning in humans. Occasionally, these pathogenic strains prompt product recalls due to food contamination. The benign strains of *E. coli* provide benefits to their hosts by producing vitamin K2 and preventing the establishment of pathogenic bacteria in the intestine. *E. coli* cells, although primarily residing in the body, can survive outside for a limited time, making them valuable indicators for testing environmental samples for fecal contamination. The main route through which pathogenic strains causes disease is the fecal-oral route, making *E. coli* a leading cause of food and waterborne human diarrhea globally, particularly in developing countries. Additionally, it is a prominent pathogen responsible for urinary tract infections. Harmful types of *E. coli* are categorized into groups such as Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enterohemorrhagic (EHEC), and Enteroinvasive (EIEC) (Gerba, 2000).

Salmonella is a mobile, non-spore-forming, Gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae and the tribe *Salmonella*e. Non-motile variations include S. Gallinarum and S. Pullorum. The genus *Salmonella* comprises two species that can cause human illness: S. enterica and S. bongori. *Salmonella* enterica, of primary public health concern, has six subspecies: S. enterica subsp. enterica (I), S. enterica subsp. salamae (II), S. enterica subsp. arizonae (IIIa), S. enterica subsp. diarizonae (IIIb), S. enterica subsp. houtenae (IV), and S. enterica subsp. indica (VI). *Salmonella* is further categorized into serotypes based on the Kaufmann-White typing scheme, introduced in 1934, which distinguishes strains by their surface and flagella antigenic properties. *Salmonella* species are commonly identified by their serotype names. For instance, *Salmonella* enterica subsp. enterica encompasses various serotypes, including *S. Enteritidis* and *S. Typhimurium*, prevalent in the U.S. Kaufmann initially proposed 44 serotypes, but by 2007, the number had expanded to 2,579 (Sakamoto et al., 2019). *Salmonella* induces two types of illnesses:

Gastrointestinal illness, characterized by nausea, vomiting, diarrhea, cramps, and fever, typically lasting a few days and resolving within a week. In healthy individuals, symptoms often subside on their own, though long-term arthritis may develop (Hasan et al., 2019).

Typhoidal illness results in high fever, diarrhea or constipation, aches, headache, lethargy, and sometimes a rash. This is a severe condition, with up to 10% mortality in untreated cases. Various foods, including meats, eggs, fruits, vegetables, and even dry items like spices and raw tree nuts, can become contaminated with the gastrointestinal strain. Typhoidal illness is usually associated with drinking water contaminated with sewage or crops irrigated with sewage-contaminated water. Pets such as turtles, reptiles, and chicks can carry *Salmonella*, which can be transmitted to anything they come in contact with, including food or the owner's face through unwashed hands.

Chapter2

Method and Materials

2.1. Study Duration and Collection of water sample

The study has been performed in the year 2023, January to 2024, March. Water samples have been collected using sterile, clean and autoclaved bottles from five different locations of Hatirjheel waterbody and twenty different locations of River Buriganga. Sample collection was carried out under aseptic conditions. In order to avoid any changes of the microbial flora present in the samples during transportation, the bottles have been stored in an ice pack. One hundred (100) water samples in total were collected. The Fall (October-January), Summer (February-May) and Monsoon (June-September) seasons were chosen for the sampling.

2.2. Sample Preparation

The collected samples were then prepared for further analysis. To begin with, 100 ml of water sample was separated from each of the sample bottles for membrane filtration. After that, this filter paper was placed on MFC agar (Fecal Coliform Agar base). This agar was used in order to isolate and identify coliform and fecal coliform present in the sample. The remaining samples were prepared for the spread plate technique on suitable selective media. Firstly, the samples were diluted using serial dilution. In summer season, dilution factors were 10^3 and 10^4. In the winter season the dilution factor was 10^2 and 10^3. For identification and isolation of the organisms, three different agar media have been used which includes Eosin methylene Blue Agar (EMB), Mannitol Salt Agar (MSA) and, *Salmonella* Shigella Agar (SS). EMB was used in order to isolate *E. coli* in the sample, MSA was used to isolate *Staphylococcus Spp*. Finally, SS was used to isolate *Salmonella spp*.

2.3. Isolation and identification of Organism

Culturing of water samples

The spread plate technique has been performed in the study for isolating and counting the viable microorganisms present in the water sample by means of spreading 100µl of samples in the solidified selective media plates depending on the size of the plate. The culture plates were

incubated for 48 hours in 37°C. After 24 hours of incubation, colony forming unit (CFU) were counted and listed on the datasheet. After 48 hours new colonies were counted again. Finally, the plates were discarded, considering the risk factors.

Subculture of organisms

The streak plate technique has been conducted in the study in order to isolate the single colonies from the cultures of microorganisms. To precede the streaking technique from EMB green sheen colored colony, from MSA yellow colored and finally, black colored colony has been taken from SS agar culture plates. The method has been performed on nutrient agar media. After that, the nutrient agar plates were kept on the incubator at 37°C for 18 to 24 hours. After 24 hours, the results have been analyzed for further procedure.

Stock of Isolates

Afterwards, from the incubated NA plates, discrete colonies were collected for stock. Here, two types of media were used for stock: T1N1 (solid) and NB (liquid). For T_1N_1, a discrete colony was stabbed in the T1N1 with the help of a sterile needle and kept in an incubator for 24 hours at 37°C. After that, 200 µl paraffine oil was added to the T1N1, sealed with parafilm tape and stored at room temperature. Furthermore, for NB, one loop full of discrete colonies was inoculated into the NB containing microcentrifuge tubes with the help of a sterile loop and kept in an incubator for 24 hr 37°C. After that, 200µl glycerol oil was added and sealed with parafilm tape and finally stored in a -20°C fridge.

2.4. Biochemical characterization:

Antibiotic susceptibility test of isolates:

The antibiotic susceptibility test (AST) has been conducted using the disk diffusion technique in order to determine if a particular antibiotic would be effective enough to inhibit bacterial growth or not. To perform this technique, single colonies from the subcultures have been taken, inoculated into 900 ml of saline water and mixed well through the help of a vortex machine. Mueller-Hinton agar was used for the antibiotic susceptibility test. The mixture has been lawned over the surface of MHA agar plates through sterile and autoclaved cotton swabs. After that, the antibiotic discs were firmly placed on the lawned surface of the sterile MHA plates. The antibiotic disks include Erythromycin, Amoxicillin, Tetracycline, Norfloxacin,

Gentamicin, Imepenum, Ampicillin, Ciprofloxacin, Kanamycin, Ceftriaxone, Clindamycin, Methicillin, Chloramphenicol, Cefoxitin, Ceftazidine, Amakicine, Cefepime, Azithromicin, Metronidazole and Linezolid. Finally, the diameters of the zones of inhibition were counted and compared after the plates were incubated for 24 hours at 37°C.

2.5. Molecular characterization of isolates:

DNA extraction

In order to separate this genetic material from the rest of the cell as well as to run the PCR, DNA extraction has been conducted in the study. To begin with, loopful colonies from the subcultures have been taken and inoculated into 400µl TE buffer containing microcentrifuge tubes. Vortex has been done to mix them well. After that, the tubes were centrifuged at 13000 rpm for 10 minutes. The supernatants were discarded and a 400µl TE buffer was added again to the remaining pellets. The mixtures were then placed in the water bath at 100℃ for 10 minutes. Following another centrifugation at 3000 rpm for 10 minutes, the supernatant was transferred to another sterilized new containing microcentrifuge tube. Finally, the tube was kept in storage at -20℃.

Polymerase chain reaction (PCR) Amplification of the genes:

As a concluding remark, Polymerase Chain Reaction (PCR) has been conducted. The PCR process involves a series of temperature-dependent reactions that selectively replicate and amplify a targeted DNA segment. In this study, PCR has been performed for methicillin resistant genes (*mecA*) and toxic shock syndrome toxin gene (TSST) genes. Following the procedure, 10µL Forward primer has been added with 90µL Nucleus free water. A 10µL Reverse primer has also been added with another 90µL Nucleus free water. The mixture has been vortexed and applied short spin. Furthermore, based on the calculation and total number of DNA chosen, forward primer, reverse primer, and nucleus free water all have been mixed together with the Master mix, vortexed and short spin has been applied. On the final step, 11µL of Master mix and 2µL of DNA have been mixed together. Finally, the PCR has been conducted considering the following condition.

Table 1 Primer list

Gene	Primers	PCR product (bp)	Sequence (5'-3')	Reference
mecA	mecA P4 mecA P7	162bp	5' TCCAGATTACAACTTCACCAGG 3' 5'CCACTTCATATCTTGTAACG 3'	(Igbinosa et al., 2016)
tsst	TSST	326bp	5' ACCCCTGTTCCCTTATCATC 3' 5' TTTTCAGTATTTGTAACGCC 3'	(Esmailkhani et al., 2018)

Gel electrophoresis

For agarose gel electrophoresis 6µl of the PCR amplicon was loaded with dye in 2% agarose gel containing ethidium bromide followed by electrophoresis in 1X TAE buffer at 80V for 1 h and observed under UV transillumination. A 100 bp ladder was used to determine the size of the PCR product for methicillin resistant genes (mecA) and toxic shock syndrome toxin gene (TSST) genes.

Chapter3

Results

3.1: Seasonal variation of colonies:

Firstly, the sampling was done from January 2023 to January 2024. Three seasons are to be considered for this project which are Fall, Summer, and Monsoon. Where October to January is considered Winter, February to May is considered Summer, and June to September is considered Monsoon season. In between this timeline, there were 107 samples collected from both Buriganga and Hatirjheel. 60 samples were taken from throughout Hatirjheel and 40 samples were collected from all over Buriganga. Then, the spread plate method was followed to count colonies on selective media. Next, the 24 hours and 48 hours of colony count were taken from the selective media plates.

Staphylococcus:

Now, the average colony count for the Summer season from Hatirjheel samples was 239.2 cfu/ml from dilution 10^4. Next, For the Monsoon season, the average colony count was 193.2 cfu/ml. Finally, for the Fall season, the average colony count was 166.452 cfu/ml

Now, from Buriganga, the colony count during Summer was 140 cfu/ml, and during monsoon, the colony count was 150.2 cfu/ml. Here is the graph of seasonal variation of the *Staphylococcus spp* colonies both from Hatirjheel and Buriganga.

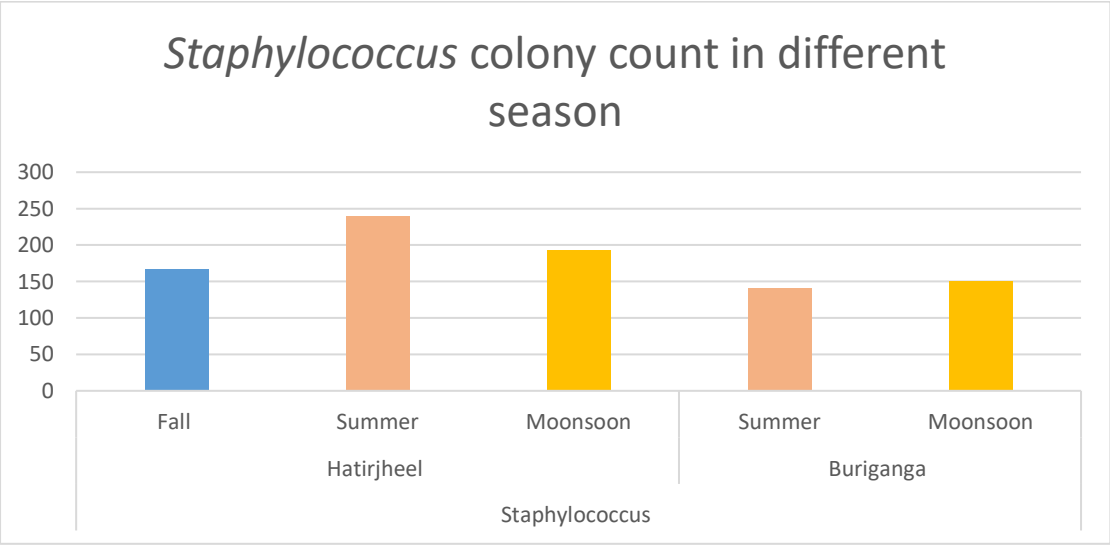

Figure 3 Seasonal variation of Staphylococcus spp. *colonies from Hatirjheel and Buriganga*

Salmonella:

The average colony count from Hatirjheel for *E.coli*, coliform and fecal coliform in the summer season was 314.6 cfu/ml. Now, for the monsoon season: 26.8 cfu/ml. Fall season: 26.436 cfu/ml.

Now, from Buriganga:

The average colony for *Salmonella* and *Shigella* in the Summer season was: 37.092 cfu/ml . Now, for the monsoon season: 0.2108 cfu/ml. Here is the graph of seasonal variation of the *Salmonella* sp. colonies both from Hatirjheel and Buriganga.

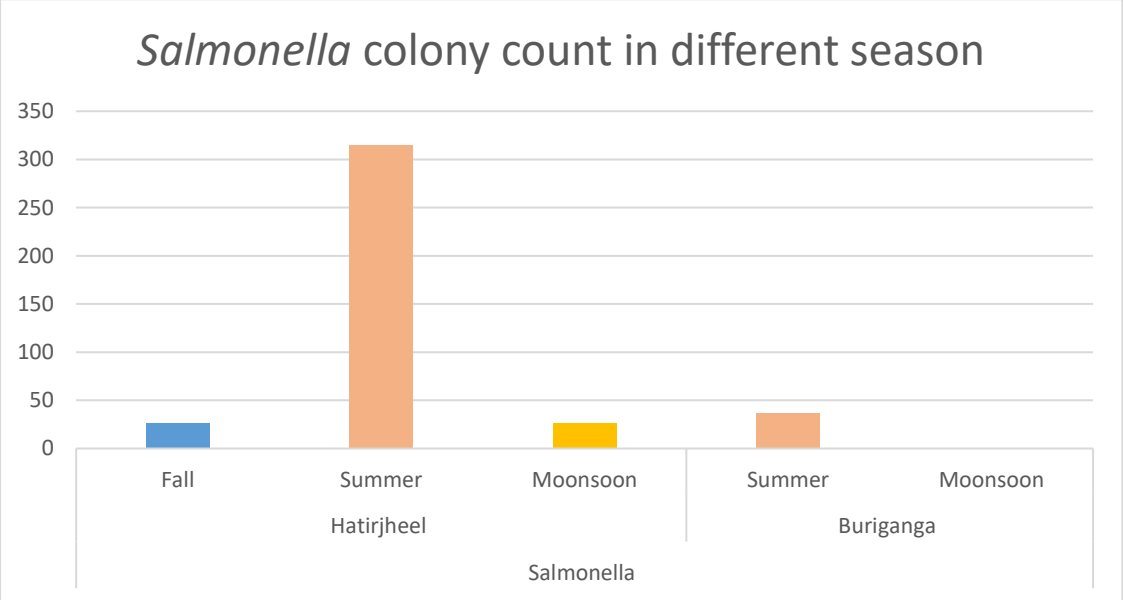

Figure 4 Seasonal variation of Salmonella sp. *colonies from Hatirjheel and Buriganga*

Escherichia coli/ Coliform/ Fecal Coliform:

The average colony count from Hatirjheel for *E. coli*, Coliform, and Fecal coliform in the Summer season was: 694.12 cfu/ml. Now, for the monsoon season: 114.4 cfu/ml. Fall season: 636 cfu/ml. Now, from Buriganga: The average colony for *E. coli*, Coliform, and Fecal coliform in the Summer season was: 45.264 cfu/ml.Now, for the monsoon season: 1042.8 cfu/ml. Here is the graph of seasonal variation of the *E. coli, Coliform, Fecal Coliform* colonies both from Hatirjheel and Buriganga.

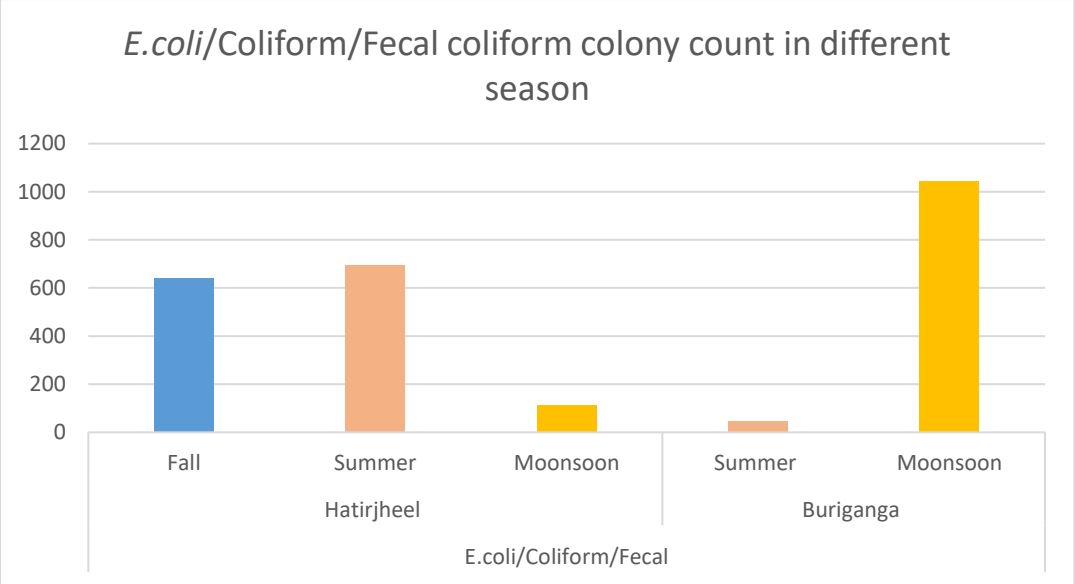

Figure 5 Seasonal variation of E. coli sp. *colonies from Hatirjheel and Buriganga*

3.2 Antibiogram:

A total of 63 *Staphylococcus* isolates from Hatirjheel and 40 isolates from Buriganga were performed for an antibiogram test against 11 antibiotics. The zone of inhibition was measured and it was then compared to the standard CLSI (Clinical & Laboratory Standards Institute) guideline to determine if they are resistant or sensitive. Among which we have compared the data depending on the seasonal variation.

Staphylococcus:

Hatirjheel/Fall:

Among the samples, isolates were 100% sensitive to Chloramphenicol. 45% were resistant to Erythromycin and 55% were sensitive to Erythromycin. 30% isolates were resistant to Clindamycin and 70%were sensitive. 0% isolates were sensitive to Cefixime and 100% were

resistant.

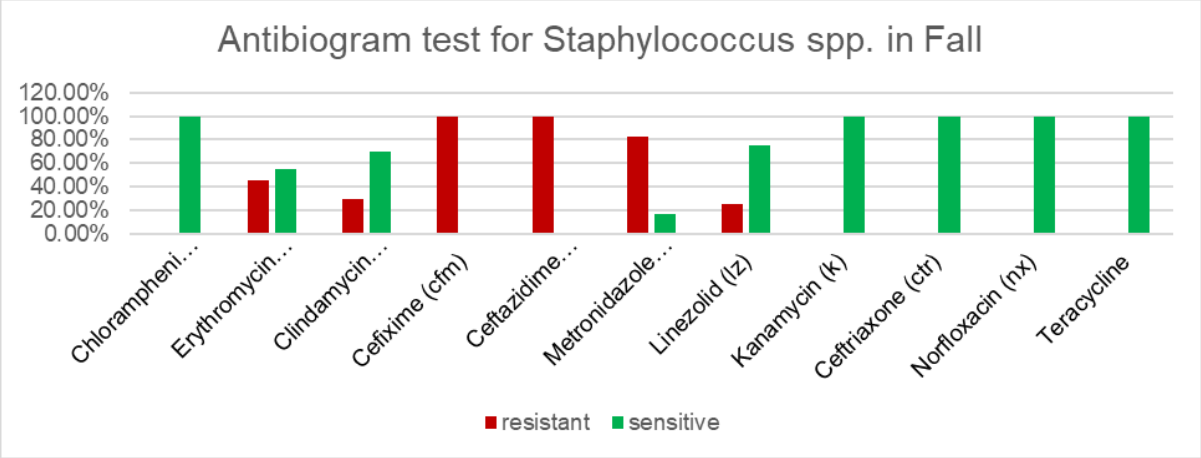

Figure 6 AST results of Staphylococcus sp. *From Hatirjheel during Fall season*

In the case of Ceftazidime 100% were resistant and 0%were sensitive. Next, Metronidazole was 83% resistant and 17% sensitive. About Linezolid, 25% was resistant and 75% was sensitive. Kanamycin was 100% sensitive. Cetriaxon and Chloramphenicol were 100% sensitive. 0% isolates were resistant to Norfloxacin and 100% were sensitive. Finally, Tetracycline was 0% sensitive and 100% resistant.

Hatirjheel/Summer:

During the summer season, Chloramphenicol 0% were resistant and 100%were sensitive. 46% of the isolates were resistant to Erythromycin and 54% were sensitive to Erythromycin. Whereas 20% isolates were resistant to Clindamycin and 80%were sensitive. 100% isolates were resistant to Cefixime and 0%were sensitive. Ceftazidime was 100% resistant and 0% sensitive.

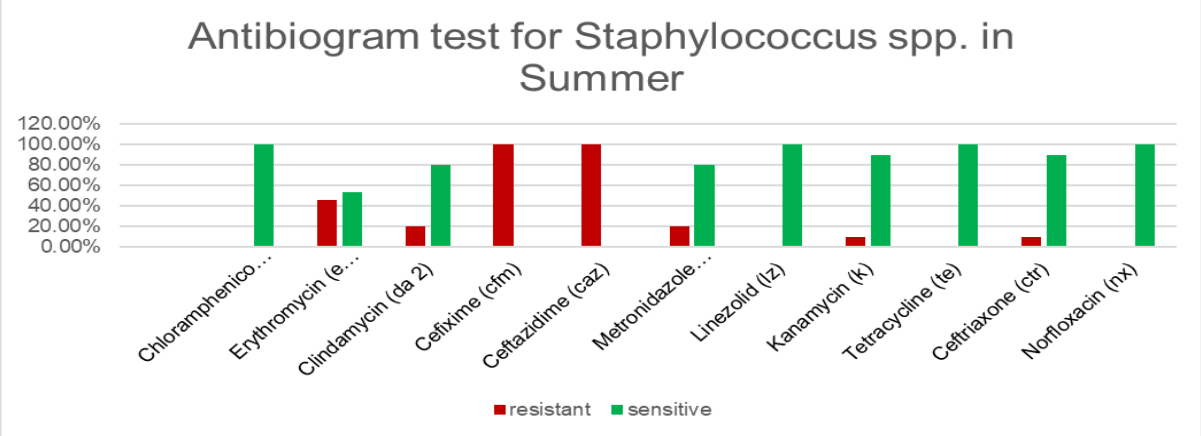

Figure 7 AST results of Staphylococcus sp. *From Hatirjheel during Summer season*

In the case of Metronidazole, 20% isolates were resistant and 80% sensitive. About Linezolid, 0% was resistant and 100% was sensitive. Kanamycin was 90% sensitive and 10% resistant. In the case of Tetracycline, 100% of the isolates were sensitive. 10% isolates were resistant and 90% sensitive to Ceftriaxone. Finally, 0% isolates were resistant to Norfloxacin and 100% were sensitive.

Hatirjheel/ Monsoon:

Among the samples, 0% isolates were resistant and 100%were sensitive to Chloramphenicol. In the case of Erythromycin, 50% were resistant and 50% were sensitive. 0% isolates were resistant to Clindamycin and 100%were sensitive. Next, 100% isolates were resistant to Cefixime and 0%were sensitive.

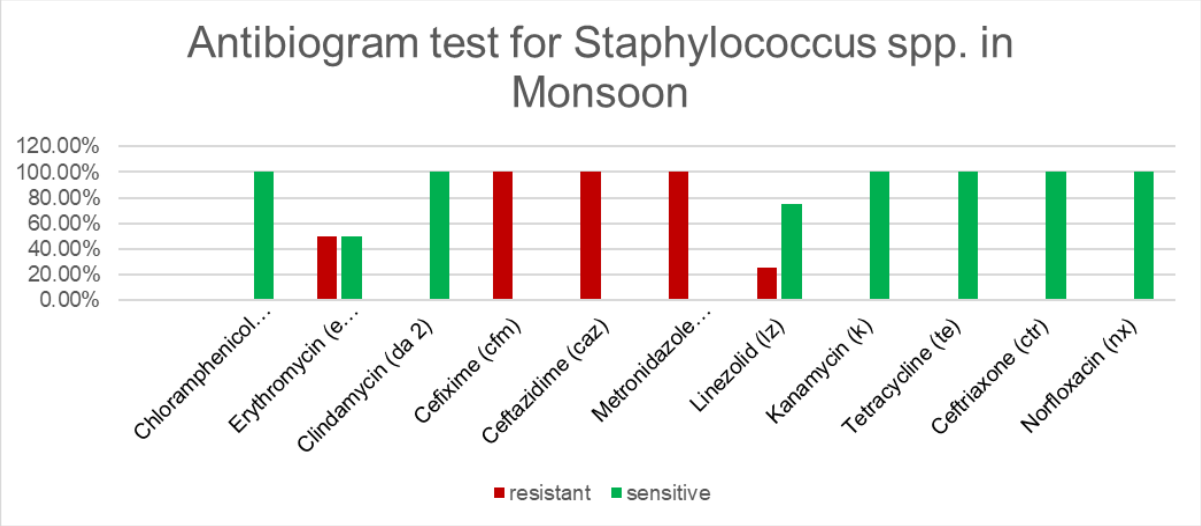

Figure 8 AST results of Staphylococcus sp. *From Hatirjheel during Monsoon season*

Then to Ceftazidime, Isolates were 100% resistant and 0% sensitive. Next,Methicillin was 100% resistant and 0% sensitive. About Linezolid, 25% was resistant and 75% was sensitive. Kanamycin was 100% sensitive and 0% resistant. On the other hand, to Tetracycline, isolates were 100% sensitive and 0% resistant. To Ceftriaxon, isolates were 100% sensitive. 0% isolates were resistant to Norfloxacin and 100% were sensitive.

Buriganga/Summer:

During the summer season, Chloramphenicol 0% were resistant and 100%were sensitive. 29% of the isolates were resistant to Erythromycin and 71% were sensitive to Erythromycin. Whereas 0% isolates were resistant to Clindamycin and 100%were sensitive. 13% isolates were resistant to Cefixime and 87%were sensitive. Ceftazidime was 100% resistant and 0% sensitive.

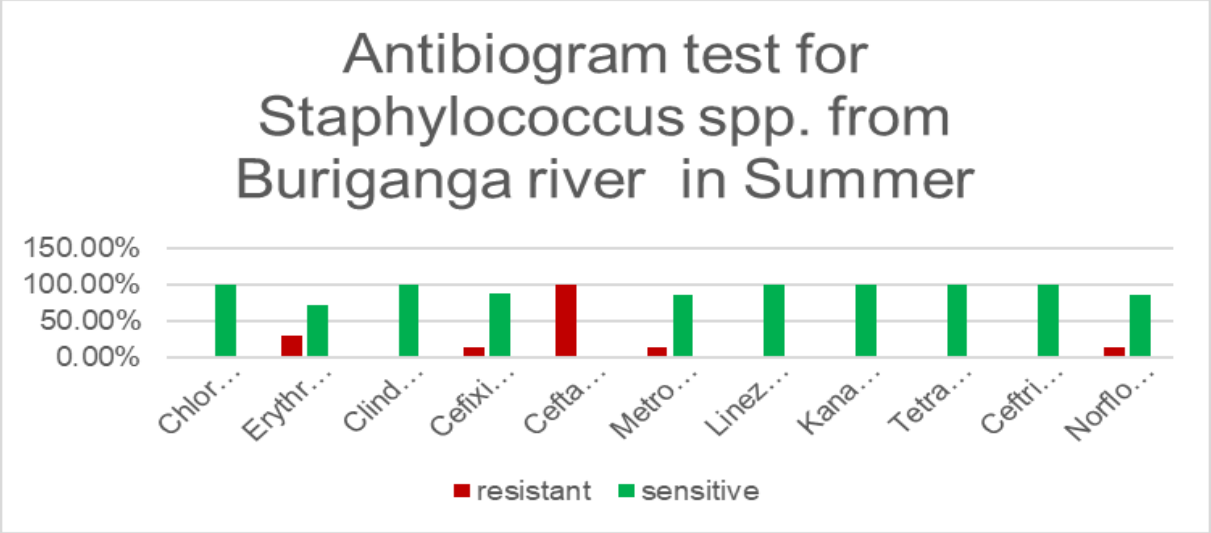

Figure 9 Antibiogram test for Staphylococcus spp. *from Buriganga River in Summer*

In the case of Metronidazole, 14% isolates were resistant and 86% sensitive. About Linezolid, 0% was resistant and 100% was sensitive. Kanamycin was 100% sensitive and 0% resistant. In the case of Tetracycline, 100% of the isolates were sensitive. 0% isolates were resistant and 100% sensitive to Ceftriaxone. Finally, 14% isolates were resistant to Norfloxacin and 86% were sensitive.

Buriganga/Monsoon:

Among the samples, 0% isolates were resistant and 100%were sensitive to Chloramphenicol. In the case of Erythromycin, 50% were resistant and 50% were sensitive. 22% isolates were resistant to Clindamycin and 78%were sensitive. Next, 14% isolates were resistant to Cefixime and 86%were sensitive.

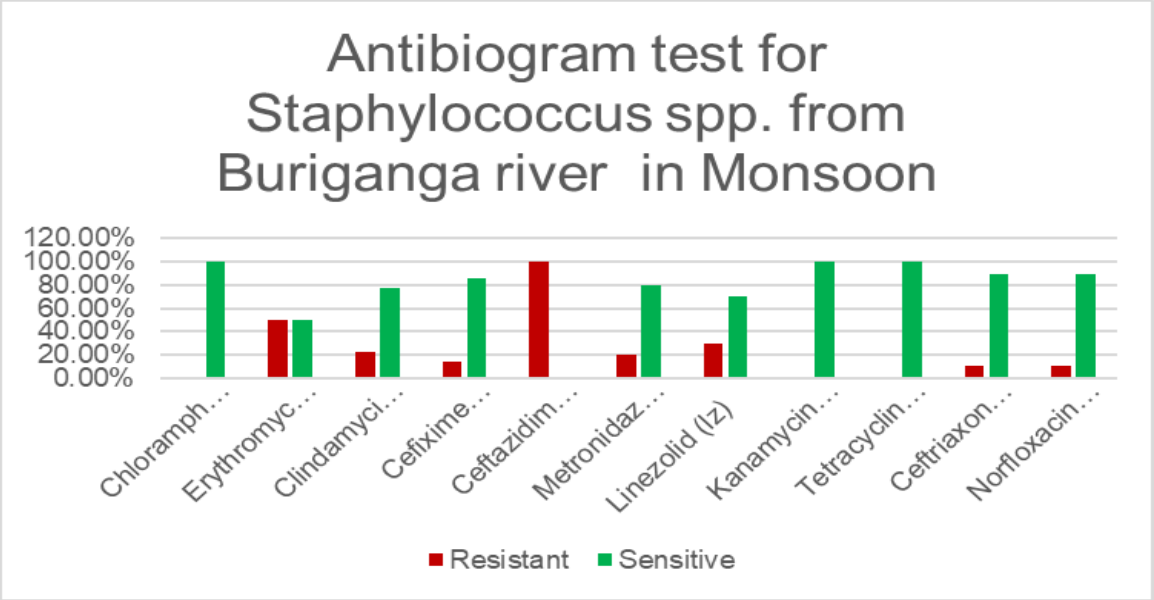

Figure 10 Antibiogram test result for Staphylococcus spp. *from Buriganga river in Monsoon*

Then to Ceftazidime, Isolates were 100% resistant and 0% sensitive. Next, Metronidazole was 20% resistant and 80% sensitive. About Linezolid, 30% was resistant and 70% was sensitive. Kanamycin was 100% sensitive and 0% resistant. On the other hand, to Tetracycline, isolates were 100% sensitive and 0% resistant. To Ceftriaxon, isolates were 90% sensitive and 10% resistant. 10% isolates were resistant to Norfloxacin and 90% were sensitive.

E. coli:

There was a total of 53 *E Coli* isolates from Hatirjheel and 40 isolates from Buriganga were performed for an antibiogram test against 9 antibiotics. The zone of inhibition was measured. Next, it was compared to the standard CLSI chart to determine if they are resistant or sensitive. Among which we have compared the data depending on the seasonal variation.

Hatirjheel/Fall:

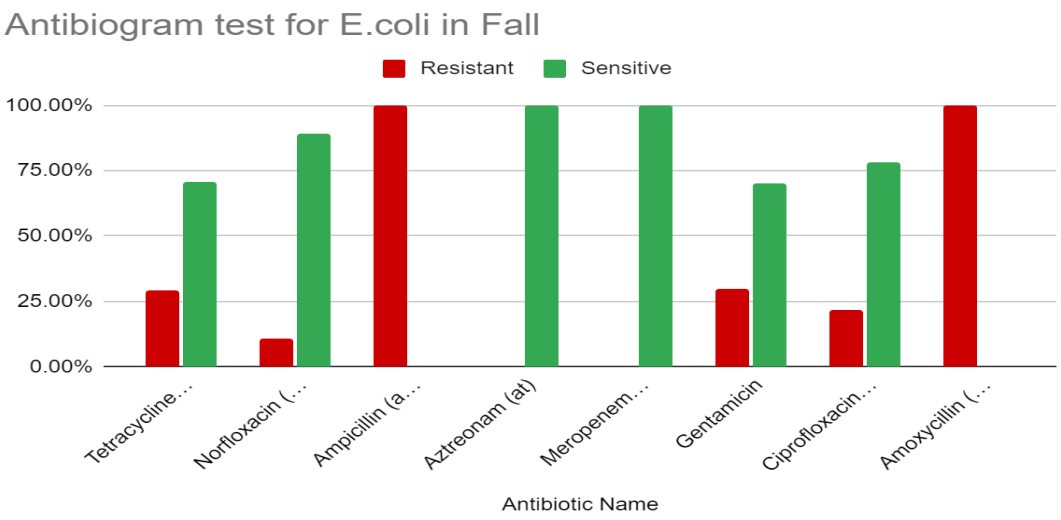

Figure 11 Antibiogram Test of *E.coli* From Hatirjheel during fall Season

During the fall season, from all isolates, Ampicillin and Amoxicillin showed 100% resistance without any sensitivity. On the other hand, Aztronum and Meropenem showed 100% of sensitivity with no resistance. 11% isolates were resistant to Norfloxacin and 89% were sensitive. In case of tetracycline, 29% isolates were resistant and 71% sensitive. 22% ciprofloxacin were resistant and 78% were sensitive. Lastly, 30% isolates were resistance for gentamicin and 70% were sensitive to it

Hatirjheel/Summer:

During the summer season, from all isolates, only Ampicillin showed 100% resistance with no sensitivity but there was no antibiotic which was 100% sensitive in this season. Also, amoxicillin showed 95% resistance and 5% of sensitivity. Tetracycline showed 15% of resistance and 85% of sensitivity. Norfloxacin showed 25% resistance and 75% sensitivity.

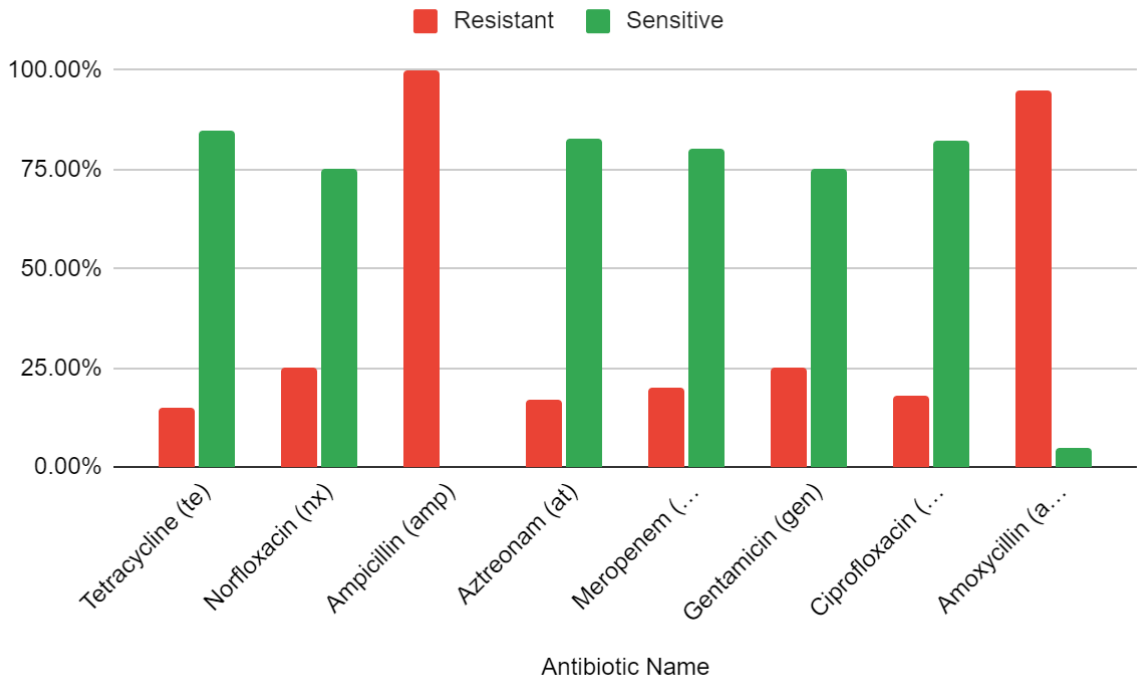

Figure 12 Antibiogram Test of *E.coli* From hatirjheel during summer Season

In case of aztreonam and ciprofloxacin, 17% and 18% isolates were resistant respectively, and 73% and 82% were sensitive. In the case of gentamicin, 25% were resistant and 75% were sensitive to it. Lastly, 20% isolates were resistant for Meropenem and 80% were sensitive to it.

Hatirjheel/ Monoson:

Among all isolates, Tetracycline, Norfloxacin, Meropenem showed 20% of resistance and 80% of sensitivity. Amoxicillin showed a high percentage of resistance which was 95% and 5% of sensitivity.

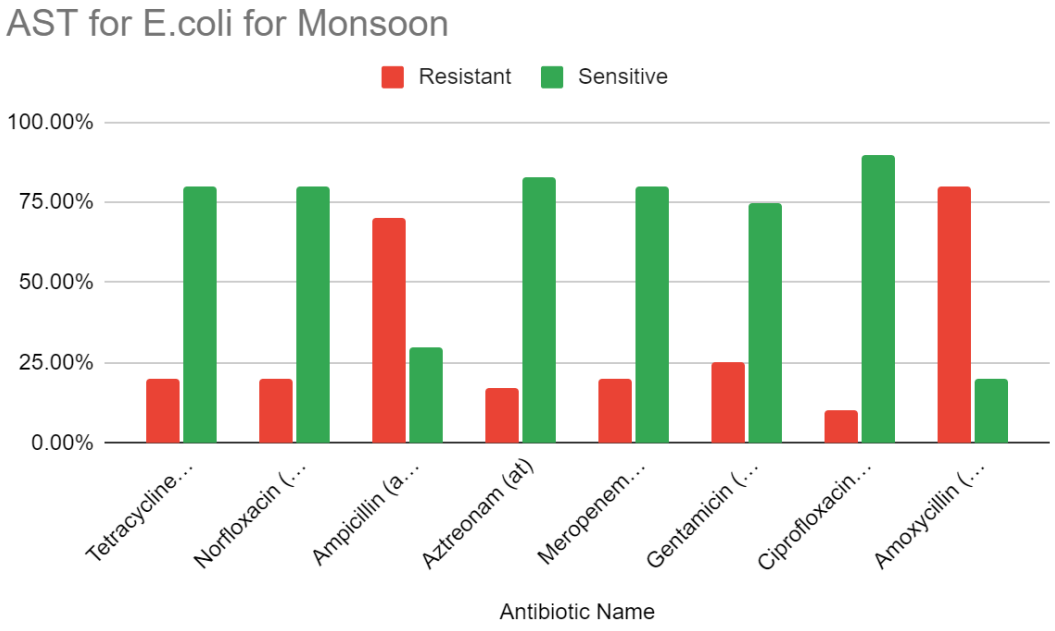

Figure 13 Antibiogram Test of E.coli *From Hatirjheel during Monsoon Season*

In the case of Ampicillin, 70% were resistant and 30% were sensitive. Aztreonam showed 17% of resistance and 83% of it. Gentamicin showed 25% resistance and 75% sensitivity. Lastly, 10% isolates were resistant to ciprofloxacin and 90% were sensitive.

Buriganga/ Summer:

Among the samples, 0% were resistant to Erythromycin and 100% were sensitive to Erythromycin. 0% isolates were resistant to Norfloxacin and 100% were sensitive. In the case of Tetracycline, Gentamicin, Ciprofloxacin and Meropenem, 0% were resistant and 100%were sensitive.

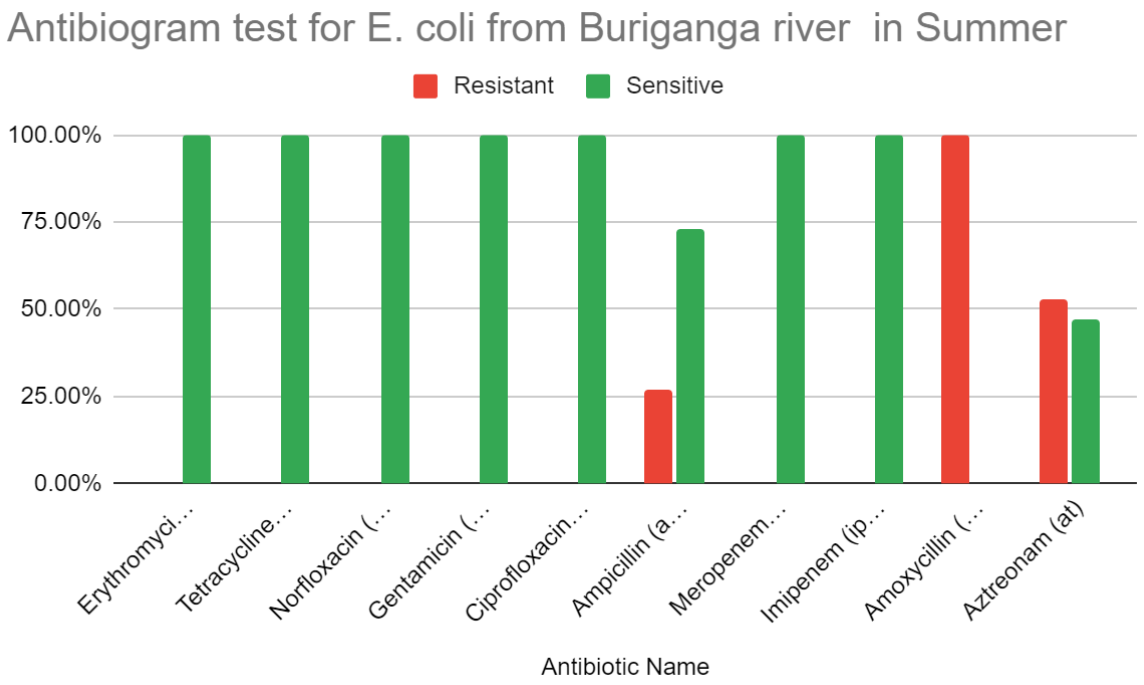

Figure 14 Antibiogram Test of E. coli *From Buriganga during Summer Season*

Isolates were 27% resistant to Ampicillin and 73% sensitive**.** 53% isolates were resistant and 47% were sensitive to Aztreonam and 0% isolates were resistant to Imipenem and 100%were sensitive. Amoxycillin was 100% resistant and 0% sensitive.

Buriganga/Monsoon:

Among the samples, 14% were resistant to Erythromycin and 86% were sensitive to Erythromycin. 0% isolates were resistant to Norfloxacin and 100% were sensitive. In the case of Gentamicin, Ciprofloxacin and Meropenem, 0% were resistant and 100%were sensitive.

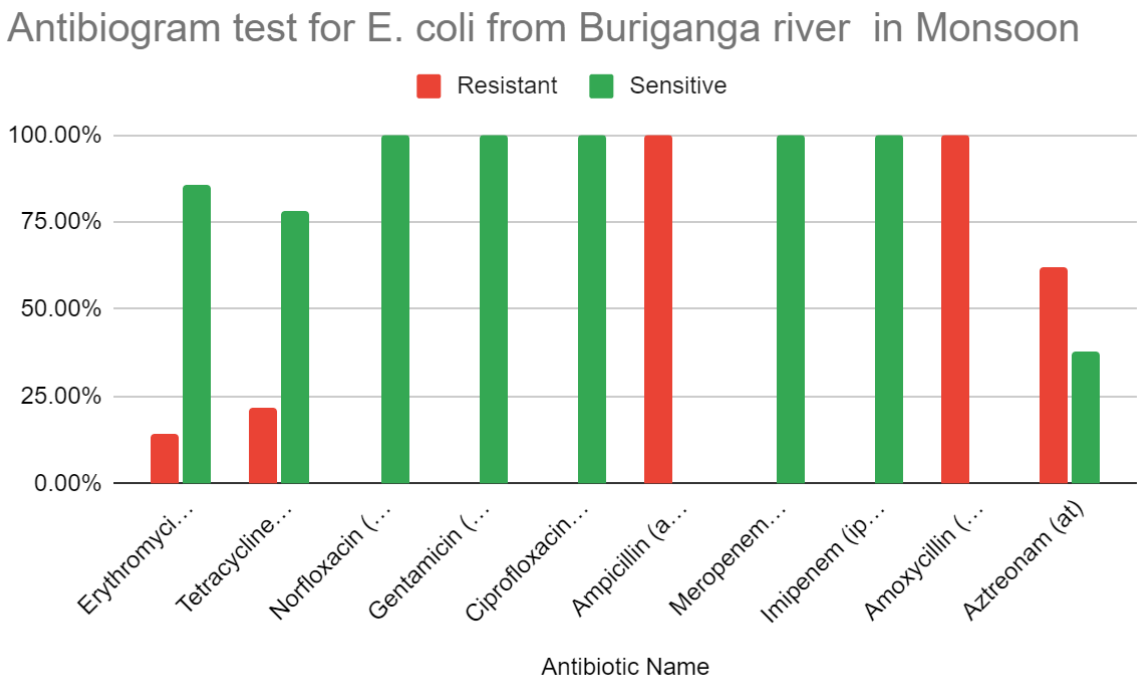

Figure 15 Antibiogram Test of *E.coli* From Buriganga during Monsoon Season

Isolates were 22% resistant and 78% sensitive to Tetracycline. Then, the Isolates were 100% sensitive to Norfloxacin. Isolates were 100% resistant to Ampicillin. 100% isolates were resistant to Imipenem and amoxicillin with 0% sensitivity.

Salmonella:

A total of 53 *Salmonella*/Shigella isolates from Hatirjheel and 24 isolates from Buriganga were performed for an antibiogram test against 9 antibiotics. The zone of inhibition was measured and it was then compared to the standard CLSI chart to determine if they were resistant or sensitive. Among these, we have compared the data depending on the seasonal variation.

Hatirjheel Fall:

The AST results of the samples collected during the Fall show that the isolates are 100% sensitive to Amikacin and Tetracycline. On the other hand, the isolates are 100% resistant to Ciprofloxacin and Ampicillin.

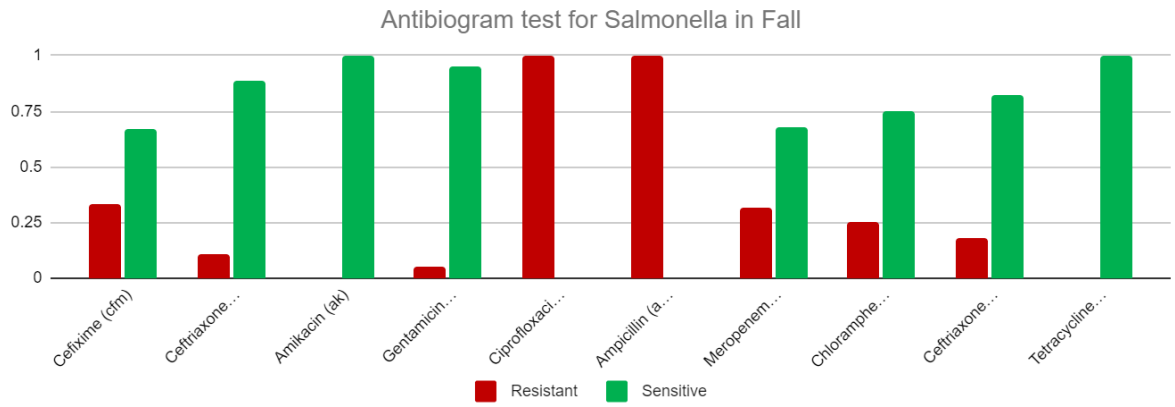

Figure 16 Antibiotic Susceptibility Test from Salmonella *from Hatirjheel during Fall.*

Furthermore, the isolates are 33% resistant and 67% sensitive to Cefixime. The isolates are seen to be 11% resistant and 89% susceptible to ceftriaxone. Here the isolates are 5% resistant to gentamicin and 95 % sensitive. Among the samples, 32% are resistant and 68% are sensitive to Meropenem. In the case of Chloramphenicol 25% are resistant and 75% are sensitive.

Hatirjheel Summer:

During the Summer, the isolates were 100% sensitive to Cefixime, Gentamicin, and Amikacin. On the other hand, they were 100% resistant to Ceftazidime. In the case of Ciprofloxacin and Meropenem, the isolates were 25% resistant and 75% sensitive.

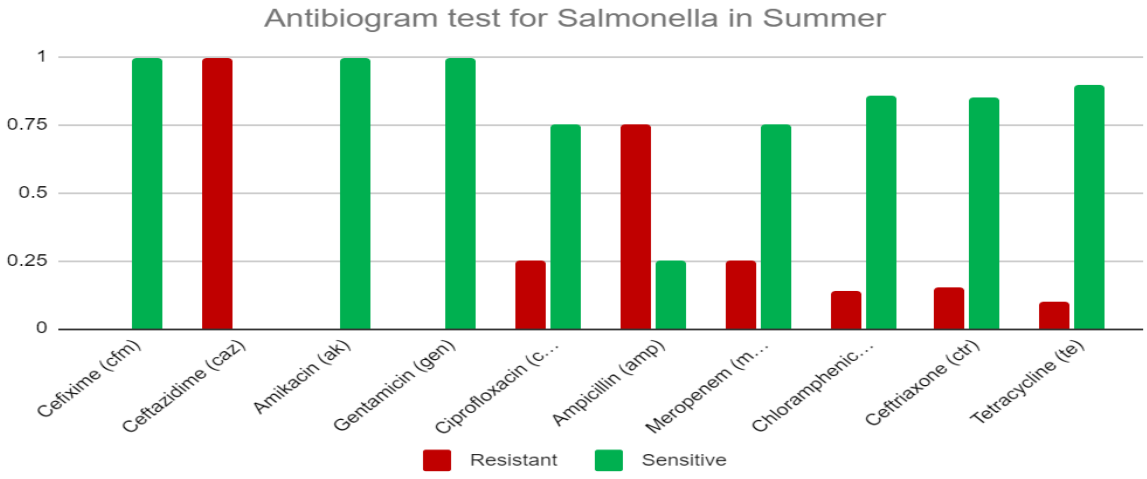

Figure 17 Antibiotic Susceptibility Test from *Salmonella* from Hatirjheel during Summer.

The isolates were 75 % resistant and 25% sensitive to Ampicillin. Also, the isolates were 14% resistant and 86% sensitive to Chloramphenicol, 15% resistant and 85% sensitive to Ceftriaxone, and 10% resistant and 90% sensitive to Tetracycline.

Hatirjheel Monsoon:

During the Monsoon, the Isolates were 100% resistant to Amikacin, Gentamicin, and Meropenem. On the other hand, the isolates were 100% resistant to Ceftazidime and Ampicillin. In the case of Cefixime, the isolates were 50% resistant and 50% sensitive.

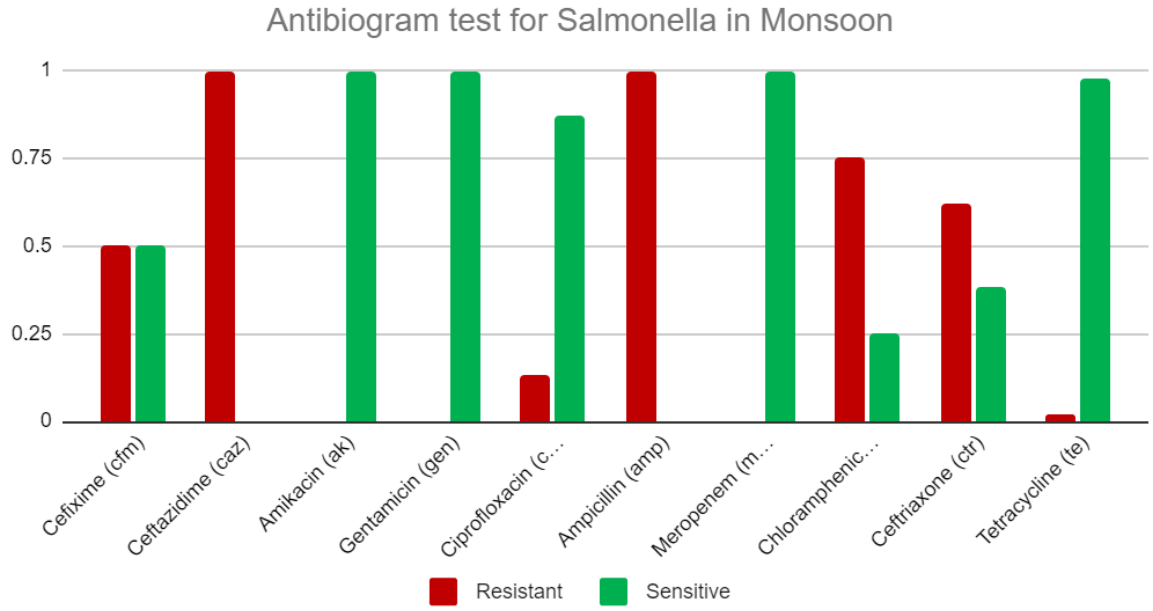

Figure 18 Antibiotic Susceptibility Test from Salmonella *from Hatirjheel during Monsoon.*

Moreover, they were 13% resistant and 87% sensitive to Ciprofloxacin. Chloramphenicol shows 75% resistance and 25 % sensitivity to the isolates. They are 62% resistant and 38% sensitive to Ceftriaxone. Lastly, the isolates were 2% resistant and 98% sensitive to Tetracycline.

Buriganga Summer:

During the Summer, the isolates from Buriganga were 100% sensitive to Gentamicin and Co-trimoxazol. On the other hand, they were 100% resistant to Cefixidime and Ampicillin. The

isolates were 9% resistant and 91% sensitive to Amikacin and Ciprofloxacin.

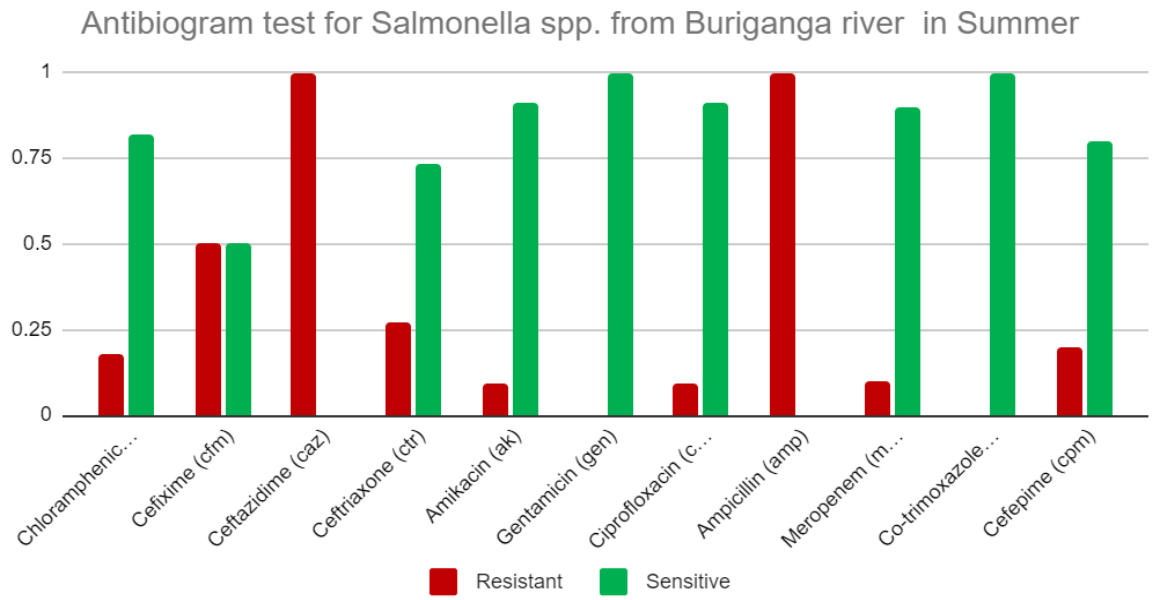

Figure 19 Antibiotic Susceptibility Test from Salmonella *from Buriganga during Summer.*

The isolates were 50% restant and 50% sensitive to Cefixime. In the case of Chloramphenicol the isolates were 18% resistant and 82 % sensitive. To Ceftriaxone, 27% resistant and 73% sensitive. To Meropenem, 10% resistant and 90% sensitive and to Cefepime the isolates were 20% resistant and 80% sensitive.

Buriganga Monsoon:

The isolates from the samples of Buriganga in Monsoon are 100% sensitive to Amikacin, Gentamicin, and Cefepime. On the other hand, they were 100% resistant to Ceftazidime and Ampicillin.

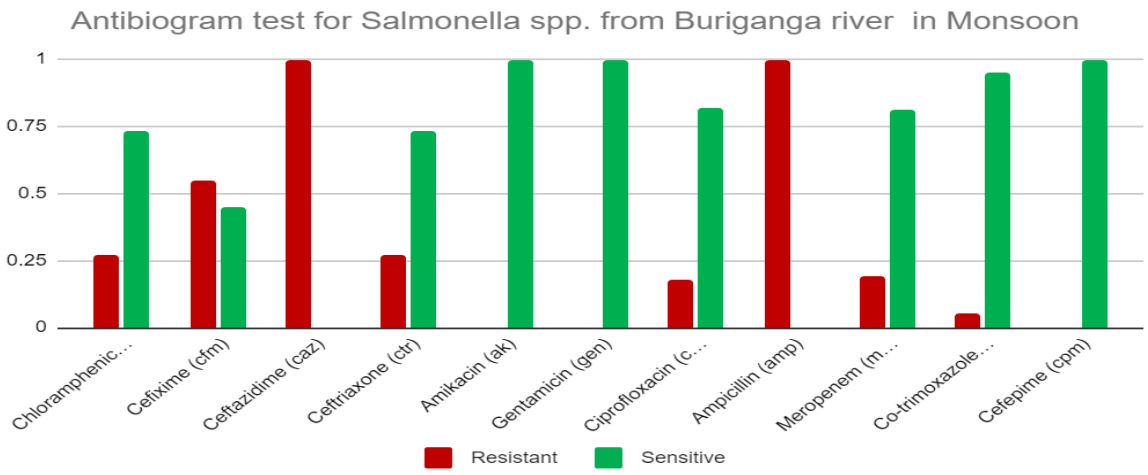

Figure 20 Antibiotic Susceptibility Test from Salmonella *from Buriganga during Monsoon.*

To Chloramphenicol and Ceftriaxone, the isolates are 27% resistant and 73% sensitive. The isolates were 55% resistant and 45% sensitive to Cefixime. To Ciprofloxacin, the isolates are 18% resistant and 82% sensitive. To Meropenem the isolates are 5% resistant and 95% sensitive.

3.3. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a molecular biology technique performed to determine the presence of each of the following (Table 1) genes; Methicillin resistant genes (mecA) , toxic shock syndrome toxin gene (tsst).

Each of the PCR tube contained 13 µl of mixture which included 6.5 µl of master mix, 1 µl of each forward and reverse primers, 2.5 µl of nuclease free water and 2 µl of the template.

For methicillin resistance genes (mec A) the amplification conditions were: 5 min at 94℃, then 30 cycles of 30 s at 94 ℃, 1 min at 59 ℃, 1 min at 72 ℃ and a final extension of 10 min at 72 ℃

The amplification condition for TSST gene was 94℃ for 3 min followed by 29 cycles of 94℃ for 1.5 min, 54℃ for 1.5 min, and 72℃ for 1 min and a final extension 72℃ for 7 min

3.4 PCR result:

Table 2 PCR result for mecA

Water bodies	Total isolates	Positive isolates	percentages
Hatirjheel	60	20	33%
Buriganga river	40	14	35%

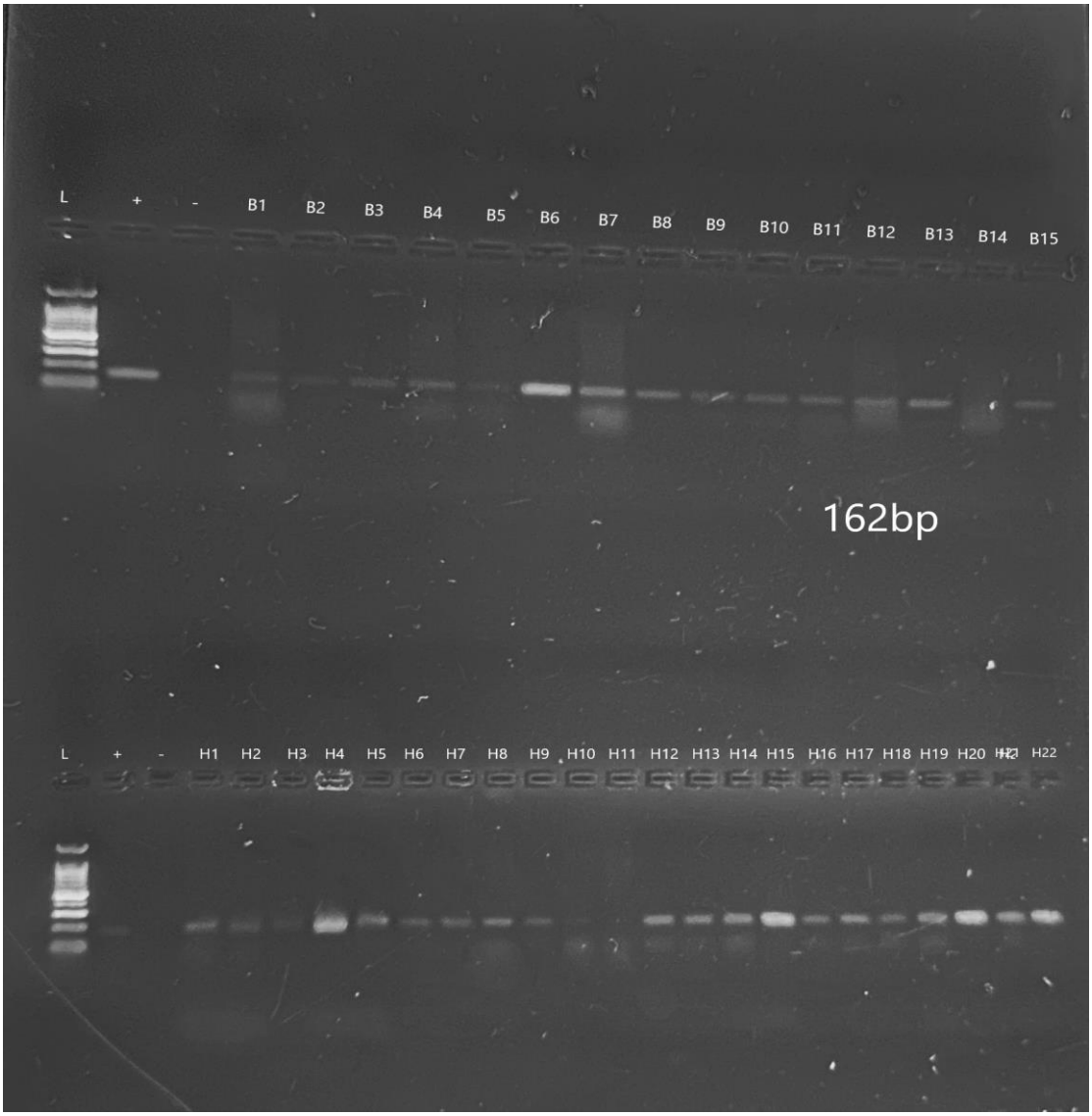

Figure 19: gel electrophoresis of Mec A gene

here the upper wells were Buriganga river samples and lower wells Hatirjheel portion. The product size of mecA gene is 162 bp where 100 bp ladder was used. Here we can see in Burihanga river there are 14 bands at B1,2,3,4,6,7,8,9,10,11,12,13,14,15. also, positive isolates for Hatirjheel shows band in H1,2,3,4,5,6,7,8,9 ,12,13,14,15,16,17,18,19,20,21,22. So, the overall positive isolate percentage for Hatirjheel 33% and Buriganga 35%.

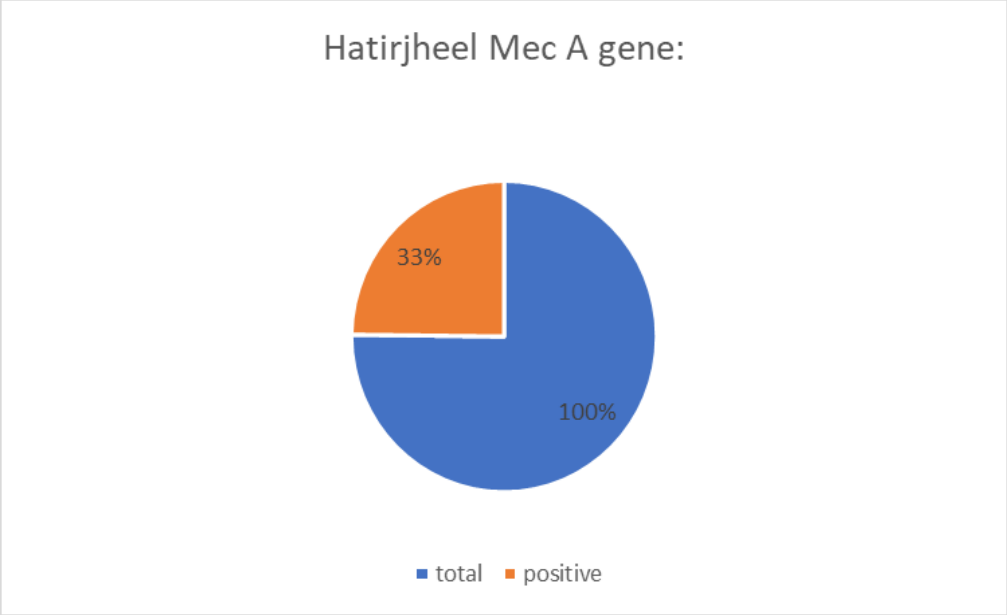

Figure 21 Presence of mecA gene in Hatirjheel

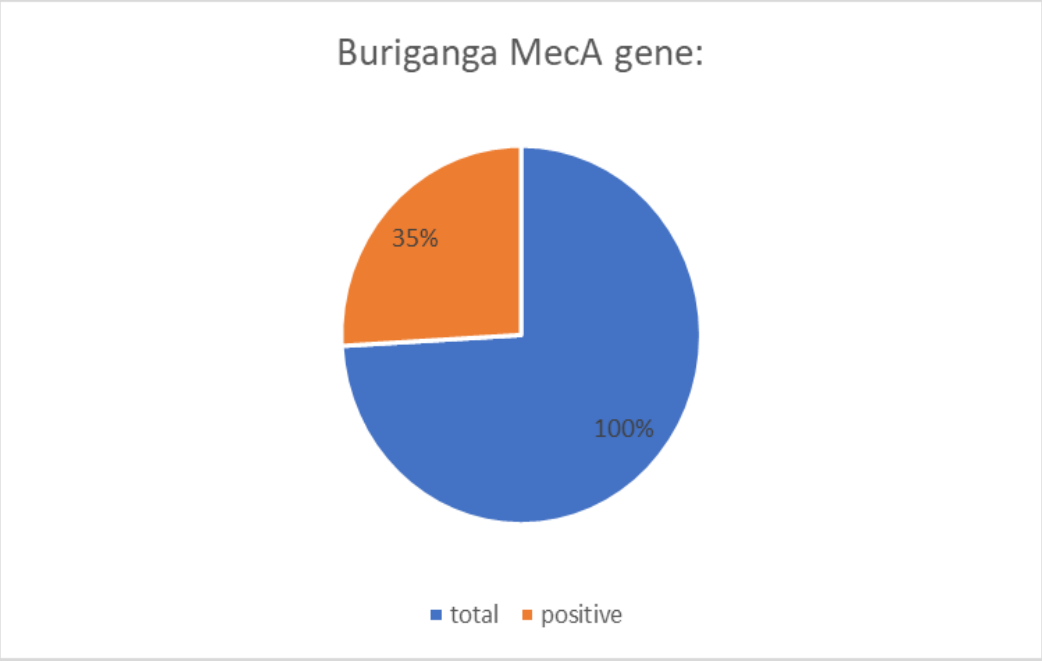

Figure 22 Presence mecA gene in Buriganga

Table 3 PCR result for tsst

Water bodies	Total isolates	Positive isolates	percentages
Hateerjheel	60	2	4%
Buriganga river	40	2	5%

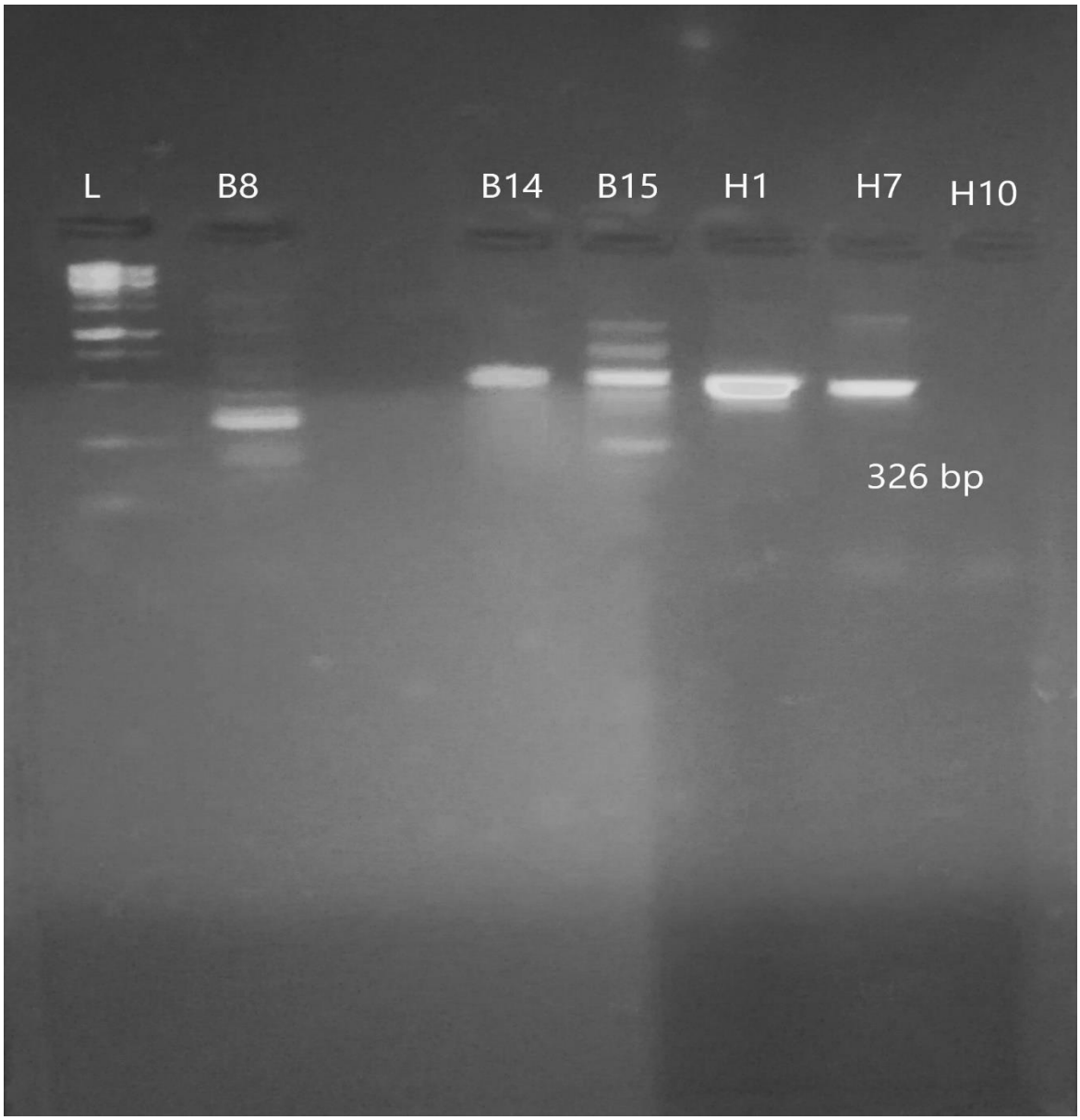

Figure 23 Gel electrophoresis of tsst gene

Here the upper portion denotes Buriganga river samples and lower portion denotes Hatirjheel portion. The product size of tsst gene is 326 bp where 100 bp ladder was used. Positive isolate in Buriganga river shows a band in B14,15.

also, positive isolates in hatirjheel show in H1,7.

so, the overall positive isolate percentage for Hatirjheel 4% and Buriganga 5%.

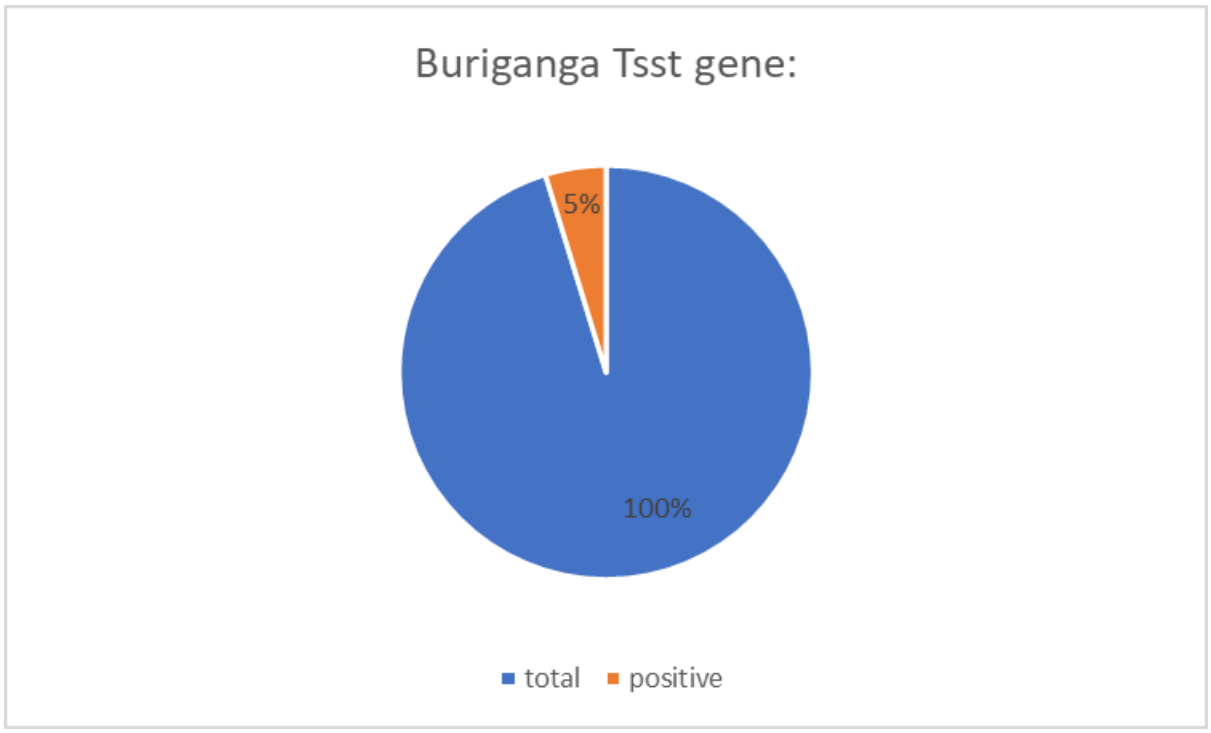

Figure 24 Presence tsst gene in Buriganga

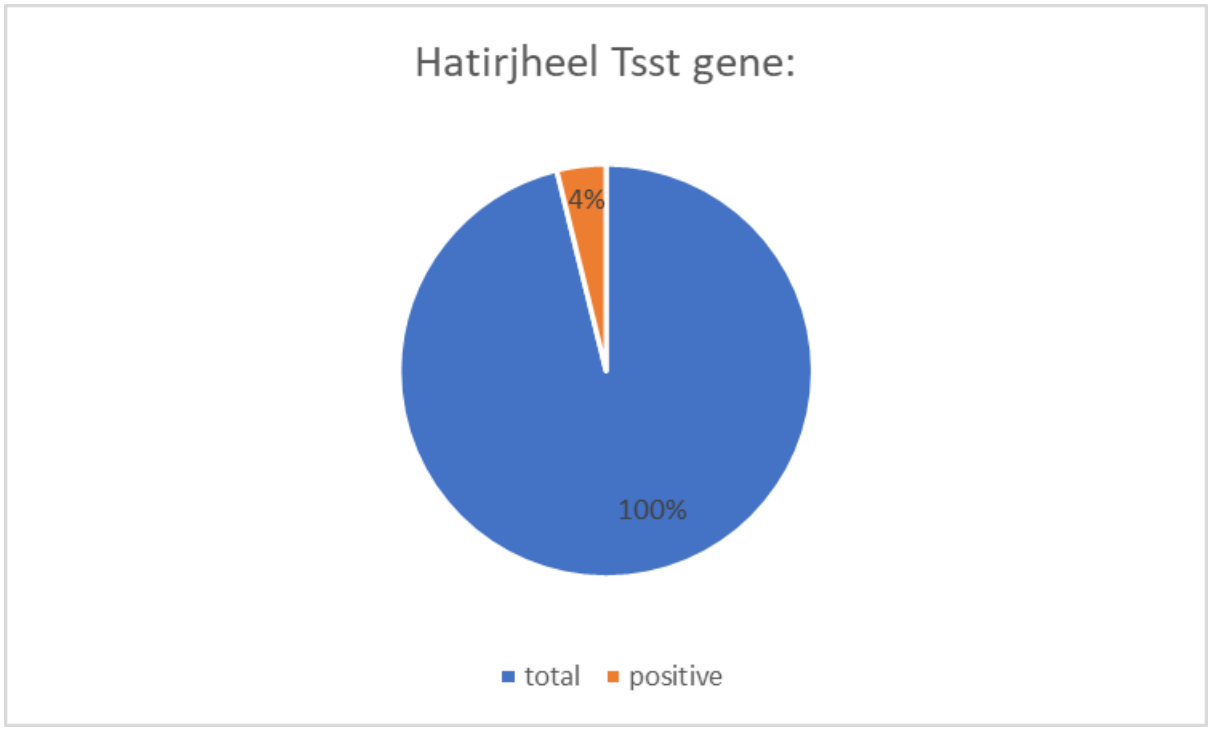

Figure 25 Presence tsst gene in Hatirjheel

Chapter 4

Discussion

The goal of conducting this study was to detect, isolate, and describe the presence of *E. Coli, salmonella, shigella*, coliform, fecal coliform, and *Staphylococcus spp* from Hatirjheel Lake and Buriganga River, two of Dhaka city's primary surface water bodies. The seasonal variation of these microorganisms is one of the major focuses of the study. From January 2023 to March 2024, 107 samples in total have been collected during the three different seasons. The investigation demonstrated three distinct seasonal variations based on average colony count during fall, summer and monsoon season. From the results obtained from Hatirjheel samples, it explained that the average colony count of *Staphylococcus spp* is the highest during summer season compared to monsoon and fall. The colony count is 239.2% on average. In the case of monsoon and fall season, more colonies of *Staphylococcus spp* were found in monsoon season and less in fall season. Considering the results obtained from Buriganga River water samples, the average colony count of *Staphylococcus spp* during monsoon is 150.2% which is higher than the summer season. The average colony count of *Salmonella, Shigella* obtained from Hatirjheel water samples was the highest (314.6%) in the summer season. However, it is nearly the same in fall and monsoon season. From Buriganga river water samples, the average colony for *Salmonella, Shigella* in the Summer season was 37.092 %, the highest however, in the monsoon season, 0.2108 %. Presence of *E. coli,* Coliform and Fecal coliform in the Summer season was the highest based on the average colony count from Hatirjheel water samples, second highest in fall season and very less during monsoon season. From Buriganga river water samples, the average colony for *E. coli,* Coliform and Fecal coliform in the Summer season was less, however, higher during monsoon season.

The results demonstrate that the average colony counts of *Staphylococcus spp, Salmonella, Shigella, E. coli,* Coliform and Fecal coliform organisms were highest during the summer season and lowest during monsoon and fall season in the case of Hatirjheel water samples. However, in case of Buriganga river water samples, the results showed slightly dissimilarity. On an average, highest number of *E. coli* has been found, after that *Staphylococcus spp,* and lowest number of *Salmonella, Shigella* found in both Hatirjheel and Buriganga river water samples. The seasonal differences in bacterial colony counts highlight the continually changing

nature of the water environment of the Buriganga and Hatirjheel waterbody where many environmental conditions may have an impact on microbial populations.

Antimicrobial resistance (AMR) remains the world's most urgent public health concern (Gajic et al., 2022). According to the World Health Organization (WHO), antibiotic resistance is rising to dangerously high levels in all parts of the world, leading to increased morbidity and mortality. Hence, the six-leading mortality-causing pathogens— *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus pneumoniae, Acinetobacter baumannii, and Shigella sonnei* -were responsible for 929,000 deaths attributable to AMR and 3.57 million deaths associated with AMR in 2019 (Gajic et al., 2022). Antibiogram for suspected *Staphylococcus aureus* showed an interesting pattern of sensitivity and resistance. Among all 11-antibiotic used on isolate, and both the location showed wide range of resistance to different antibiotic. In summer hatirjheel lake isolates showed resistance to Erythromycin, Clindamycin, Cefixime, Ceftazidime, Methicillin and Ceftriaxone as follows 46%, 20%,100,100%,20% and 10%. The resistance level of Ceftazidime and Methicillin jumped to 100% during monsoon. On the other hand, during summer in Buriganga it showed lower level of resistance, eg: for Erythromycin 29% and Methicillin 14%. Whereas Ceftazidime showed 100% resistance as Hatirjheel. Additionally, during monsoon in Buriganga river isolates showed notable increased resistance including Clindamycin 22%, Metronidazole20%, and Linezolid30% Notably, Ceftazidime shows a significant increase in resistance from Summer to Monsoon in Hatirjheel, and Linezolid shows emergence of resistance in the Monsoon season in both locations.

Similarly, Antibiogram for suspected *E. coli* showed an interesting pattern of sensitivity and resistance. Hatirjheel showed alarming resistance to Amoxicillin and Ampicillin in both seasons following 95% and 100%. Whereas Buriganga showed resistance in Amoxicillin wich is 100%. During monsoom Hatirjheel showed 20% resistance to Meropenem whereas buriganga showed 100% resistance to Imepenem in the same season. For instance, there are some antibiotics which showed sensitivity in both seasons are Norfloxacin, Tetracycline, Gentamicin, Ciprofloxacin. During both seasons Hatirjheel isolate showed more resistance to antibiotics than Buriganga.

Antibiogram for suspected *Salmonella* spp. Samples showed the most resistance to antibiotics. During monsoon in Hatirjheel it clearly showed the most resistance with five antibiotics Amikacin, Gentamicin, Meropenem, Ceftazidime, and Ampicillin at 100% resistance. This is

very alarming because Meropenem is a broad spectrum antibiotic which is considered to be the most strongest antibiotic.In summer the resistance showed 25% resistance to meropenem for instance showed high resistance to Ceftazidime 100%. On the other hand, Buriganga river showed 100% to Cefixidime and Ampicillin in both seasons. For the other antibiotics it showed moderate to high levels of sensitivity. Hatirjheel displays a broad spectrum of antibiotic resistance in the Monsoon compared to its summer season. What is more concerning is that Buriganga never reached the level of Hatirjheel in both seasons. The High resistance to some antibiotics is a public health concern. It suggests the presence of multidrug resistant bacteria in these waters, which could pose a risk to humans and animals.

According to Journal of chemical microbiology, High-level resistance to methicillin is caused by the mecA gene which are the major causes of nosocomial infections worldwide (Igbinosa et al., 2016). The result in PCR shows 33% positive isolates in Hatirjheel and 35% positive isolates in Buriganga river which indicates the presence of mec A gene. mecA gene is responsible for the production of PBP2a that allows the resistance to methicillin and other β-lactam antibiotics. All staphylococci isolates were analyzed for methicillin resistance using mecA gene-specific primers. Around 15% staphylococci harbored mecA gene and 1% harbored mecC gene. mecA and mecC genes are found in SCCmec mobile genetic elements and widely distributed in MR-CoNS. All MR-CoNS and MS-CoNS were analyzed for integrons. The integron system works as a natural cloning and expression system which allows bacteria to incorporate gene cassettes and convert them to functional genes. This cloning and expression feature responsible for the dissemination of resistance genes, cause the effortless spread of resistance genes and the rapid evolution of resistance to a wide range of unrelated antibiotics among diverse bacteria. More than 100 different types of antibiotic resistance gene cassettes are harbored by integrons which facilitate the adaptations that extend beyond antibiotic resistance and pathogenicity. Using the crucial recombination platform (the integrase and attI site), integrons have the apparently limitless capacity to exchange and accumulate functional gene cassettes, which permits rapid adaptation to selective pressure and may ultimately ensure increased fitness and advantage to the host (Rowe-Magnus et al., 2003). Hence, all types of other mobile DNA elements such as conjugative plasmids, transposons, insertion sequences, and even entire chromosome, would probably be vast reservoirs of integron, lending support to the long-standing concept of a single massive genetic pool that is available and shared among bacteria (Rowe-Magnus et al., 2003).

The tsst gene is responsible for the production of toxic shock syndrome toxin. Toxic shock syndrome is a complicated and life-threatening infection, mostly associated with *Staphylococcus* aureus (Esmailkhani et al., 2018). In the result, the water bodies of Hatirjheel show 4% positive isolates of tsst gene and 5% positive isolates in Buriganga river. S. aureus strains resistant to numerous antimicrobial drugs are commonly isolated from nosocomial infections. The spreading of multi-drug resistant strains is one of the major challenges in healthcare all around the world, owing to difficult treatment (Igbinosa et al., 2016).

Findings of this study indicate that the subject water bodies are contaminated with the risks associated with Dhaka city surface waters. It is essential to not take the presence of these microorganisms lightly as they are potentially infective.

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Appendix

Media Composition:

The composition of all media used in the study is given below:

Mannitol salt agar:

Ingredients	Amount(g/L)
peptones	10
Beef extract	1
D-mannitol	10
Sodium chloride	75
Phenol red	0.025
Agar	15
Distilled water	1000ml
Final pH (at 25^0C)	7.4 ±0.2

Nutrient Agar:

Ingredients	Amount(g/L)
Peptone	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5

Agar	15
Final pH (at 25^0C)	7.4 ±0.2

Muller-Hinton Agar:

Ingredients	Amount(g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5
Starch	1.5
Agar	17
Final pH (at 25^0C)	7.4 ±0.2

Blood Agar Base:

Ingredients	Amount(g/L)
Beef extract	10
Tryptose	10
Sodium chloride	5
Agar	15
Final pH (at 25^0C)	7.3±0.2

Saline:

Ingredients	Amount(g/L)

Sodium chloride	9.0