Prevalence and Characterization of Methicillin Resistant *Staphylococcus* spp. in Surface Water of Dhaka

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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Declaration

I hereby declare that the thesis project titled **"Prevalence and Characterization of Methicillin Resistant *Staphylococcus* spp. in Surface Water of Dhaka"** has been written and submitted by me, Adiba Masnun and has been carried out under the supervision of Mahbubul Hasan Siddiqee, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Abstract

Dhaka city being the capital of Bangladesh faces problem with rapid urbanization based on rural-to-urban migration often results in the contamination of water bodies with bacteria pathogenic to humans. This study aims to determine the prevalence of environmental *Staphylococcus* spp. contamination in water with industrial wastes, specifically focusing on *Staphylococcus aureus*. A total of 35 water samples were collected from both Hatirjheel Lake and Buriganga River, representing Dhaka city's water bodies. Then they were processed for isolation of cultureable *Staphylococcus* spp. strains. For this, the collected water samples were first plated on to Mannitol Salt Agar (MSA). One pure colony from each positive plate was then selected for further processing. Presumptive isolates were then confirmed using biochemical methods. To determine whether they are *Staphylococcus aureus* or not. After that hemolytic activity on the samples were observed by inoculating them on Blood Agar and it showed 100% hemolysis for isolates from Hatirjheel and 82.35% for those from Buriganga. For determining the virulent activity of the strains, coagulase test was done and 72.22% coagulase positive were observed for Hatirjheel-isolates and 71% Buriganga-isolates. Antibiotic susceptibility test showed similar results for Hatirjheel Lake and Buriganga River, except for methicillin-resistance. Resistance to methicillin was found in 5.56% of the Hatirjheel-isolates whereas 35.50% of the Buriganga-isolates were MRSA. This study reveals that both Hatirjheel Lake and Buriganga River are contaminated with *Staphylococcus* spp.that may lead to serious health hazard in near future.

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Chapter 1

Introduction

1.0 Introduction

Water is the most common liquid on earth and is certainly vital for all living forms of life. The quality of water is of great importance as it has a huge influence on public health by preventing ill-health. Outbreaks of infectious water-related diseases and serious epidemics may occur due to poor microbiological water quality (World Health Organization, 1995).

Dhaka is the largest city in Bangladesh and the center of trade and economy. It is one of the most polluted cities in the world and one of the issues concerned is the management of municipal waste. Garbage, refuse, sludge and discarded material and the wastages produced by industry, hospital, or household community activities are the sources of solid wastages. Four types of waste streams i.e. Domestic (49%), commercial (21%), industrial (24%), and hospital (6%) constitute the total solid wastes of Dhaka city. There are three types of wastes produced by hospital and pharmaceutical industries- Infectious waste, Sharp Waste and Non-infectious Waste (Islam, 2016)

The most important water recreational sites in Dhaka include Dhanmondi Lake, Ramna Lake, Gulshan Lake and Hatirjheel Lake. Among them Hatirjheel covers a large part of Dhaka city. The low-lying areas behind Sonargaon Hotel, stretching from the eastern side of the Tongi-Diversion Road up to the Rampura Bridge on the Pragati Shwaraniare surrounded by Hatirjheel Lake (Sohail-us-samad, 2009). The Hatirjheel lowlands provide very essential hydrological functions. The water of Hatirjheel Lake is used for transportation through water taxi service, small boating arrangements are done for general people, road side vendors often use its water and children of slum area bathe or play on this Lake. It drains and detains storm water from a large area (about 30 km^2) of Dhaka city. A number of major storm sewer outfalls basically supply storm water discharges into these lowlands. Although it is common that both domestic and industrial wastewater connections to the storm sewer network are mostly illegal in Dhaka. As a consequence, the storm sewers mainly carry domestic sewage as well as industrial wastewater during dry season. This has significantly degraded the environmental condition of Hatirjheel and reduced its capacity to work as a retention basin for storm water (Sohail-us-samad, 2009).

The main river flowing beside Dhaka city is Buriganga River. Country boats and launches carry human as well as food items (fruits, vegetables) on Buriganga River. As a result, the bank of the river is always crowded with people. They frequently use the water to wash their hands and face. Local vendors also use the water to wash utensils. It is not uncommon that people, who come from villages with no or little knowledge about drinking clean water, may drink the river water. Because of the tanneries in the riverbank of Buriganga and for using the river as dumping ground for the liquid and solid wastes, the river is now under threat of extinction.

If the value of pH of water is greater than 7, it represents the basic characteristics of water which is harmful for the skin. The average value of pH of Buriganga is 12. According to the experts, major reason of the pollution in Buriganga is the Hazaribagh Tanary. Dhaka WASA is in lack of sufficient recycling plants and dumps seepage wastes in the river. Buriganga is now in serious trouble as well as the surrounding environment. This is partly a maintenance problem but an environmental problem was created by the discharging of wastewater with excessively high solids content and a dangerous level of chromium among other heavy metals and toxic materials. Health impact from the disposal of wastes from the tanneries is either death or increased probabilities of death and sufferings by illness including skin diseases, fevers, headaches, respiratory diseases etc. (Bhowmik, 2007).

Although the water of these two water bodies is used on a regular basis, they are failed to provide clean and healthy water to mass population. Generally, the wastes are dumped beside the roadside areas. Eventually the wastes are washed away with rain water or are manually thrown alongside the waster bodies (Lake, pond, and river) in Dhaka. As a result, wet lands in Dhaka are polluted with numerous unexpected waste materials.

Staphylococcus is a genus of Gram positive, nonspore-forming cocci that are often found as normal human microbiota of the skin and nasal cavity. *Staphylococcus aureus* is the most potent species among the many others. It is a common member of normal microbial flora of human skin. It is found 20-30% of adults in nasopharynx at any one time. A study conducted by Aktar et al (2013) in Dhaka, Bangladesh, investigated that 27% of *Staphylococcus aureus* was present in

skin and soft tissue infections. It has two mechanisms to cause disease: one is by multiplication and spreading widely in tissue, another is by producing extracellular enzymes and toxins. Multiplication in tissue can result in boils, skin sepsis, post-operative wound infections, enteric infections, septicemia, endocarditis, osteomyelitis and pneumonia. Whereas, producing and releasing enterotoxin can lead to gastrointestinal diseases and can be indicated by projectile vomiting, diarrhea, fever, abdominal cramps, electrolyte imbalance and loss of fluids; such as toxic shock syndrome toxin-1 causes toxic shock syndrome.

Since *S.aureus* is found on the skin and mucous membranes of humans and animals, the presence of this organism in the environment is likely to have been caused by contamination by humans or animals. Environmental water can receive *Staphylococcus aureus* through human contacts such as swimming pools, spa pools and other recreational waters. They can be detected in drinking water supply as well (WHO, 2001).

The exposure of the "superbug" bacterium known as MRSA (Methicillin-resistant *Staphylococcus aureus*) and its increased prevalence has raised questions as to the routes of transmission related to disease. Evidence of the bacteria surviving wastewater, tap water and drinking water biofilms and various reports on MRSA infections has created an alarm in the public and an authorized discussion is needed to identify whether or not MRSA infections occur from tap water exposures. The possibility of dying if infected with MRSA infection is 5%. MRSA was identified as a national health crisis by 2005 in the USA. Approximately 19,000 deaths each year in the US due to MRSA; nearly 73% mortality rate for seniors (Staph & Backgrounder, 2013).

MRSA has been discovered from different untreated and improperly treated water bodies, in which it can survive for days to week. MRSA has been found in 83% of raw sewage sample in the US. It is suggested that the tap water contain wide range of MRSA (Staph & Backgrounder, 2013).

Whether aquatic environment is a potential media to spread MRSA or not is constantly evaluated. The knowledge of where MRSA is persisting in water, how well it is surviving and the success of current treatment are major considerations. An important source of Staph and

MRSA contamination are bathers, where during the first 15 minutes of water immersion an average person sheds a million Staph bacteria (Staph & Backgrounder, 2013). MRSA can be spread at recreational water facilities and other places by direct and indirect contact with infected persons. Direct contact can happen when someone touches another person's MRSA infection. Indirect contact can happen when someone shares items (like towels or razors) or touches surfaces (like hand rails or locker room benches) contaminated with MRSA. MRSA is most likely to spread when it comes into contact with an uncovered cut or scrap. Since *S. aureus* is found on the skin and mucous membranes of humans and animals, the presence of this germ in the environment is likely to have been caused by contamination by humans or animals.

In this experiment, two main water bodies selected in Dhaka City are Hatirjheel Lake and Buriganga River. The aim of this study was to determine the presence of *Staphylococcus* spp. in the two water bodies and observe their antibiotic resistance pattern in Hatirjheel as well as Buriganga. According to the hypothesis, the water will be contaminated with *Staphylococcus* spp. and the contaminants will have different characteristics in Hatirjheel and Buriganga.

Chapter 2

Methods and Materials

2.0 Materials and Method

2.1 Water Sampling

Water samples were collected aseptically in autoclaved plastic bottles from different parts of Hatirjheel Lake and Buriganga River. Small boats were used to collect water samples from different locations. The sample collection procedure started from the mid-October of 2017 and lasted till the end of January 2018. Total of 40 samples were collected, 20 from Hatirjheel Lake and 20 from Buriganga River.

2.2 Processing

The water samples were processed for isolation of cultureable *Staphylococcus aureus* strains. For this, the collected water samples were diluted in 10-fold (10^{-1} and 10^{-2}) serial dilutions and plated on MSA, selective agar for *Staphylococcus aureus*, using spread plate method. These colonies were then subcultured on NA to isolate single colonies. In order to preserve the samples, colonies were transferred from NA to NB. After 24 hours incubation at 37°C glycerol was added to NB and stored at -20°C for later use.

2.3 Identification

The isolated colonies were then tested for following biochemical tests to detect whether they are *Staphylococcus aureus.*

GRAM Staining

To establish whether the bacteria are gram positive or gram negative, gram staining was done.

Biochemical tests

Various biochemical tests were performed to accurately identify the presumptive bacteria chosen previously. Most of the methods were done according to the microbiology laboratory manual. The biochemical test performed were Catalase, Oxidase, IMViC test (Indole production test, Methyl red test, Voges- Proskauer test, and Citrate utilization test), Triple sugar iron agar test and Urease test.

Catalase test was done to determine the ability of the bacteria to degrade hydrogen peroxide by producing the enzyme catalase(Reiner, 2013). The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain and the reagent used for this test was 1% Kovacs oxidase (Shields & Cathcart, 2013). Indole production test was done to determine the ability of the bacteria to degrade the amino acid tryptophan by the enzyme tryptophanase. 5 drops of Kovac's reagent was added to culture tube (MacWilliams, 2013). Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of high concentration of acid end products. The Voges-Proskauer test determines the capability of some organisms to produce acetyl methyl carbinol from glucose metabolism. 5 drops of methyl red indicator were added for MR test(Cappuccino. & Sherman, 2014)and 12 drops of Barritt's reagent A (5% alpha-naphthol) and 3 to 5 drops of Barritt's reagent B (40% KOH) was for VP test in cultured broth tube. Citrate utilization test was done to determine the ability of organisms to utilize citrate as a common carbon source and Simmons citrate agar were prepared for this test (Cappuccino. & Sherman, 2014). Triple sugar iron agar test is used to determine whether organisms utilize glucose and lactose or sucrose fermentatively and produce hydrogen sulfide (H_2S). Urease test determine the ability of an organism to produce an exoenzyme, called urease, that hydrolyzes urea to ammonia and carbon dioxide (Brink, 2010).

2.4 Hemolytic test

Hemolytic test otherwise known as Blood Agar culturing method is used for identification of forms of hemolysis from pathogenic microorganisms. Generally pathogenic microbes secrete an enzyme known as "Hemolysin", an exotoxin by nature and disrupt membrane of the host likely erythrocytes. The mechanism of action of hemolysin is that it disrupts RBCs and increases the content of free iron and is also involved in dermonecrosis and vasoconstriction. Sodium Citrate anticoagulant was added to a fresh autoclaved Duran bottle before blood is placed in the bottle.
Blood agar base was prepared and autoclaved. The agar was cooled and 5ml of blood was added to 100ml of Blood agar base. Vigorous shaking was done to prepare Blood agar and then the agar was poured into fresh petri dish. Using sterile loop, small amount of each bacteria from 24-hours

old pure culture was streaked onto blood agar. The agar plates were kept in incubation for 24 hours at 37°C. After 24 hours incubation the colonies created clear zones when they produced beta hemolysis, greenish clear zone appeared when the bacteria partially broke blood cells, they are known as alpha hemolysis and if bacteria were not able to break down blood cells then they indicated gamma hemolysis(Cappuccino. & Sherman, 2014).

2.5 Coagulase test

Coagulase is an enzyme which can clot blood plasma and convert into gel like consistency. On this basis, microorganisms can be classified as coagulase positive or coagulase negative. On a clean glass slide, autoclaved saline was placed at two ends of the slide and test microorganisms were applied. A drop of plasma was added on to it. Clot formation observed were noted within 5-10 secs is considered to be positive for coagulase (Cappuccino & Sherman, 2014).

2.6 Kirby-Bauer Disc Diffusion

The Kirby-Bauer (K-B) disk diffusion test is the most common method for antibiotic resistance/susceptibility testing. It was used to test whether the experimental bacteria were susceptible to specific antibiotics as recommended by Clinical Laboratory Standard Institute (CLSI) using commercial antimicrobial disks. The antibiotic disks used in this study were: Clindamycin(2µg), Kanamycin(30µg), Linezolid (30µg), Cefoxitin (30µg), Erythromycin (15µg), Ceftazidime (30µg), Tetracycline(30µg), Norfloxacin (10µg), Methicillin(5µg).

MH agar plates (one for each organism to be tested) were allowed to come to room temperature. A sterile inoculating loop or needle was touched to four or five isolated colonies of the organism to be tested. The organism was suspended in 10 ml of sterile saline. The saline tube was vortexed to create a smooth suspension. The turbidity of this suspension was adjusted to a 1.0 McFarland standard by adding more organism if the suspension was too light or diluting with

sterile saline if the suspension was too heavy. The suspension was used within 15 minutes of preparation. A sterile swab was dipped into the inoculum tube. The dried surface of a MH agar plate was inoculated by streaking the swab three times over the entire agar surface; the plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculums. The lid was left slightly ajar, allowing the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step. Each antibiotic impregnated disk must be pressed down with sterile forceps to ensure complete contact with the agar surface. Within 15 minutes after the disks were applied, the plates were inverted and placed in an incubator at 37°C. After overnight incubation, the plates were examined for zone of inhibition and the diameter of the zone of inhibition was measured to the nearest whole millimeter by a ruler. The zone diameters for individual antimicrobial agents were then translated into susceptible, intermediate, or resistant categories according to the CLSI guidelines.

Chapter 3

Results

3.0 Results

3.1 Identification

Biochemical Tests were conducted on the isolated samples and the following table describes the results. Besides biochemical tests, coagulase and hemolysis tests have also been done to discover the virulence factors and hemolytic properties of the isolated strains respectively, which are included in the table as well. The table is given in the Supplementary.

From the Figure 3.1 it is evident that the total isolates of 35, 14 of them were presumptively *S.aureus*. Among them 8 isolates were from Hatirjheel and 6 were from Buriganga. The remaining 60% isolates mainly include *S.hemolyticus* (3 from Hatirjheel, 3from Buriganga), *S.kloosi* (2 from Jatirjheel and 2 from Buriganga)and other species incorporating *Staphylococcus gallinarum*, *Staphylococcus arlettae*, *Staphylococcus devriesei* ,*Staphylococcus massiliensis*, *Staphylococcus chromogenes*.

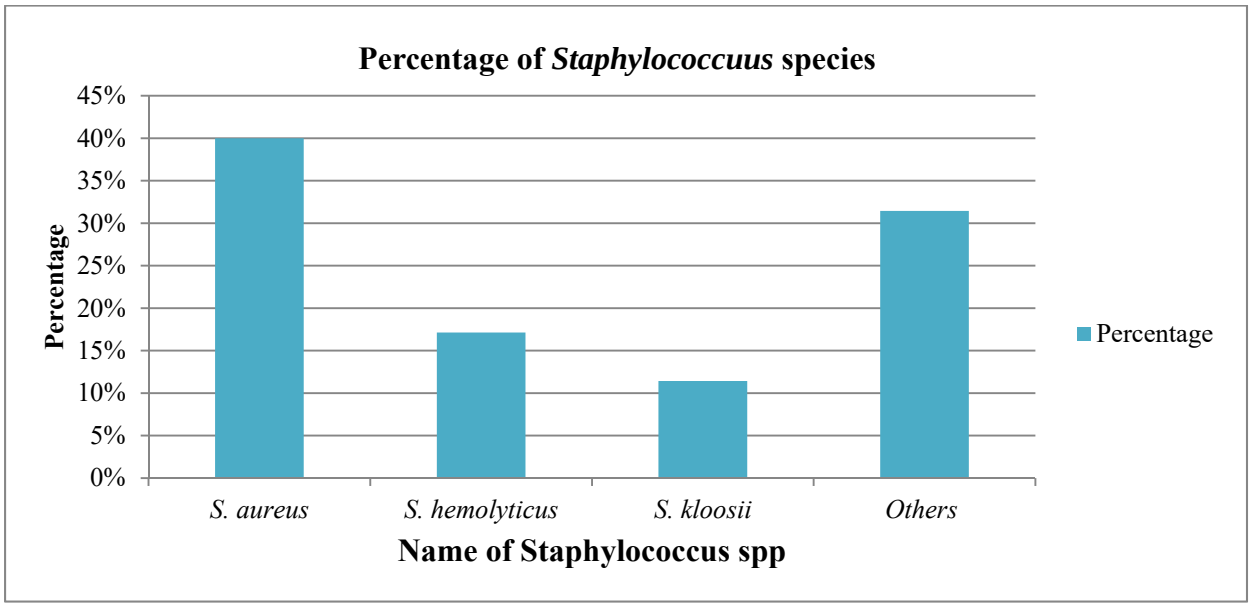

Figure 3.1: Percentage of *Staphylococcus* species

From the 40% of the *S.aureus* detected from the total isolates, Hatirjheel showed the greater amount of presence compared to Buriganga (Figure 3.2).

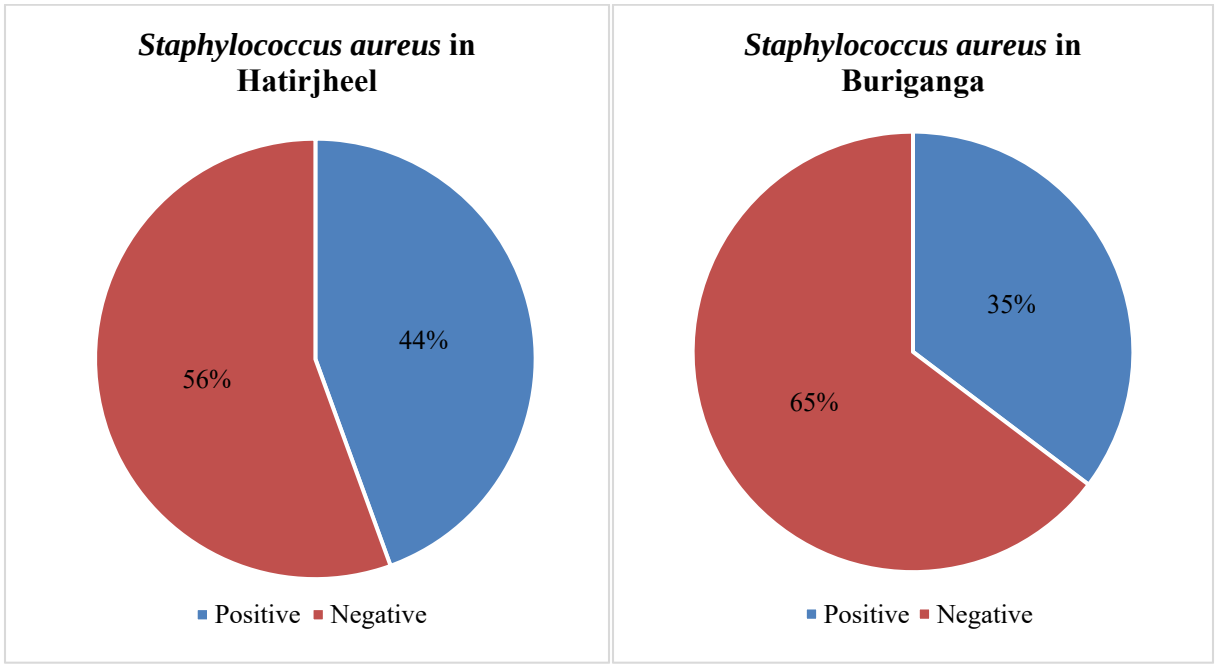

Figure 3.2: Presence of *Staphylococcus aureus* in Hatirjheel and Buriganga

The results from the Figure 3.3 suggest a significant variation among the coagulase and hemolysis characteristics in Hatirjheel and Buriganga. Both Coagulase and Hemolysis characteristics were higher in Hatirjheel than Buriganga.

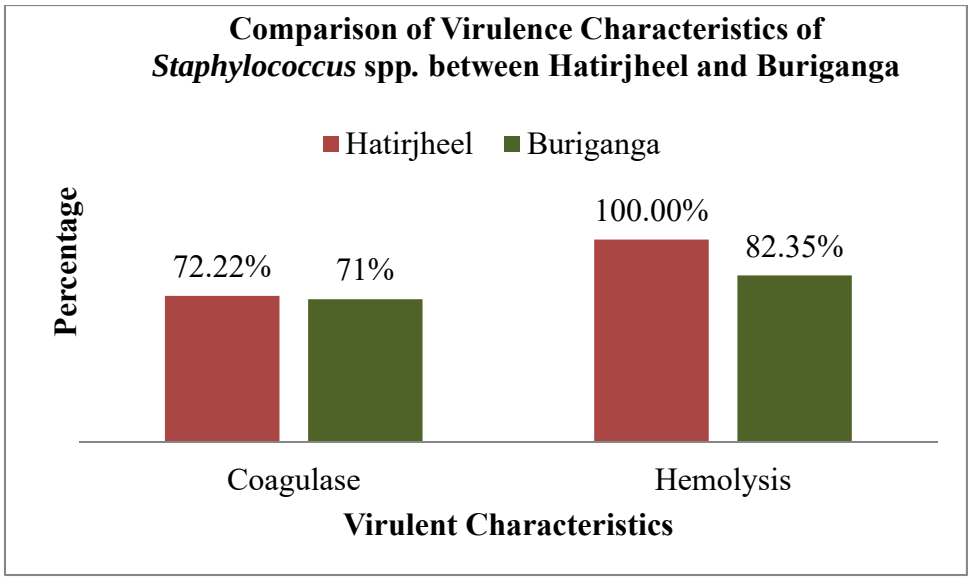

Figure 3.3: Graphical Translation of Virulent Characteristics of *Staphylococcus* spp. in Hatirjheel and Buriganga

The hemolysis pattern in Hatirjheel and Buriganga (Fig 3.4) indicated a notable difference. Alpha hemolytic Staphylococcus were present more in Hatirjheel than in Buriganga. In case of beta and gamma hemolysis, the results displayed a notable difference between Hatirjheel and Buriganga. Isolates that showed gamma hemolysis were all present only in Buriganga, while Hatirjheel did not have one.

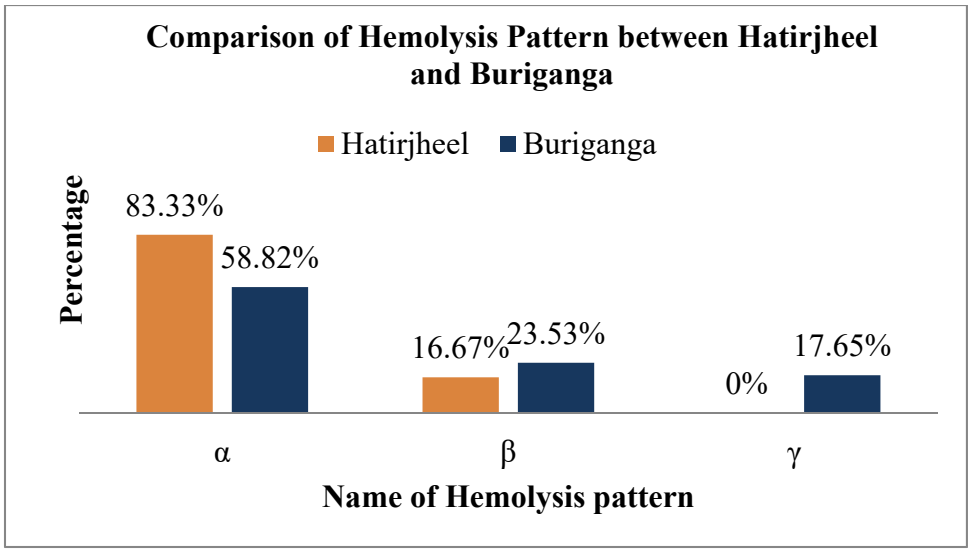

Figure 3.4: Comparison of Hemolysis Pattern between Hatirjheel and Buriganga

3.2 Antibiogram

Total 35 isolates, 18 from Hatirjheel and 17 from Buriganga were tested for antibiotic susceptibility against 9 antibiotics. The zones of inhibition were measured and compared with the standard CLSI chart to determine if they are resistant, intermediate or sensitive. Patterns of antibiogram showed slight difference between Hatirjheel Lake and Buriganga River.

The percentage of antibiotic resistance among 9 antibiotics represented in table 3.1.

Table 3.1: Antibiotic Resistance Pattern Together in Hatirjheel & Buriganga

Name of Antibiotics	Percentage of Resistance
Clindamycin	17.14%
Kanamycin	2.86%
Linezolid	2.86%
Cefoxitin	97.14%
Erythromycin	42.86%
Ceftazidime	77.14%
Tetracycline	2.86%
Norfloxacin	2.86%
Methicillin	20%

Out of 35 isolated, only 7 were MRSA, rest of the 28 isolates were MSSA. Only 1 isolate from Hatirjheel displayed MRSA positive strain, on the contrary, 6 isolates from Buriganga displayed MRSA positive strains.

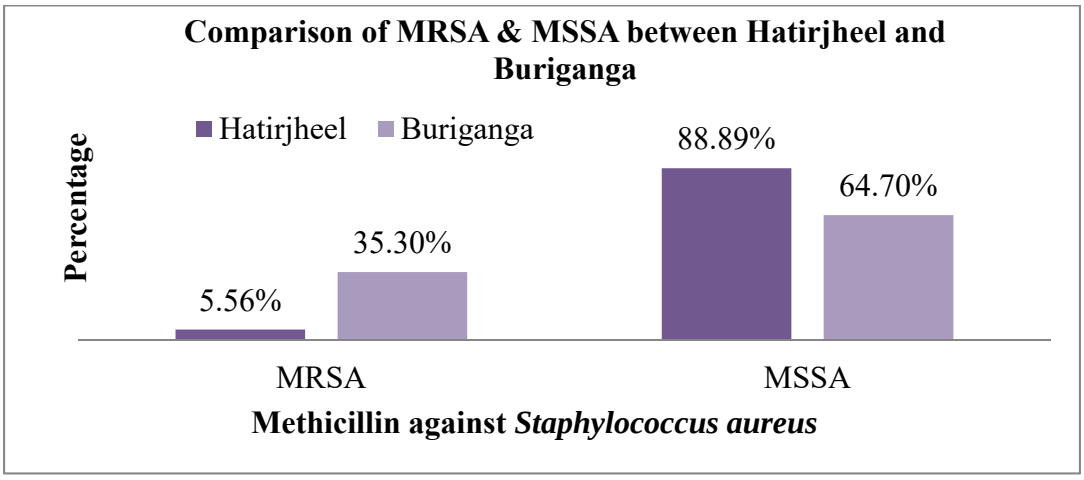

Figure 3.5: Graphical Representation of MRSA & MSSA between Hatirjheel and Buriganga

The Multidrug Resistance pattern between Hatirjheel and Buriganga was determined on the basis of resistance against two or more antibiotic classes.

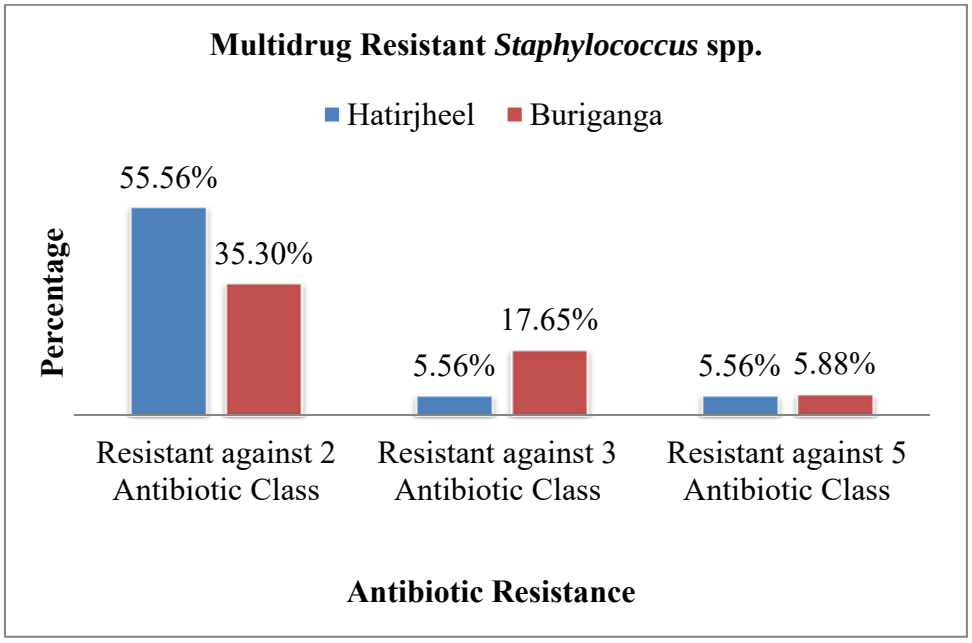

Figure 3.6: Graphical Translation of Multidrug Resistance Pattern of Hatirjheel and Buriganga

Total three categories were designed (Fig 3.6) in this study on the basis of antibiotic resistance among different groups or classes of antibiotic: 1) resistant against 2 classes of antibiotic, 2) resistant against 3 classes of antibiotic, and 3) resistant against 5 classes of antibiotic. Maximum resistance was observed among *Staphylococcus* spp for first category (resistant against 2 groups of antibiotics) compared to other two categories. In the first category, Hatirjheel and Buriganga showed 55.56% and 35.30% respectively. However, presumptive isolates of *Staphylococcus* spp. of Buriganga showed 17.65% higher than Hatirjheel 5.56% for the second category. Almost similar antibiotic resistance was observed for Hatirjheel (5.56%) and Buriganga (5.88%). The percentage of antibiotic resistance was gradually decreased among three categories from first to third. Sharp decrease was observed for Hatirjheel isolates compared to Buriganga isolates.

The resistance pattern of presumtive *Staphylococcus* spp.against 9 antibiotics for both Hatirjheel Lake and Buriganga river was represented in bar diagram (Fig 3.7) .

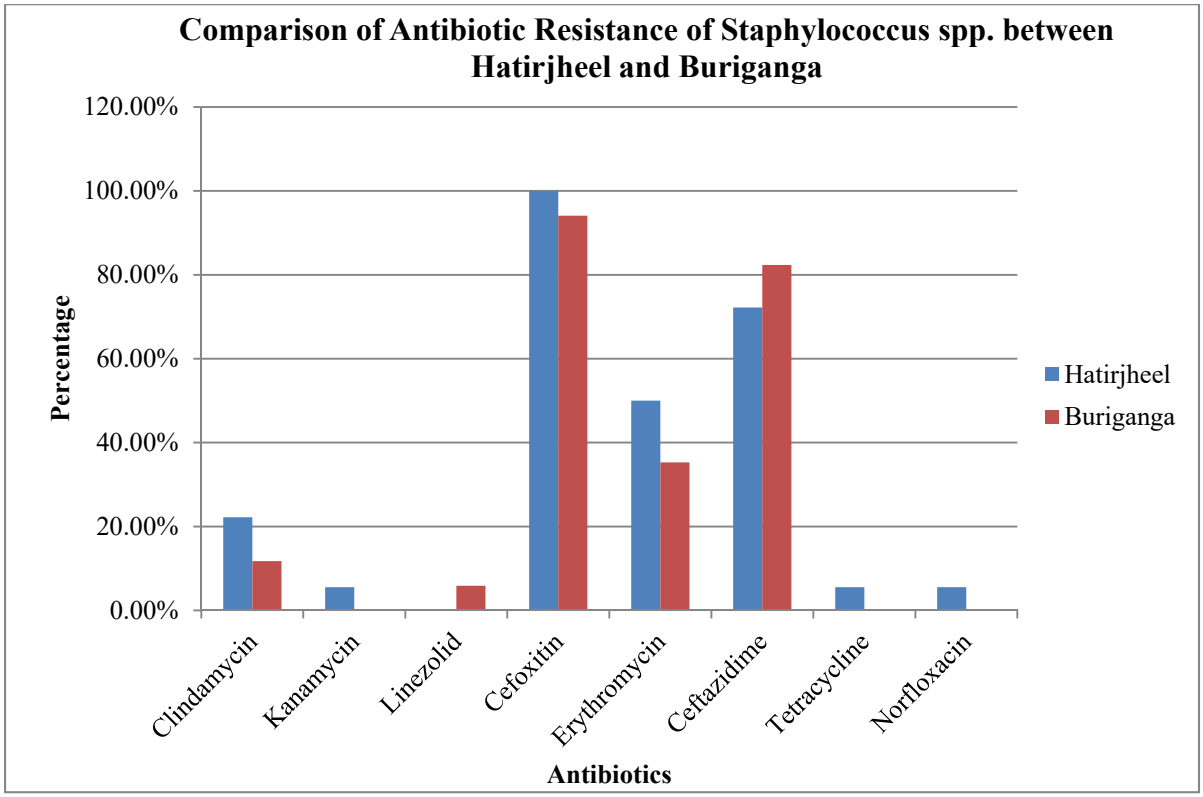

Figure 3.7: Graphical Representation of Antibiotic Resistance of Presumptive *Staphylococcus* spp. between Hatirjheel and Buriganga

It can be depicted from Fig 3.7 that Kanamycin, Tetracycline, and Norfloxacin showed 5.55% resistant to *Staphylococcus* spp. in Hatirjheel Lake, but 0% resistant towards Buriganga. The resistance pattern of Cefoxitin, Ceftazidime, and Erythromycin was potential among all other tested antibiotics because they showed high resistance for *Staphylococcus.* spp. Higher resistance to Cefoxitin and Erythromycin was observed for Hatirjheel isolates compared to Buriganga. However, presumptive *Staphylococci* isolates of Buriganga showed higher 82.35% resistance to Ceftazidime compared Hatirjheel 72.22%. While only Cefoxitin showed total 100% resistant in Hatirjheel Lake's isolates.

3.3 Statistical Analysis:

For statistical analysis, a chi-square test was performed to analyze the association of MRSA strains in Hatirjheel and Buriganga samples. A p-value of ≤ 0.05 was considered as statistically significant.

Chapter 4

Discussion

4.0 Discussion

The purpose of this study was to isolate and identify the presence of *Staphylococcus aureus*from two of the most notable water bodies in the Dhaka city; Hatirjheel Lake and Buriganga River. *Staphylococcus aureus* cause a range of illness impetigo, boils, cellulitis, folliculitis, scalded skin syndrome, to life-threatening diseases such as toxic shock syndrome, endocarditis, bacteremia, and sepsis. In Bangladesh, the occurrence of MRSA was alarming due to indiscriminate use of antibiotics. In 2002, the prevalence of MRSA on clinical *S. aureus* isolates was 47.2% (Rahman, 2002). In 2011, 43.48% MRSA was investigated on clinical samples (Hossain, 2011). This is why it is important to know if there are the presence of *Staphylococcus aureus* and possible MRSA in two major Water bodies of Dhaka.

For identification of *Staphylococcus* spp., Biochemical tests were performed. From the total isolates of 35, 40% of them were presumptively *S.aureus*. Those isolates that gave around 90.8% similarity for *S.aureus* subspecies aureus- were considered as *S.aureus* positive by ABIS online. However, rest of the 60% isolates predominantly include *S.hemolyticus*(17.14%), *S.kloosi*(11.42%) and other species incorporating (31.44%) *Staphylococcus gallinarum*, *Staphylococcus arlettae*, *Staphylococcus devriesei*, *Staphylococcus massiliensis*, *Staphylococcus chromogenes*.

From 40% of *S.aureus*, there were 57% from Hatirjheel and 43% were from Buriganga. The result showed that the Hatirjheel Lake contain high amount of *S.aureus* presence than in Buriganga River. This might be because Haitjheel has comparatively low water content than Buriganga.On top of that, the water that drains in Hatirjheel is stuck in one place. In one study it is mentioned that Hatirjheel is considered a closed aquatic system receiving storm water, sewage water and rain water (Sohail-us-samad, 2009). *Staphylococcus aureus* was one of the two most concentrate opportunistic pathogens present in urban storm water, according to a study, ranging from 10 to 1000 organisms/ml (Percival Lane, 2013).

After the completion of identification of the organisms, their antibiogram was performed in order to determine their resistance pattern.

Two virulent factors, such as: Coagulase and Hemolysiswere analyzed. This analysis suggested that Hatirjheel Lake had 72.22% coagulase positive *Staphylococccus* spp., whereas Buriganga Lake had 71%. Research showed coagulase-positive staphylococci (CoPS) are more resistant to antibiotics compared to coagulase-negative staphylococci (CoNS) (Sajadi, Kaboosi, & Ghadikolii, 2017)**.** However, this research showed mixed result. Both coagulase positive and negative staphylococci showed the most resistance against antibiotics. In this study coagulase-positive staphylococci demonstrated low resistance to tetracycline and kanamycin in oppose to above mentioned research paper (Sajadi et al, 2017). Hanselman et al (2009) in their study discovered that 27.7% of *S.aureus* was CoPS and 3.3% of MRSA was CoPS. However, in this study 56% *S.aureus* were CoPS and 14.28% of MRSA was CoPS.

A study suggested that CoNS can be important as an emerging pathogen (Moraveji, Tabatabaei, Shirzad Aski, & Khoshbakht, 2014). Another study claimed that CoNS is a reservoir of resistance genes that can be transferred to other pathogens such as *S.aureus* (May, Klein, Rothman, & Laxminarayan, 2014). To date, very limited number of studies has been done to discover the potentials of CoNS.

When the total percentage of Hemolysis pattern in*Staphylococcus* spp. between Hatirjheel and Buriganga was calculated, their result showed 100% and 82.35% hemolysis positive respectively. By the results it could be evident that Hatirjheel Lake contains *Staphylococcus* spp. that is all hemolysis positive and Buriganga River had some non-hemolytic *Staphylococcus* spp. Different hemolysis patterns of *Staphylococcus* spp. gave different results between Hatirjheel and Buriganga. Hatirjheel showed 83.33% α-hemolysis, on the other hand Buriganga showed 58.82%. 16.67% & 23.53% β-hemolysis were shown by Hatirjheel and Buriganga respectively. In terms of γ-hemolysis, Hatirjheel showed no gamma hemolysis but Buriganga showed 17.65% hemolysis. Thus, only α-hemolysis was higher in Hatirjheel Lake but both β-hemolysis and γ-hemolysis were higher in Buriganga River.

One study showed that hemolysin is one of the significant virulence agents of CoPS and CoNS. All isolates that showed non-hemolytic activity were CoNS (Moraveji et al, 2014). This fact was also evident in this study as non-hemolytic isolates were all CoNS. Till now; there are scarce information about hemolytic pattern in CoNS. Human isolates showing CoPS mostly produced

alpha-hemoysin although very few produced beta-hemolysin (Hummel et al, 1992, Aarestrup et al, 1999). Glandstone & Glencross (1960) examined that CoPS produced high yields of alpha-hemolysin than beta-hemolysin. In this experiment CoPS produced more alpha-hemolysis than beta-hemolysisin Hatirjheel. It means this study results support the claim. This might be because of the sewage and storm water discharges accumulate in Hatirjheel is greatly from human source. CoPS from Buriganga showed more β-hemolysis than Hatirjheel. According to Aarestrup et al., (1999) and Larsen et al. (2002), a high percentage (75-97%) of *S.aureus* strains isolated from bovine mastits produce beta-toxin, whereas only 10-15% of human isolates express this factor. Hazaribagh Tannery is one of the prime reasons of pollution in Buriganga River, suggested by the experts(Bhowmik, 2007).It might be a big reason for showing more β-hemolysis in Buriganga than Hatirjheel because the river water receives huge number of *S.aureus* from tannery discharges.

This study presented a difference between MRSA and MSSA. According to the study report, MRSA observed in Buriganga (35.50%) was higher than in Hatirjheel(5.56%) samples ($P \leq 0.05$). This automatically displayed more MSSA in Hatirjeel(88.89%) compared to Buriganga(64.70%). The reason Buriganga having a higher rate of MRSA than Hatirjheel might be because of the hospitals and clinics located near the river($P \leq 0.05$). From a field survey it was mentioned that Sir Salimullah Medical College, also known as Mitford Hospital had been taking the complete advantage of its location near Buriganga River by throwing waste dumping materials on it (Rahman, Ahmed, & Ullah, 1999)**.** Chintis *et al* (2004) blamed the improper management of hospitals wastes as a reason for spread of MRSA in his study. This could be an evidence for the hypothesis in this study that showed higher presence of MRSA in Buriganga than Hatirjheel ($P \leq 0.05$).

Methicillin-resistant *Staphylococcus aureus* may be nosocomial or community-acquired. Studies showed that antimicrobial resistance is wider for nosocomial-acquired MRSA (Maranan, 1997). Healthcare workers (HCWs) are most likely to be important in the transmission of MRSA, but more frequently act as vectors, rather than being the main sources of MRSA transmission (Albrich et al, 2008). The most important mode of MRSA transmission is through contamination of the hand. Healthcare workers' sanitation plays an immense role here. If they do not clean their hands properly after handling an MRSA patient then it might transfer to other patients, even to

him. The water they use for washing hands may also transmit MRSA, if not properly treated. Colonized HCW are most often transiently colonized, but they may become persistent carriers if they have chronic dermatitis or sinusitis and this may lead to prolonged MRSA transmission (Coia et al, 2006). Other authors suggested that MRSA incidence is low in the community (Abudu, 2001). MRSA is defined as community-acquired if the positive culture was obtained outside hospital settings within two days of hospital admission, and if the subject had no prior history of hospitalization within the preceding two years (Salmenlina, 2002).

The overall resistance pattern of *Staphylococcus* spp. showed 97.14% resistant to Cefoxitin which was the highest among all 9 antibiotics. Ceftazidime showed the second highest resistance pattern (77.14%). Erythromycin showed the third highest resistance pattern (42.86%). Kanamycin, Linezolid, Tetracycline and Norfloxacin showed the lowest resistance (2.86%).

Multidrug resistant (MDR) Staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA) is a serious problem in the treatment and control of Staphylococcal infections (Livermore, 2000; Zapun, Contreras-Martel, & Vernet, 2008).

In this study, isolates exhibited multidrug resistance Staphylococci, defined as resistance to two or more antibiotic classes. The only MRSA isolated from Hatirjheel showed resistance against 4 antibiotics. Among the 6 MRSA from Buriganga three of them were resistance towards 3 antibiotics, two of them were resistance towards 4 antibiotics and only one showed resistance towards 6 antibiotics.

Comparison of antibiotic resistance among Hatirjheel and Buriganga showed that isolates of Hatirjheel showed 100% resistant towards Cefoxitin which showed 94.11% resistant for Buriganga. Clindamycin and Erythromycin showed 22.22% and 50% resistance in Hatirjheel respectively. Linezolid showed complete susceptibility in Hatirjheel whereas showed 5.88% resistance in Buriganga. Ceftazidime showed more resistance in Buriganga (82.35%) than Hatirjheel. Overall Hatirjheel showed more resistant to antibiotics. Water from major storm sewer outfall, direct rainfall and runoff from surrounding areas fill up the Hatirjheel Lake. One investigation on Hatirjheel Lake showed heavy chromium pollution(Hashem, M.A., Nur-A-Tomal, M.S., Abedin, M.J., Bushra, 2017).

Conclusion and Future Direction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are more and more prevalent in the hospital environment and represent a challenge to infection control practices. Considering the fact that MRSA has been exposed to water bodies according to this study result, CA-MRSA should be considered as a serious threat as HA-MRSA. There is an enormous lack of research on the presence of MRSA in water bodies, although the surface water of lakes and rivers inside Dhaka are polluted with them.

In the future this work could be extended by doing molecular work to determine the mode of resistance among MRSA strains. Plasmid Profile should be done to discern whether the antibiotic resistant is chromosome mediated or plasmid mediated. Horizontal gene transfer can be a means that can act as a vector to transfer their genes to other members of the same bacterial species, as well as to bacteria in another genus or species. Skin diseases are occurring to people living near Hatirjheel and Buriganga which are unheard and untreated. But it could not gain the attention of mass population since the diseases probably cured within a short time period. But what should be a matter of great concern is that the people affected with these short-term skin diseases can act as a carrier to transfer diseases to healthy individuals which would be a more serious threat.

Further a survey can be conducted on the slums living near Hatirjheel Lake and Buriganga River to have a more vivid picture of affected individuals. There could be a single or multiple source of Staphylococcal contamination in water bodies. The reasons should be identified along with the sources.

Finally, campaigns should be arranged for general people to train them to practice some personal hygiene to prevent skin infections. Hands must be kept clean by washing thoroughly with soap and water or using an alcohol-based hand sanitizer. Cuts and scrapes should be kept clean and covered with a bandage until healed. Any kind of contact with other people's wounds or bandages should be avoided. Personal items such as towels, washcloths, razors, bars of soap, nail clippers, clothing, uniforms, and sheets should not be shared with other individuals.

Chapter 5

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Appendix

Media compositions

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

All the media used were from Himedia

Mannitol Salt Agar:

Composition	Amount(g/L)
Proteose peptone	10
Beef Extract	1
Sodium chloride	75
D-Mannitol	10
Phenol red	0.025
Agar	15
Final pH (at 25°C)	7.4±0.2

Nutrient Agar:

Composition	Amount(g/L)
Peptone	5
Sodium chloride	5
Beef Extract	1.5
Yeast extract	1.5
Agar	15
Final pH (at 25°C)	7.4±0.2

Mueller- Hinton Agar:

Composition	Amount(g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5

Starch	1.5
Agar	17
Final pH (at 25°C)	7.4±0.1

Blood Agar Base:

Composition	Amount(g/L)
Beef Extract	10
Tryptose	10
Sodium chloride	5
Agar	15
Final pH (at 25°C)	7.3±0.2

Coagulase:

Composition	Amount(g)
Coagulase Plasma	0.1

Saline:

Composition	Amount(g/L)
Sodium chloride	9.0

Motility, Indole, Urease Agar:

Component	Amount(g/L)
Peptone	3%
Sodium Chloride	0.5%
Urea	2%

Mono Potassium Phosphate	0.2%
Phenol Red	0.0005%
Agar	0.4%
pH	7

Simmons Citrate Agar:

Component	**Amount(g/L)**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Sodium citrate	2
Sodium chloride	5
Bromothymol blue	0.08
Agar	15
Final pH (at 25°C)	6.8±0.2

Triple Sugar Iron (TSI):

Component	**Amount(g/L)**
Beef extract	3
Peptone	20
Yeast extract	3
Lactose	10
Sucrose	10
Dextrose monohydrate	1
Ferrous sulphate	0.2
Sodium chloride	5

Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12

Indole Broth:

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

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

Component	Amount(g/L)
Buffered peptone	7
Dextrose	5
Dipotassium phosphate	5
Final pH (at 25°C)	6.9±0.2

Reagents

Gram's iodine (300 ml)

To 300 ml distilled water, 1 g iodine and 2 g potassium iodide was added. The solution was mixed on a magnetic stirrer overnight and transferred to a reagent bottle and stored at room temperature.

Crystal Violet (100 ml)

To 29 ml 95% ethyl alcohol, 2 g crystal violet was dissolved. To 80 ml distilled water, 0.8 g ammonium oxalate was dissolved. The two solutions were mixed to make the stain and stored in a reagent bottle at room temperature.

Safranin (100ml)

To 10 ml 95% ethanol, 2.5 g safranin was dissolved. Distilled water was added to the solution to make a final volume of 100 ml. The final solution was stored in a reagent bottle at room temperature.

Kovac's Reagent (150 ml)

To a reagent bottle, 150 ml of reagent grade isoamyl alcohol, 10 g of pdimethylaminobenzaldehyde (DMAB) and 50 ml of HCl (concentrated) were added and mixed. The reagent bottle was then covered with an aluminum foil to prevent exposure of reagent to light and stored at 4°C.

Methyl Red (200 ml)

In a reagent bottle, 1 g of methyl red powder was completely dissolved in 300 ml of ethanol (95%). 200 ml of destilled water was added to make 500 ml of a 0.05% (wt/vol) solution in 60% (vol/vol) ethanol and stored at 4°C.

Barrit's Reagent A (100 ml)

5% (wt/vol) a-naphthol was added to 100 ml absolute ethanol and stored in a reagent bottle at 4°C.

Barrit's Reagent B (100 ml)

40% (wt/vol) KOH was added to 100 ml distilled water and stored in a reagent bottle at 4°C.

Catalase Reagent (20 ml 3% hydrogen peroxide)

From a stock solution of 35 % hydrogen peroxide, 583 µl solution was added to 19.417 ml distilled water and stored at 4°C in a reagent bottle.

Urease Reagent (50 ml 40% urea solution)

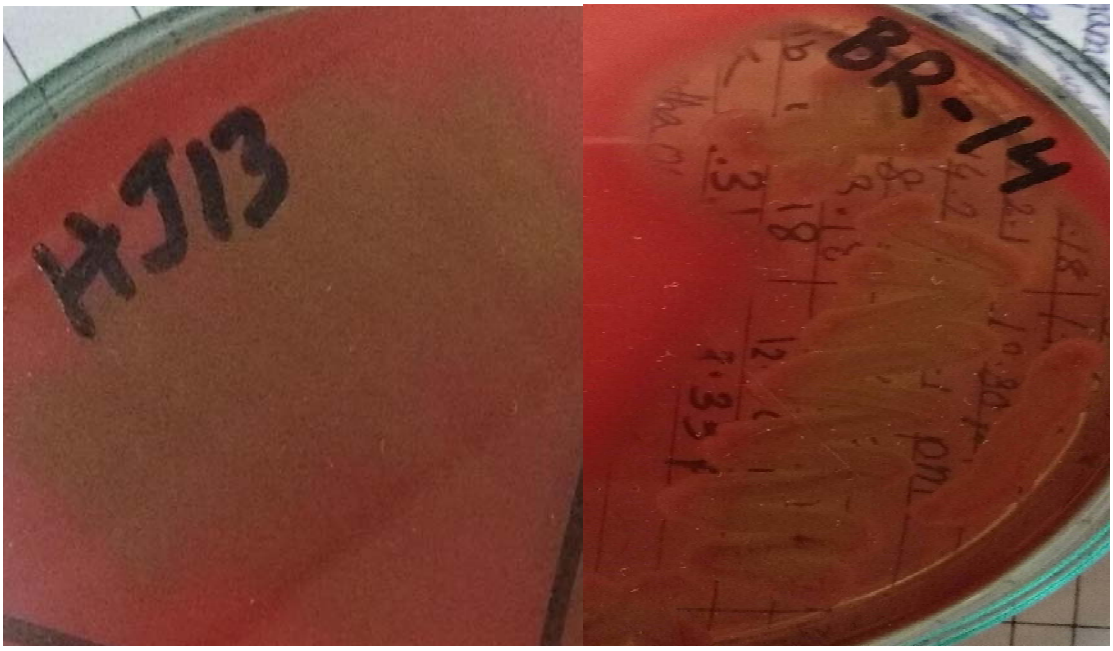
To 50 ml distilled water, 20 g pure urea powder was added. The solution was filtered through a HEPA filter and collected into a reagent bottle. The solution was stored at room temperature.

Supplementary

Results of Biochemical, Coagulase & Hemolysis Test

Sample No	Sample	Gran Staining	Catalase	Oxidase	IMViC				Urease	TSI				Coagulase	Hemolysis
					Indole	MR	VP	Citrate		Slant	Butt	Gas	H2S		
0	Control	+	+	-	-	-	+	-	-	A	A	-	-	+	β
1	HJ-1	+	+	-	+	+	-	-	-	-	-	-	-	-	α
2	HJ-4	+	+	-	+	-	-	-	-	-	-	-	-	-	α
3	HJ-5	+	+	-	-	+	-	-	-	A	A	-	-	-	α
4	HJ-6	-	-	-	-	+	-	-	+	A	A	-	-	-	α
5	HJ-7	-	+	-	+	-	-	-	+	A	A	-	-	+	α
6	HJ-8	+	+	-	-	-	-	-	+	A	A	-	-	+	β
7	HJ-9	+	+	-	-	-	-	-	+	A	A	-	-	+	β
8	HJ-10	+	+	-	-	-	-	-	+	A	A	-	-	+	α
9	HJ-11	+	+	-	-	-	-	-	+	K	A	-	-	+	α
10	HJ-12	+	+	-	+	-	-	-	-	-	-	-	-	+	α
11	HJ-13	-	-	-	-	-	-	-	-	A	A	-	-	-	α
12	HJ-14	+	+	-	-	-	-	-	+	A	A	-	-	+	β
13	HJ-15	+	+	-	-	-	-	+	+	K	A	-	-	+	α
14	HJ-16	+	+	-	+	-	-	-	+	-	-	-	-	+	α
15	HJ-17	+	+	-	-	-	-	-	+	A	A	-	-	+	α
16	HJ-18	+	+	-	-	-	-	+	+	A	A	-	-	+	α
17	HJ-19	+	+	-	-	-	-	-	+	A	A	-	-	+	α
18	HJ-20	+	-	-	-	-	-	-	-	A	A	-	-	+	α

Sample No.	Sample	Gram Stainig	Catalase	Oxidase	IMViC				Urease	TSI				Coagulase	Hemolysis
					Indole	MR	VP	Citrate		Slant	Butt	Gas	H2S		
19	BR-1	-	+	-	-	-	-	-	+	A	A	-	-	+	α
20	BR-2	+	+	-	-	-	-	-	+	A	A	-	-	+	α
21	BR-3	-	+	-	-	-	-	-	-	A	A	-	-	-	γ
22	BR-4	+	+	-	-	-	-	-	-	A	A	-	-	+	β
23	BR-5	+	+	-	+	-	-	-	+	-	-	-	-	+	α
24	BR-6	+	+	+	+	-	+	+	-	-	-	-	-	+	β
25	BR-7	-	+	-	+	+	+	-	+	A	A	-	-	+	α
26	BR-8	+	+	-	-	+	+	-	+	A	A	-	-	-	α
27	BR-9	-	+	-	-	-	-	-	-	A	A	-	-	+	α
28	BR-11	+	-	-	-	-	-	-	-	A	A	-	-	-	α
29	BR-12	+	+	-	-	-	-	-	+	A	A	-	-	+	α
30	BR-14	+	+	-	-	-	-	-	+	A	A	-	-	+	β
31	BR-15	-	+	-	+	-	-	-	-	-	-	-	-	-	γ
32	BR-16	+	+	-	-	-	-	-	+	K	A	-	-	+	α
33	BR-17	+	+	-	-	-	-	-	+	K	A	-	-	+	β
34	BR-18	-	+	-	-	-	-	-	+	A	A	-	-	-	γ
35	BR-19	-	+	-	-	-	-	-	+	A	A	-	-	+	α



α Hemolysis **β Hemolysis**

γ Hemolysis

Figure: α, β, γ Hemolysis

Figure: Coagulase test in Buriganga River and Hatirjheel Lake.

Antibiotic Resistance Pattern in Hatirjheel & Buriganga

Sample	Clindamycin	Kanamycin	Linezolid	Cefoxitin	Erythromycin	Ceftazidime	Tetracycline	Norfloxacin	Methicillin
HJ-1	R	S	S	R	R	R	S	S	S
HJ-4	R	S	S	R	S	R	S	S	S
HJ-5	R	S	S	R	S	S	S	S	S
HJ-6	S	I	S	R	R	I	S	S	S
HJ-7	S	S	S	R	R	R	S	S	S
HJ-8	S	S	S	R	S	R	S	S	S
HJ-9	S	S	S	R	S	S	R	S	S
HJ-10	S	S	S	R	R	R	S	S	I
HJ-11	S	S	S	R	S	R	S	S	S
HJ-12	I	S	S	R	R	R	S	S	S
HJ-13	S	S	S	R	R	R	S	S	R
HJ-14	S	S	S	R	S	R	S	S	S
HJ-15	S	S	S	R	R	R	S	S	S
HJ-16	S	S	S	R	R	R	S	S	S
HJ-17	R	R	S	R	R	S	S	R	S
HJ-18	S	S	S	R	S	S	S	S	S
HJ-19	S	S	S	R	S	R	S	S	S
HJ-20	S	S	S	R	S	R	S	S	S
BR-1	S	S	S	R	R	I	S	S	R
BR-2	S	S	S	R	S	R	S	S	R
BR-3	R	R	S	R	R	R	S	S	R
BR-4	S	S	S	R	S	R	S	S	S

Sample	Clindamycin	Kanamycin	Linezolid	Cefoxitin	Erythromycin	Ceftazidime	Tetracycline	Norfloxacin	Methicillin
BR-5	S	S	S	S	S	R	S	S	S
BR-6	R	S	S	R	S	R	S	S	S
BR-7	S	S	S	R	S	R	S	S	S
BR-8	I	S	S	R	S	R	S	S	S
BR-9	S	S	S	R	I	S	S	S	S
BR-11	S	S	S	R	R	R	S	S	R
BR-12	S	S	S	R	S	S	S	S	S
BR-14	I	S	S	R	S	R	S	S	S
BR-15	S	S	R	R	I	R	S	S	R
BR-16	S	S	S	R	S	R	S	S	R
BR-17	S	S	S	R	R	R	S	S	S
BR-18	S	S	S	R	R	R	S	S	S
BR-19	S	S	S	R	R	R	S	S	S

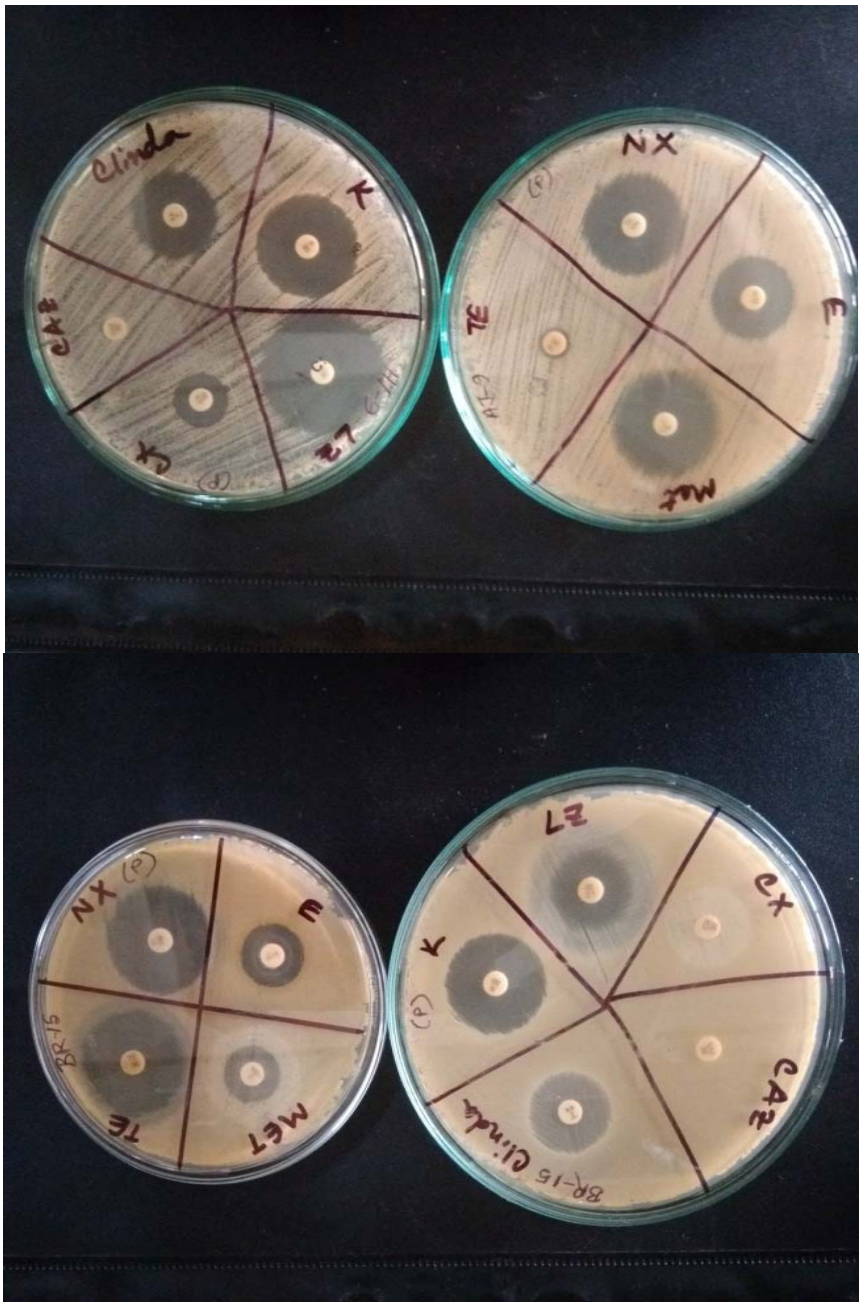


Figure: Antibiogram plates for Buriganga River and Hatirjheel Lake.