Prevalence and Antimicrobial Resistance Pattern of *Staphylococcus aureus* in the Hospital Waste Water and Adjacent Community Water in Dhaka city

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

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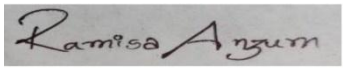
Declaration

It is hereby declared that

The thesis submitted titled **"Prevalence and Antimicrobial Resistance Pattern of *Staphylococcus aureus* in the Hospital Waste Water and Adjacent Community Water in Dhaka city "**

1. is our very own work which is written for the purpose of our BSC degree at BRAC university.
2. The thesis does not contain any previous data from any third party, whereas the information taken from any external sources has been cited and references have been provided accurately.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have enriched our paper with all the main sources of help.



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The thesis titled **"Prevalence and Antimicrobial Resistance Pattern of *Staphylococcus aureus* in the Hospital Waste Water and Adjacent Community Water in Dhaka city"** submitted by

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Ethics Statement

Samples from certain locations were obtained for this study's completion while taking all necessary safety procedures. The BRAC University Life Sciences Laboratory served as the site of all investigations. It should be mentioned that neither human nor animal models were used in this investigation.

Abstract:

Staphylococcus aureus is an important pathogen that has the potential to cause infections both in nosocomial and community settings. In terms of morphology, *Staphylococcus aureus* is a gram-positive bacteria that causes life-threatening diseases starting from skin infections to food poisoning. The organism was initially known to be a leading nosocomial pathogen, but epidemiologically unique variants started to prevail in the community water as well. *S. aureus* has shown resistance to many antibiotics, resulting in significant mortality and morbidity.

From December 2022 to June 2023, 68 samples both from hospital and community settings were collected. From the collected samples, 33 PCR-confirmed *Staphylococcus aureus* isolates were found. However, 31 positive isolates were derived from community tap water but surprisingly only 2 positive isolates were derived from community tap water. It was observed that all the positive isolates were derived from community water and found to be highly resistant to aztreonam and clindamycin, whereas the isolates also showed resistance to a specific class of antibiotics (Tetracycline, Clindamycin, Ciprofloxacin, Erythromycin, and cefepime).

Our research paper will give an overview of how Staphylococcus aureus is emerging in hospital effluents and consecutively spreading to the community tap water thus resulting in causing nosocomial infections. A more detailed knowledge of the virulence and pathogenicity of *Staphylococcus aureus* will enable us to familiarize ourselves that how ARGs have emerged in the strain of Staphylococcus aureus, which led to its spreading in adjacent community water. It was speculated that these ARBs and ARGs were spread from hospital facilities through the hospital's untreated wastewater.

Keywords: *Staphylococcus aureus*, ARGs, ARBs, Hospital wastewater.

Dedication

"The feeling of awed wonder that science can give us is one of the highest experiences of which the human psyche is capable." – Richard Dawkins

We would like to dedicate the thesis to our parents. Words can hardly express my gratitude and admiration for them. They have taught us how to be independent and determined. They have helped to grow the confidence in us. They have never been taught to give up on the way of progression. They are the inspiration and motivation for us. Thanks a lot for the support we have always received from them.

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List of Acronyms

MRSA – Methicillin Resistant *Staphylococcus aureus*

PBP2a – Penicillin Binding Protein

SCCmec- Staphylococcal cassette chromosome mec

MDR – Multi Drug Resistance

HA-MRSA – Health Associated Methicillin Resistant *Staphylococcus aureus*

CDC – Centre for Disease Control and Prevention

NI – Nosocomial Infection

ARB – Antibiotic Resistant Bacteria

HGT – Horizontal Gene Transfer

WWTP – Waste-water Treatment Plans

CA-MRSA – Community-acquired Methicillin Resistant *Staphylococcus aureus*

VRSA – Vancomycin Resistant *Staphylococcus aureus*

PCR – Polymerase Chain Reaction

MIC – Minimum Inhibitory Concentration

HWWs – Household Well Water System

ARGs – Antibiotic Resistance Genes

AR – Antibiotic Resistance

DNA – Deoxyribonucleic Acid.

DNCC – Dhaka North City Corporation

AMR – Anti-microbial Resistance

NICRH – National Institute of Cancer Research & Hospital

DSH – Dhaka Shishu (Children) Hospital

TSB – Tryptic Soy Broth

MSA – Mannitol Salt Agar

NA – Nutrient Agar

MHA – Muller Hilton Agar

LB – Luria Bertani broth

PBS – Phosphate-Buffered Saline

TE – Tris – EDTA

EDTA – Ethylenediamine Tetra acetic Acid

MCT – Micro-Centrifuge Tubes

TBE – Tris-borate-EDTA

UV – Ultra Violate

Bp – Base-pair

CLSI – Clinical and Laboratory Standards Institute

AST – Antibiotic Susceptibility Test

HAI – Healthcare Associated Infection

SSSI – Skin and Skin Structure Infection

RNA – Ribonucleic Acid

Chapter 1

Introduction

1.1 Background

Staphylococcus aureus is an opportunistic pathogen as it can cause many skin diseases, from soft tissue infections to more severe diseases, including toxic shock syndrome, necrotizing pneumonia, and endocarditis.

Staphylococcus aureus is a skin microbiota and is also found in the nasal passages of 20-30% of healthy people and animals (Enright, 2008). In short, *Staphylococcus aureus* is a skin microbiota. But, *Staphylococcus aureus* has become one of the pathogens responsible for causing nosocomial infection. It is concerning that, some strains of *Staphylococcus aureus* are showing resistance to many antibiotics, making the therapy and prevention for Staphylococcal infections more critical. The initial part of our study discusses the spread of *Staphylococcus aureus* in hospitals.

Spread of *Staphylococcus aureus* in hospitals and community water

Staphylococcal infection can be developed by anyone, however, a specific aged of people are more vulnerable to this infection. These include people with chronic conditions such as diabetes, cancer, lung diseases, and so on. In hospitals, the risk is higher because many patient immune system is weak/compromised as well as it is also risky for the patients in the intensive care unit (ICUs), patients who had surgeries, and so on. The main source of *S. aureus* in hospitals is septic lesions and carriage sites of patients and personnel. In practical terms, septic lesions and the wounds of patients are the main sources of *S.aureus* in hospitals. These are the areas where the microbes tend to grow and spread from one patient to another patient. As a result, by direct or indirect route of transmission, *S.aureus* gets discharged into the hospital wastewater. Also, *S.aureus* forms biofilms which makes it more persistent in

water systems and resistant to antibiotics (Otto 2006; Hoiby *et al.,* 2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) consisting of almost 50% isolates is responsible for causing infections in healthcare settings globally.

Staphylococcus aureus is globally considered the major leading community-based bacterial agent. It is worth mentioning its importance as a human pathogen due to its potential to cause infections and its capacity to thrive in any environmental conditions and multiple antimicrobial resistance (WHO, 2017). Both methicillin-resistant and susceptible strains of *Staphylococcus aureus* are currently responsible for causing community-based infections throughout the world. It is happening because of its enhanced virulence, colonization potential, antibiotic resistance, and transmissibility. Household reservoirs are mainly encouraging them. The likelihood of recurring infections emerges when staphylococci become an ongoing problem in houses, either as colonizers or as environmental contaminants.

The widespread use of antibiotics to prevent or treat hospital-acquired infections in humans leads to the selection of resistant strains. Patient urine and or feces contain between 10 to 90 percent of the used antibiotics as metabolites (Berkner *et al.,*2014). Due to the widespread use of antibiotics in hospitals, antibiotic concentrations are much higher in hospital wastewater compared to domestic wastewater (Bisseux *et al.,* 2014). A particular strain for the selection of antibiotic-resistant strains is created by the wide population of different bacteria, antimicrobial agents, and nutrients in the sewage environment which gives rise to newly resistant mutant bacteria (Kummerer,2009). Moreover, the resistant genes can be transmitted to the neighbouring bacteria present in the sewage. Although wastewater treatment technology has advanced, resistant microbes are not eliminated from hospital sewage during the wastewater treatment process (Liu et al., 2008). Thus, hospital wastewater has become a particular concern.

1.2 The emergence of Antibiotic resistance and its spread to hospital effluents to community settings:

Since the 1940s, antibiotics have been the most important drugs for treating infections. But some bacteria have acquired resistance to almost all kinds of available antibiotics, making them capable of causing diseases that may hamper human health and creating a huge public health concern. Antibiotic-resistant bacteria (ARB) have evolved and spread across human and animal populations worldwide due to selective advantage brought on by the misuse of antibiotics (Xiong *et al*.,2015).

The hospital environment has been recognized as one of the main sources of antimicrobial resistance (AMR) due to the widespread use of a broad spectrum of antibiotics and the discharge of antibiotic-resistant genes (ARGs). These antibiotic-resistant genes are getting released into the environment through hospital effluents (Brown *et al.,* 2015). Some antibiotic-resistant pathogens (MRSA, CRE, VRE) may cause serious risks to human health (Xiao *et al.,* 2017). The potential effluent source is hospital sewage water disperses many ARG and ARB; they thrive in the environment as a result it speeds up the proliferation of multiple antibiotic-resistant bacteria.

Antibiotics play a role in treating most bacterial infections, but some strains are getting very difficult to treat and other therapeutic alternatives are failing as well. The term ESKAPE refers to six pathogens with increasing multi-drug resistance and increased virulence. The main six pathogens responsible for the majority of nosocomial infections are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumon*ia, *A. baumani, P. aeruginosa* and Enterobacter species (Rice et al., 2008). Bacterial evolution is the cause of AMR, it is growing increasingly, and still, we are missing new drugs to combat these emerging superbugs.

In addition, wastewater contains a large number of antimicrobial compounds, including partially metabolized antibiotics and detergents which altogether act as selective inhibitors of ARB proliferation (Wellington *et al.,* 2013; Berendonk *et al.,* 2015; Martinez and Baquero, 2017). The nutrient-rich environment provides an ideal niche for ARB proliferation through the HGT (Horizontal gene transfer) of ARGs (Pilmis *et al.,* 2020). The high microbial load of the distribution system can also promote ARB proliferation. The sewage from healthcare settings may also contain discarded or unused partially metabolized medicinal products excreted through clinical patient feces and urine creating additional selective pressure on ARB within the sewage networks (Rodriguez *et al.,* 2014).

Chapter 2

LITERATURE REVIEW

2.1 *Staphylococcus aureus*

*Staphylococcus aureu*s is one of the species of the genus Staphylococcus and a member of the family Micrococcaceae. Staphylococci are Gram-positive and catalase-positive coccoid organisms that grow within the range of 7–48 degrees Celsius, with an optimum of 35-40 degrees Celsius, and have the capability to metabolize glucose oxidatively or fermentatively. It is 0.5 to 1.5 micrometers in diameter, and when seen under a microscope, these cells create clusters that resemble unique "bunches of grapes" (Balaban & Rasooly, 2005). On media, these organisms can be grown up to 10% salt and the colonies often show gold or yellow color colonies. These organisms can grow aerobic or anaerobic at temperatures between 18°C and 40°C.

Staphylococcus aureus is an important nosocomial infection that causes serious infections in humans (Lowy,1998). Infections are common in both community-acquired and hospital-acquired cases and the preventive measures against the emergence of MRSA (Methicillin-resistant *Staphylococcus aureus*) still remain a challenge (Boucher &Corey, 2008). *Staphylococcus aureus* (including antibiotic-resistant MRSA) is present on the skin and mucous membranes, and humans are known to be the main reservoir of these organisms (Boucher & Corey, 2008). It is observed that 15% of the population tends to have persistent *S.aureus* colonization in the anterior nares. Whereas, some populations tend to have high *S.aureus* colonization up to 80%; such as the workers in hospitals, hospitalized patients, and people with weakened immune systems.

Additionally, *S. aureus* can lead to foodborne intoxications, particularly when meat and other food are mishandled by dairy-based goods (Lindsay,2008). The wide variety of virulence

factors that *S.aureus* creates, contains various matrix-binding proteins that make the *S.aureus* capable to adhere to host surfaces, and a varied set of secreted toxins and hydrolytic enzymes that ensures its immense invasive potential, are primarily responsible for its capability to create diseases.

2.2 Emergence and Evolution of MRSA

Anti-microbial therapy has always proved ineffective for controlling *Staphylococcus aureus* and no effective treatment has ever been able to evolve throughout the last 50 years. In the year 1961, Methicillin-resistant *S.aureus* (MRSA), the first clinical isolate was described. Since then, MRSA started to spread gradually and in the 1970s it started to cause serious problems in hospitals all over the world. Over the past forty years, several MRSA clones have emerged, spread, and developed (Hiramatsu, 1995). These clones have shown exceptional success in changing their genetic material through mutagenesis and external genetic uptake only by allowing themselves to respond to the increased use of antibiotics and selection pressure (*Kuroda et al.* 2001).

MRSA is known to carry the mecA gene of *S. aureus* strains which codes for PBP2a, an extra penicillin-binding protein. Penicillin-binding protein (PBPs) works as crucial enzymes for the formation of bacterial cell walls but it is made inactive by beta-lactam antibiotics, which reduces their ability to fight bacteria. Even though they have a weak affinity for PBP2a, these antibiotics still allow bacteria to survive in the presence of beta-lactam antibiotics because this enzyme continues to function as an important PBP and avoids being inactivated. MRSA is resistant to almost all beta lactam antibiotics due to the presence of mecA (Fuda &Suvorov, 2004). Due to plasmid transfer of the penicillinase gene (blaZ) and clonal spread of resistant

strains, more than 80% of *S. aureus* isolates by the late 1960s were penicillin-resistant, regardless of their origin in the community or a hospital (Chambers, 1984).

As was already mentioned, the gene mec A, which codes for PBP2a, is present in MRSA isolates. The gene is a component of the staphylococcal cassette chromosome mecA (SCCmecA), a 21–60 kb mobile genetic element. Two theories explain the origin of MRSA. According to the single clone hypothesis, the mobile genetic material only ever entered the S. Aureus population once, leading to the development of a single MRSA clone that has subsequently spread globally. The second and most widely accepted theory states that MRSA strains have undergone multiple iterations through the horizontal transfer of the mobile genetic element into phylogenetically diverse MSSA precursor strains (Enright & Robinson, 2002).

2.3 Virulence factor of *Staphylococcus aureus*:

Staphylococcus aureus possesses a variety of virulence factors. The factors make the organism to be successful as a pathogen to create infections in humans and animals. Virulence factor help in attachment to host cells, the breakdown of the host immune system, tissue invasion, sepsis and facilitate toxin-mediated syndromes. This is the underlying cause of staphylococcal infections that persists despite weak host immune responses (Kim et al.,2016). Based on their mechanism of action and role in pathogenesis, Staphylococcal virulence factors are classified below:

Factors	Characteristics
Helping attachment to host tissues	
Microbial surface components recognizing adhesive matrix molecules (MSCRAMM)	Cell surface proteins that interact with host molecules such as collagen, fibrinogen, and

	fibronectin thus elicit the tissue attachment. Staphylococcal protein A, fibronectin-binding proteins A and B, collagen-binding protein, and clumping factors A and B belong to this family. They contribute to host immune invasion. (Vasquez *et al.,* 2011).
Breaking/ evading the host immunity	
Polysaccharide microcapsule	Withstands phagocytosis and killing by polymorphonuclear phagocytes (Nilsson,1997)
Protein A	It acts as a superantigen, binds to the Fc region of an immunoglobulin, suppresses opsonization, and restricts the host immune response (Hong *et al.,*2016).
Alpha toxin (Alpha hemolysin)	It was the first bacterial exotoxin to be recognized as a cell membrane pore former which causes cell leakage and death.
Chemotaxis inhibitory protein of S.*aureus* (CHIPS)	CHIPS are known as an extracellular protein that inhibits the chemotaxis functioning of neutrophils and monocytes (Postma *et al*.,2004)
Tissue invasion:	
Extracellular adherence protein (Eap)	An exoprotein that binds to the host cell matrix, plasma protein, and endothelial cell

	adhesion molecule ICAM-1. It also contributes to adhesion and invasion and also has immune modulatory activity (Edwards *et al.*, 2012).
Protease, lipase, nucleases, phospholipase C, metalloproteases(elastase), and staphylokinase	These enzymes contribute to tissue destruction as well as help in bacterial penetration into tissues.

Table 1: *Staphylococcal* virulence factors

2.4 Pathogenesis:

The process of *S.aureus* infections involves five stages. These are:

1) Colonization 2) Local infection 3) Systematic dissemination and or sepsis 4) Metastatic infections 5) toxinosis

The microorganism can colonize the anterior nares and be persistent for weeks or months without causing infection. Colonization causes infection under some particular reasons. Such as: if anyone stays in the hospital for a long time, immunocompromised patients, surgery, use of invasive medical devices, and so on. A local skin abscess occurs when the pathogen is injected into the skin at the site of infection. It can further spread and cause many serious infections such as epilepsy, cellulitis, bullous impetigo, and wound infections. Pathogens can enter the blood and cause sepsis. Next, it can take a bigger form and cause diseases like endocarditis, osteomyelitis, and pyogenic arthritis. Moreover, certain syndromes can also occur in the absence of bloodstream infection. These are toxic shock syndrome, scalded skin syndrome, and food-borne gastroenteritis.

2.5 Hospital wastewater as a primary source for ARGs and ARBs:

HWW (Hospital wastewater) serves as a suitable growth medium for a wide variety of disease-causing microorganisms such as bacteria, fungi, and parasites. Hospital wastewater also contains some resistant bacteria and antibiotic residues which can suppress the growth of susceptible bacteria, thereby increasing the number of resistant bacteria in water bodies. Resistant bacteria that enter the environment acts as a carrier of infectious genes or carriers of antibiotic resistance genes (ARG) that causes a serious threat to public health hazard (Asfaw *et al.,* 2017). HWW Is being discharged directly into the community. Wastewater contains disease-causing parasites which also increase the risk of skin infections and other harmful diseases in humans (Asfaw et al., 2017). To process or treat the wastewater of a hospital, the development of certain advanced techniques is a must thing to do. Only then, the release of harmful contaminants into the environment will be lessened.

Moreover, as antibiotics are introduced by various human activities, the environment has become a potential reservoir of ARGs and ARBs, resulting in the emergence and transmission of resistance genes. The scenario of ARG emergence and prevalence is increasing at an alarming rate, and 70% of all nosocomial infections are showing resistance to at least one of its antibiotic families. HWW is an important source of ARGs and ARBs and such effluents are highly contagious due to their infectious and toxic properties (Wiederhold,2017). Resistance to antibiotics has already been viewed as a health problem but it is now reported that the nonclinical setting is an important factor in the spread of resistant genes (Berglund, 2015). Sewage treatment plants act as a hotspot for the emergence and spread of ARGs and ARBs in ecosystems (Rizzo *et al.,* 2013). Even after processing the wastewater, aquatic ecosystems are suitable sites for the acquisition and dissemination of such genes, as some

antibiotics and ARGs are not completely removed from the waterbodies. According to the estimation provided by WHO, ARBs are responsible for 25,000, 23,000, and 38,000 deaths annually in the European Union, the United States, and Thailand (WHO,2014). Many human pathogens such as *E. Coli, A.baumannii, Enterococcus sp., Klebsiella pneumonia, S. aureus, Pseudomonas aeruginosa, Serratia marcescens, and Citrobacter.* And other Enterobacter species exist, and there are increasing reports of the emergence and distribution of these pathogens in different environmental settings.

Studies on HWW have shown that the most common disease-causing bacteria belong to the genus Bacillus, accounting for 80-90%, with Staphylococci and Streptococci varying between 5-10% (Oyeleke & Istifanus, 2009). *Staphylococcus aureus* is the most common pathogenic Gram-positive bacterium with a high degree of multidrug resistance (MDR). Studies have shown the emergence of MDR bacteria varies between o.58% and 40% depending on HWW size and origin (Chitnis et al., 2000). Nosocomial infections have led to an increase in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

Pathogenic microorganisms present in HWW have impacted human health for decades, and the number of antibiotic-resistant microorganisms has also increased significantly with time. Outbreaks of antimicrobial resistance have been observed globally and are becoming a challenge to both public and animal health. The usage and discard of different antibiotics in different environments result not only in the prevalence of his ARGs in the environment but also in the spread and emergence of resistant strains. This has led to increased resistance to pathogens in humans, making it more challenging to fight the infections they cause and leading to high mortality. However, due to the increasing impact of antimicrobial resistance on human health, monitoring its prevalence and spread in the environment is limited and needs to be scaled up.

2.6 Horizontal gene transfer

Staphylococcus aureus is an opportunistic human pathogen which has a unique ability to develop resistance against antibiotics very quickly. *S. aureus* contains variety of virulence and resistance genes that are embedded in mobile genetic elements, according to genomic research. It is understood that the two primary mechanisms behind the spread of resistant phenotypes are the clonal dissemination of resistant genotypes and the dissemination of genetic factors among the bacterial population.

Different antibiotic-resistance genes and virulence factors have been linked to mobile genetic elements (MGEs) in the case of *S. aureus* (Lindsay,2010). The existence of these components in the genome of *S. aureus* suggests that the acquisition and transfer of genetic material within the bacterial population may be crucial for *S. aureus* adaptability and evolution indicating that horizontal gene transfer (HGT) was important in the evolution of *S. aureus*.

HGT conducts the movement of genetic material between unicellular and/or multicellular organisms other than by the transmission of DNA from parent to offspring. It is an important factor that plays a vital role in the evolution of many organisms. Transformation, conjugation, and phage transduction are three well-known HGT processes in Gram-positive bacteria that can exchange genetic material (Morikawa K, *et al.,*2012).

2.6.1 Conjugation:

The incQ family comprises the majority of the conjugative *staphylococcal* plasmids examined. The *staphylococcal* conjugative plasmid known as pGO1, which is also regarded as the model for this kind of plasmid, is one of the most well-known examples (Thomas & Archer,1989). The basic machinery required for conjugation includes the oriT, a nickase protein (nes), and the tra operon, and all the conjugative genes are situated on a 14.5 kb tract. This plasmid exhibits strong parallels to other Gram-positive conjugative plasmids, such as

the *staphylococcal* pSK41 plasmid (Berg et al.,1998), the lactococcal pMRC01(Schwarz *et al.,* 2001), and the enterococcal pRE25 in terms of the genetic structure of the tra operon (Dougherty *et al.,*1998).

The pGO1/pSK41 family of *staphylococcal* plasmids has a significant resemblance concerning the arrangement of conjugative genes, and they also contain an identical IncQ-type relaxase and a nickase gene (nes) that causes the nick at the oriT (Berg et al.,1998). These plasmids are self-conjugative and can recruit tiny, non-conjugative coresident plasmids. They may also be connected to resistance to antiseptics, penicillin, trimethoprim, bleomycin, tetracycline, macrolides, lincosamide, streptogramin B, and other aminoglycosides (Ramsay et al., 2016). They are also linked to resistance to gentamicin (and other aminoglycosides). These plasmids played a role in the evolution of Staphylococcal species in environments rich in antibiotics, and they have most recently been linked to resistance to the two main antibiotics used to treat MRSA, linezolid and vancomycin.

2.6.2 Natural transformation:

A group of DNA-uptake proteins that are produced in the bacterial membrane are necessary for natural transformation because they function to absorb environmental DNA. Once DNA has been absorbed into the cytoplasm, it can be used as a source of nutrients, a template to repair damaged genetic material or to improve bacterial fitness by creating variety or introducing novel features (Chen, 2005)

S. aureus was considered a non-transformable species until natural transformation was demonstrated in 2012(Morikawa *et al.,* 2012). Long DNA fragments can be transferred naturally through a process called transduction that is too large for bacteriophages (Thomas & Neilson,2005). The long staphylococcal cassette chromosome mec (SCCmec) type II element was successfully transferred via transformation, which gave rise to the theory that the

natural transformation may also be responsible for the exchange of large DNA portions between various *S. aureus* clonal complexes (Lindsay, 2014).

A method unique to each species controls how competence develops. The alternative sigma factor H (SigH) functions as the primary competence regulator in *S. aureus.* The competence machinery genes (comG and comE operons), which are crucial for the emergence of natural transformation, are activated by SigH (Morikawa *et al.*, 2012). Additionally, the SigH regulon's expression is improved by the transcription factor ComK (Fagerlund *et al.,*2014). In a small population, SigH is expressed by two different processes. A "short-junction duplication" can reorganize the sigH gene, and an inverted repeat (IR) motif in the 5'-UTR of sigH mRNA can

regulate it post-transcriptionally. Though the ribosome-binding site is thought to be hindered by the IR, the activation process is yet unclear.

2.6.3 **Transduction:**

In transduction, chromosomal and extrachromosomal DNA from the donor bacteria is transferred to the recipient bacteria using a moderate bacteriophage as a carrier so that the recipient bacteria can pick up new features (Chiang *et al.,* 2019). The coexistence of phages and ARGs in the same bacterial species and ecological setting raises the possibility that phages may contribute to the transmission of drug-resistance genes (Barcelo,2018). *Staphylococcus aureus* exhibits higher resistance transduction (Foster,2017). Methicillin-resistant *Staphylococcus aureus* (MRSA) gains resistance from other bacterial species by phage-mediated transduction, which confers the mecA gene (Craft *et al.,* 2019). Phage φ80α can promote the transfer of resistance genes to S. Aureus species that are resistant to phages as well as the multidrug-resistant *S. aureus* strain USA300 (Micoli, 2021).

2.7 Knowledge gap in the existing literature:

Our study indicates that HWW is an important reservoir of ARGs and that present current wastewater treatment processes are not that efficient in removing ARGs. However, a lot of research has been done on the different aquatic systems, but a major knowledge gap still exists.

Moreover, there's a major lack of concern about hospital wastewater management and its impact on public health in Bangladesh. As a result, it has contributed to the deadly spreading of hospital pathogens which is ultimately becoming a risk for human mankind. Therefore, it continues to be out of focus while developing the control and preventive measures to lessen the impact of ARGs ad ARBs on public health because there aren't many studies related to this problem."

2.8 The novelty of our study:

Nosocomial infection has become a major threat to our public health and the pathogens spreading from hospital effluents are considered to be responsible for it. It has become a serious problem when antibiotics started acquiring resistance and sharing their ARGs among species by horizontal gene transfer.

Staphylococcus aureus is known to be a nosocomial pathogen and is currently the common cause of infection in many hospital patients. The resistance of this highly pathogenic microorganism to antibiotics along with its increasing prevalence in nosocomial infections is a cause for serious concern. Our research aims to establish a benchmark for estimating the burden of antibiotic-resistant *Staphylococcus aureus* that hospitals are responsible for releasing into the environment as well as to create awareness among the public.

2.9 Aims, Objectives, and Hypothesis

The main goal of our study was to estimate the prevalence of antibiotic-resistant *Staphylococcus aureus* in hospitals and adjacent areas in Dhaka North City Corporation. The study's initial foundation was that hospitals' untreated wastewater can be a potential reservoir for the dissemination of antibiotics which resulted in the emergence of ARGs and ARBs in the environment. The study also intended to evaluate the AMR pattern of *Staphylococcus aureus* compared to other strains and examine the load of ARGs disseminated from hospitals to the general public.

Chapter 3

Methods and Materials

3.1 Selection of our sampling location

The sample collection site for our research project was selected in the Dhaka Metropolitan area. The study was carried out considering a specific time frame which was from June 2022 to December 2023. Accordingly, three hospitals were chosen along with its adjacent community household located in Dhaka. Considering the time frame, samples have been collected every month from each study site. The National Institute of Cancer Research & Hospital (NICRH), Dhaka Shishu (Children's) Hospital, and DNCC Dedicated Covid19 Hospital, all in Mohakhali, Dhaka-1212, have been chosen as the study sites. Our study focused on hospital wastewater as our main sample and later water from the adjacent community households was collected within the range of 300m. The samples mostly consisted of tap water from the nearby community and hospital effluents (wastewater), bringing attention to the problem of hospital effluents being dumped into the environment without being properly treated, which could lead to fatal disease outbreaks. These locations were thus appropriate for carrying out this study.

Figure 1: Sampling sites (GIS map).

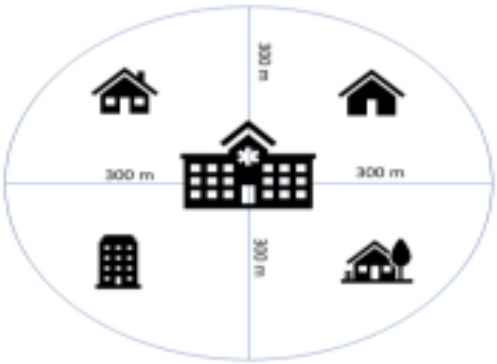

Figure 2: Illustration of the sample site collection

3.2 Sample Collection:

Samples were collected from hospital effluents and its nearby adjacent community tap water. From each hospital, one effluent wastewater tap water and four community tap water were collected every month consecutively. The preferred time for our sample collection was chosen during the first time in the day and all the possible steps were taken before our sample collection. The lab equipment associated with our sample collection was autoclaved beforehand. The essentials needed for our sample collection were one pair of gloves, a sterile sample collection bottle(500ml), and sterile falcon tubes(50ml). Moreover, sterile ice boxes and ice packs were used to carry the samples from the sampling site to the lab.

A suitable falcon tube(50ml) was used to collect the wastewater sample from hospitals and accordingly, a separate sterile water collection bottle(500ml) was used to collect water from the community. Next. The falcon tube along with a water collection bottle was transferred to the icebox. The icebox's cover was then sealed. The hand gloves were disposed of safely and hands were washed with ethanol to combat contamination. Lastly, the samples were safely transported to our laboratory for further processing.

3.3 Sample processing

For processing the sample filter apparatus, Test tubes (10 ml), falcon tubes (50 ml), sterile filter paper (0.45 ul), modified TSB (15%), normal physiological saline (0.9% NaCl), modified MSA (7.5% NaCl), and a pair of gloves were used. Modified TSB containing (Tryptone soya bean broth + 15%Nacl) was used to enrich our sample collected from community tap water. The membrane filtration method was followed for all the community tap water samples. Approximately 50ml of water was poured into the filter apparatus and filtered with 0.45 microliter. Next, with the help of a sterile tweezer, the filter paper was transferred to the falcon tubes containing modified TSB.

Later, the falcon tubes containing enriched samples were placed inside a beaker and it was kept inside the shaker incubator for 48 hours at 37 degree Celsius. For our result detection, we observed the turbidity inside the falcon tubes; turbidity indicates the growth of our preferred bacteria. The next day, each sample was diluted up to 8-fold in normal saline (0.9%NaCl). Then, taking a sample of 0.1ml using a pipette was poured on MSA agar plates and spread evenly. The MSA agar plates were labeled as 10-², 10-□, and 10-□. After spreading, the plates were labeled and stacked with masking tape and were kept inside the incubator at 37°C for 24 hours.

Wastewater from hospital sewage was serially diluted up to 8 folds inside the laminar simultaneously. In the same way, the 0.1m of the raw sample was taken from our diluted hospital sample and was spread on 10-², 10-□, 10-□ labeled MSA plates evenly.

3.4 Sample enrichment and growth on selective media:

The main purpose of our study was to differentiate *Staphylococcus aureus* from hospital effluents and community tap water. It is very obvious that, along with *S. aureus*, other

bacteria may be present in the sample water as well. Thus, three of the modified media were used to focus on the growth of *S. aureus* only. Mannitol Salt Agar (Hi-Media), Tryptic Soy Broth (Hi-Media), and Nutrient Agar (Hi-Media) were used in our study. Samples from community tap water were enriched in a TSB broth containing 15% NaCl, as bacterial loads are drastically low in community tap water. For the growth preferences of *Staphylococcus spp.,* additional sodium chloride was used. Mannitol Salt Agar is a selective media for growing *S. aureus*, and it also helps to differentiate among other species of Staphylococci. Given that *S. aureus* is capable of fermenting mannitol, mannitol salt agar with a 7.5% NaCl concentration has been chosen as a selective medium.

3.5 Colony Morphology, selection, and Analysis

After the incubation period, all MSA plates were taken out from the incubator to observe bacterial growth. The pink MSA plates turned yellow which means that the *Staphylococcus aureus* can ferment mannitol. Standard plate count methods were followed to count the total colony forming unit/ml. From every sample, 6-8 yellow/white, pink colonies (surrounded by yellow zones) were selected for streaking on MSA plates and were further incubated at 37°C for 24 hours. After that, from the MSA plates, the colonies were cultured into fresh NA media.

3.6 Molecular detection

3.6.1 DNA Extraction

DNA extraction is a process to separate DNA from cell membranes, proteins, and other biological components from a sample by using physical or chemical processes. At first, DNA isolation was done by Friedrich Miescher in 1869 (Cytol,2019). For DNA extraction, the boiling method was used in this research. At first, 150µL 1x TE (Tris- EDTA) buffer was

taken in an autoclaved microcentrifuge tube. Then, a loopful of colonies from fresh culture media (Nutrient agar) was taken and mixed within it. A short vortex was given. After that, the mixture was heated at 95°C for 15 minutes, using the dry block heater machine. Then, for five minutes, the cells were centrifuged at 13000 rpm. After centrifugation, the genomic DNA-containing supernatant was transferred to a fresh set of MCT (micro centrifuge tubes) and kept at -20°C.

3.6.2 Primer preparation for PCR using a stock solution

In this study, *Staphylococcus aureus* was molecularly identified using two sets of primers. TStaG422 primer was used to identify the genus Staphylococcu*s*, and the NUC primer was used to identify the species, *S. aureus*. Both of these primers, TStaG422 and NUC, were in stock solutions that could be found in the lab (Hussain et al., 2016). 10 µL of 100 mM forward and 10µLof 100 mM reverse primers were taken in two distinct MCTs to prepare 100 µL of working solutions (10 µM) of TStaG422 primer from 100 µM. Each tube was then filled with molecular-grade, nuclease-free water for the remaining 90 µL. The addition of the nuclease-free water was followed by a gentle re-pipetting and a short spin for 20 seconds. The working solution for nuc primers was created using the same procedure.

Gene	**Primer Sequence**	**Target Organism**	**Product Size (bp)**	**Reference**
TStaG422	5′-GGCCGTGTTGAACGTGGTCAAA TCA-3′ 5′-TIACCATTTCAGTACCTTCTGGTA A-3′	*Staphylococcus spp.*	370	(Martineau *et al.,* 1998)

NUC	5'-GCGATTGATGGTGATACGGTTAGCCAAGCCTTGACGAACTAAAGC- 3'	*Staphylococcus aureus*	279	(Martineau *et al.,* 1998)

Table 2: Primer sequences used for the amplification by PCR

3.6.3 Preparation of PCR controls

Each time the polymerase chain reaction (PCR) was run, a positive control had been used as a quality control measure throughout the entire molecular detection of the Staphylococcus aureus process. Throughout the research, a true positive isolate of Staphylococcus aureus that met laboratory standards was selected as the positive control, and a nuclease-free water and master mix mixture was used as the negative control.

3.6.4 PCR Assay

The polymerase chain reaction may effectively detect bacterial isolates at the molecular level by amplifying specific genes under specific conditions. As samples were collected every week from the chosen areas, the PCR-based detection of *Staphylococcus aureus* by amplifying the TStaG422 and NUC genes was often carried out in this research.

The PCR assay was carried out in PCR tubes, and the 13 µL PCR mixtures contained 2 µL of DNA template, 7.5 µL of 2X emerald PCR Master Mix (Takara Bio), 0.6 µL of each pair of primers (10 µM), and 2.3 µL of Nuclease-free water. To ensure adequate mixing and prevent bubbles from developing gentle re-pipetting and spinning were carefully carried out.

The following program (modified) was used to run the PCR in an Applied Biosystems (Thermo-Fischer) thermal cycler: initial denaturation at 94°C for 5 minutes, followed by 30

cycles of denaturation at 94°C for 1 minute, primer annealing at 55°C (TsTaG422) & 56°C (NUC) for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes (Dominguez et al., 2020). During each PCR, a positive control (containing DNA from the real isolate) and a negative control (containing the PCR reaction mixture only) were used as quality assurance checks. The TStaG422 and NUC primers were both employed with these PCR programs.

3.6.5 Gel electrophoresis

The target genes, which included amplified products, were visualized and confirmed to have been amplified using the traditional agarose gel electrophoresis method. The PCR products were electrophoresed at 110 V for 50 minutes in 2% agarose gel in TBE buffer (40 mM Tris, 20 mM boric acid, 1 mM EDTA, pH 8.0) with 2% agarose gel. 3 µL DNA ethidium bromide dye diluted to 0.5 µg/mL was used to stain the gel. The UV trans-illuminator was used to see through the electrophoresed Gel, and all of the photos were taken, labeled properly, and archived. When the band matched and was observed at the predicted size of 370 bp (TStaG422) and 279 bp (NUC), which shows the presence of Staphylococcus aureus in the original isolate (Effendi *et al.,*2019). 100 bp-sized DNA ladders were utilized to compare the amplicons with the predicted band size.

3.6.6 Antimicrobial Susceptibility Testing

To analyze the pattern of antibiotic resistance, antimicrobial susceptibility testing was needed for all the PCR-confirmed isolates. It was carried out by the Kirby-Bauer disc diffusion method and CLSI recommendations. Following CLSI recommendations, 14 antibiotic discs from the various groups were chosen to test each group's susceptibility. Antibiotics that were

used are Erythromycin, Tetracycline, Cefixime, Cefipime, Ceftriaxone, Ciprofloxacin, Imipenem, Amoxyclav, Amikacin, Gentamicin, Vancomycin, Clindamycin, Chloramphenicol and Aztreonam. To create a bacterial suspension, the PCR-confirmed isolates were subcultured into nutrient agar plates and cultivated overnight at 37°C. Fresh loop-full pure bacterial cultures were suspended by dipping them in 0.9% normal saline before being compared to a 0.5 McFarland turbidity standard. A sterile cotton swab was used to collect the bacterial isolate's suspension, and Mueller Hinton Agar (MHA) plates were used to create the bacterial lawn. To ensure complete diffusion of the discs on the agar surface, the antibiotic throughout then gently picked up and placed onto the MHA agar plate using sterile forceps. The plates were then stacked, labeled, and kept at 37°C for 18– 24 hours in the incubator. The MHA plates were taken out from the incubator after the incubation period to be seen, and the interpretation for the zone of inhibition was compared by the CLSI recommendations standards.

Antibiotic Name	**Antibiotic Class**	**Zone Interpretation (mm)**
AMC(Amoxyclav)	Penicillin	S>=18-,R<=13,I=14-17(mm)
TE(Tetracyclin)	Tetracyclines	S>=19,I=15-18, R<=14(mm)
DA(Clindamycin)	Lincomycin	S>=21,I=15-20,R<=14(mm)
CTR(Ceftriaxone)	Cephalosporin	S>=21,I=14-20, R<=13(mm)
CIP(Ciprofloxacin)	Fluoroqionolone	S>=21,I=16-20,R<=15 (mm)
Ipm(Imipenem)	Carbopenem	S>=16,I=14-15, R<=13(mm)

E(Erythromycin)	Macrolides	S>=23,I=14-22, R<=13(mm)
AK(Amikacin)	Aminoglycosides	S>=21,I=18-20,R<=17(mm)
CPM(Cefepime)	Beta-lactam	S>=18,I=15-17,R<=14(mm)
GEN(Gentamycin)	Aminoglycosides	S>=15, I-13-14, R<=12(mm)
VA(Vancomycin)	Glycopeptide	s>=12, I=10-11, R<=13(mm)
C(Chloramphenicol)	Chloramphenicol	S>=18,I=13-17, R<=12(mm)
AT(Aztreonam)	Beta-lactam	-
CFM(Cefixime)	Cephalosporin	-

Table 3: Antibiotics disc list used in this study with CLSI interpretation

Chapter 4

Results and Observations

4.1 Isolation of *Staphylococcus aureus*

From December 2022 to June 2023, a total of 68 samples were taken from our research sampling locations throughout many phases. 52 samples of tap water from communities near hospitals and 16 samples of hospital wastewater make up the total number of samples. A total of 211 isolates had been taken from the hospital and community samples. 48.53% of the sample size, or 15.64% of the isolates or 33 PCR-confirmed *Staphylococcus aureus* isolates, were obtained from the 68 samples (2 from hospital effluents and 31 from community tap water). All of the presumed isolates were sent for PCR-based molecular detection, the industry standard for microbial identification, after being presumptively chosen based on their colony shape on MSA agar plates.

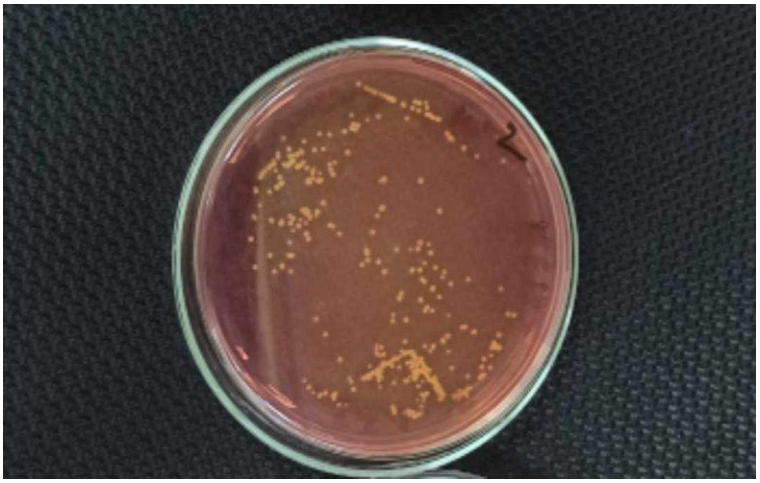

Figure 3: Presumptive colonies of *Staphylococcus aureus* (yellow colonies) on MSA plate

4.2 PCR-based identification of *Staphylococcus aureus*: result interpretation

A gel containing amplified products was successfully electrophoresed, and after that, it was seen under an ultraviolet (UV) illuminator, and the desired band size was determined. When

an isolate displays the predicted band size after being compared to the DNA ladder and a positive control, it is considered to be positive. The PCR-amplified products have been shown using a UV illuminator in the given figures below:

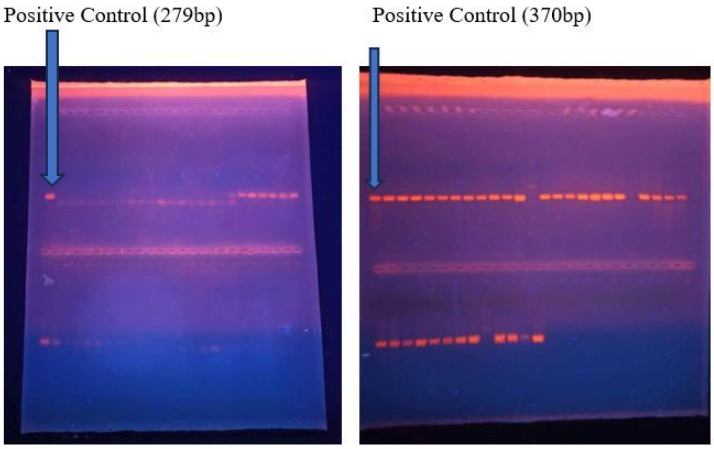

Figure 4: Nuc PCR for detecting the *Staphylococcus aureus* & TStaG422PCR for detecting the Genus *Staphylococcus*

4.3 Month-wise distribution of *S. aureus* isolates

Specifically, from December 2022 to June 2023, the study's focus was on monitoring *S. aureus* trends throughout that time. PCR-based confirmation of presumptively chosen *S. aureus* was done, and when they matched the sets of characteristics (morphology, amplified band size), they were counted as positive results. In terms of isolating *S. aureus* from the designated sampling sites, December 2022 is the highest pick. A total of 10 *S. aureus*, or 30.30% of all of our PCR-confirmed isolates, were collected from the sampling sites in December 2022. Additionally, it has been noted that 27.27% of *S. aureus* were acquired in February 2023 and 21.21% in May 2023, respectively, and that the remaining 6.06% were acquired in March 2023 & June 2023, also rest 9% from April 2023. The distribution is illustrated by the chart in the figure below.

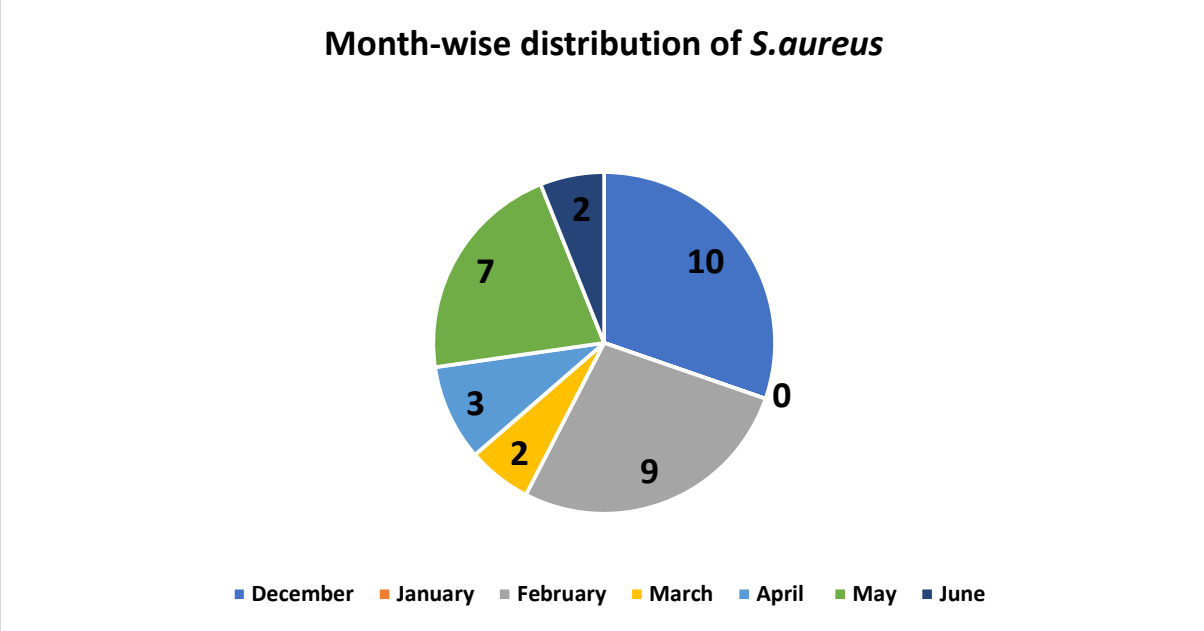

Figure 5: Month-wise distribution of PCR-confirmed *Staphylococcus aureus*

4.4 Sampling site based distribution of *S. aureus*

The study focused on three key public spaces, including hospitals and the communities around them. The DNCC Dedicated Covid-19 Hospital (DNCC-DCH), Mohakhali, Dhaka-1212, Dhaka Shishu (Children) Hospital (DSH), Shaymoli, 1207, and National Institute of Cancer Research & Hospital (NICRH) served as the sampling sites for this research. The general population can easily reach these locations to meet their basic medical needs. Data analysis revealed that 48.48% of the isolates came from the DNCC Dedicated Covid-19 Hospital (DNCC-DCH), located in Mohakhali, Dhaka-1212. With 30.30% of the total isolates, the National Institute of Cancer Research & Hospital (NICRH) had the second-highest number. The Dhaka Shishu (Children) Hospital (DSH) also provided 21.21% of our isolates for the duration of the study. The following figure illustrates the data.

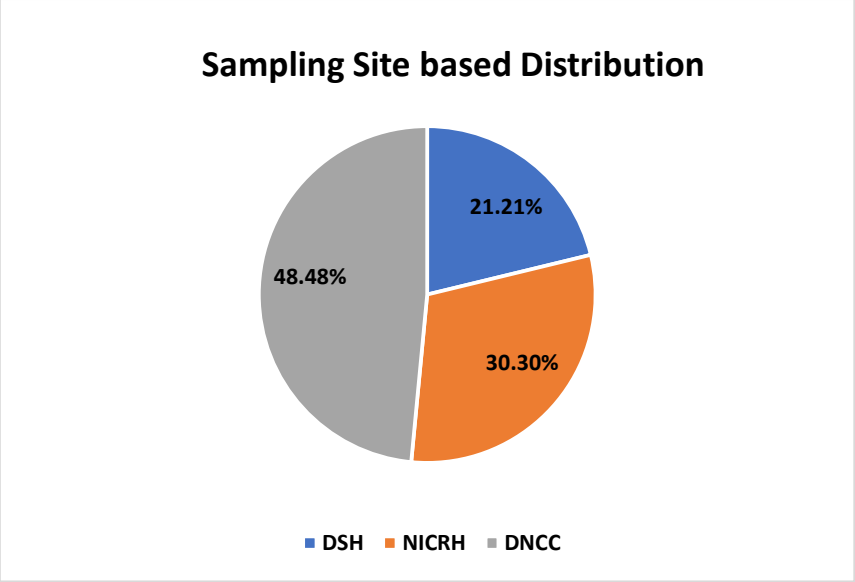

Figure 6 : Sampling site based distribution of *S. aureus*

4.5 Result of Antimicrobial Susceptibility Test

After the MHA plates had been incubating, it was noticed and analyzed that the isolates were either resistant, intermediate, or sensitive to the antibiotic-impregnated discs. Results (resistant, intermediate, or sensitive) were interpreted in accordance with the CLSI recommendations. Figure illustrates this observation below:

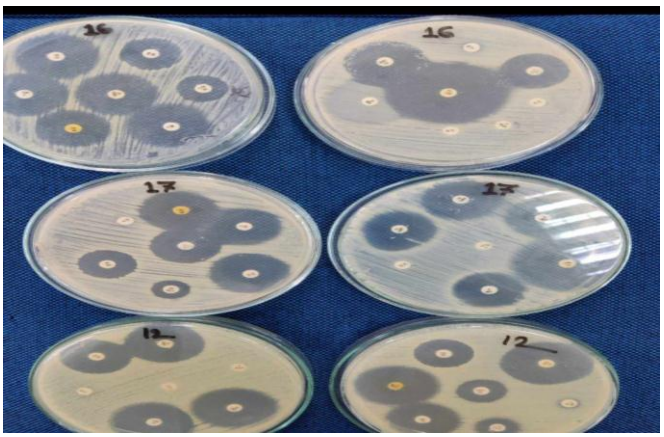

Figure 7: Antimicrobial resistance pattern of *Staphylococcus aureus*

4.6 Antimicrobial resistance pattern of total isolates

After analyzing the data from the Antimicrobial Susceptibility Testing, it was found that all the isolates were resistant to Aztreonam and Cefixime. It was also observed that 87.87% of the isolates were resistant to Erythromycin. 48.48% of the isolates were resistant to Ceftriaxone. 33.33%, 24.24%, 21.21%, 15.15% of the isolates were resistant to Clindamycin, Ciprofloxacin, Cefepime & Tetracycline, respectively. Also, 33.33%, 15.15% , 12.12% ,9.09% and 6.06% of the isolates showed and intermediate zone against Ceftriaxone, Cefepime, Clindamycin, Ciprofloxacin and Tetracycline, respectively. However, in terms of sensitive patterns, Amoxyclav, Amikacin, Gentamicin, Imipenem, Vancomycin and Chloramphenicol showed the highest(100%) susceptibility against all of the isolates followed by Tetracycline (78.78%) , Ciprofloxacin (66.67%) , Clindamycin (54.54%), Cefepime (63.,63%), Ceftriaxone (18.18%) and Erythromycin (12.12%) showed their susceptibility.

Antibiotics	Resistant	Intermediate	Susceptible
Aztreonam	**100%**	**0%**	**0%**
Cefixime	**100%**	**0%**	**0%**
Erythromycin	**87.87%**	**0%**	**12.12%**
Ceftriaxone	**48.48%**	**33.33%**	**18.18%**
Clindamycin	**33.33%**	**12.12%**	**54.54%**
Ciprofloxacin	**24.24%**	**9.09%**	**66.67%**
Cefepime	**21.21%**	**15.15%**	**63.63%**
Tetracycline	**15.15%**	**6.06%**	**78.78%**
Amoxyclav	**0%**	**0%**	**100%**
Amikacin	**0%**	**0%**	**100%**
Gentamicin	**0%**	**0%**	**100%**

Imipenem	**0%**	**0%**	**100%**
Vancomycin	**0%**	**0%**	**100%**
Chloramphenicol	**0%**	**0%**	**100%**

Table 4: Antimicrobial resistance pattern of total isolates

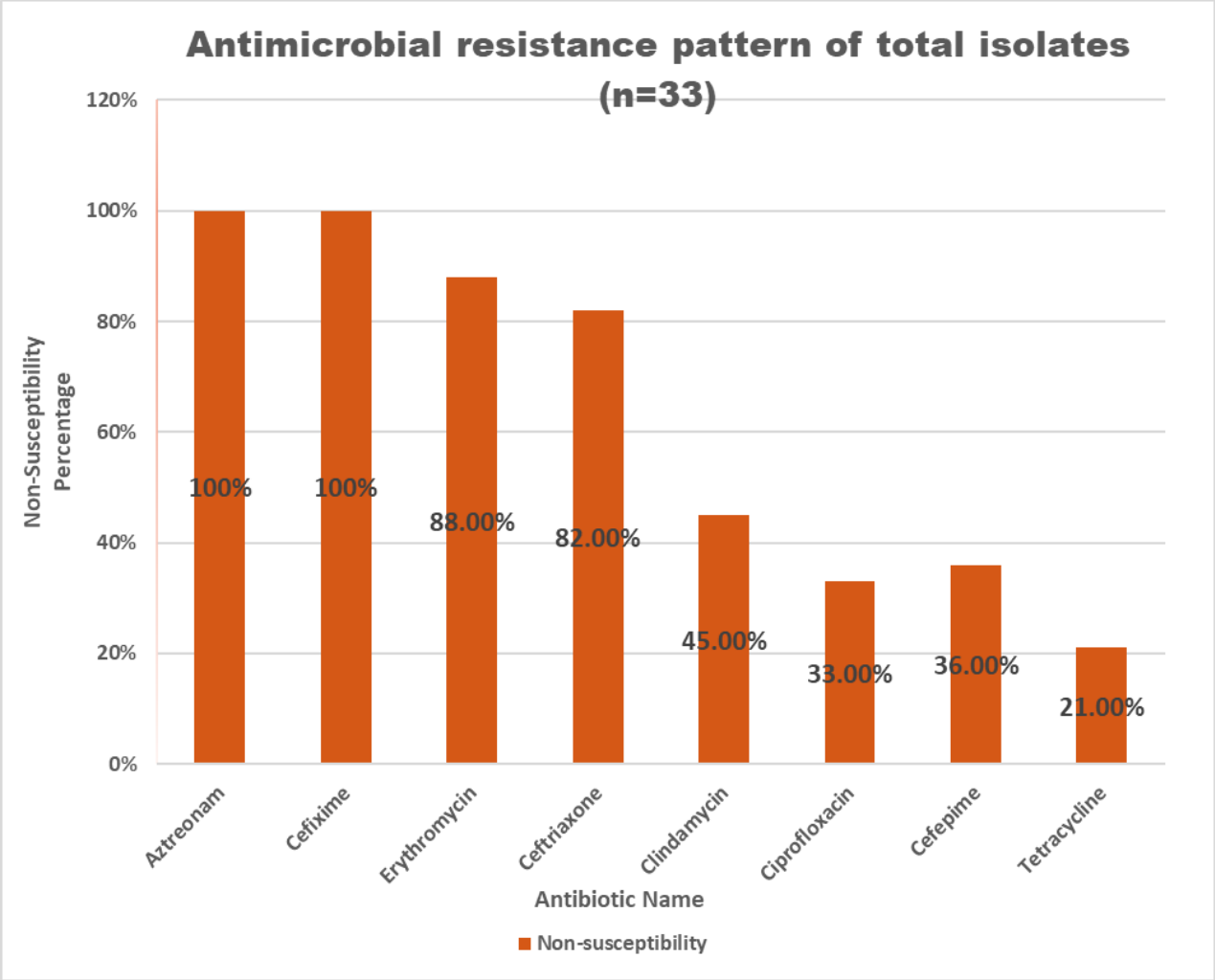

Figure 8: Antimicrobial resistance pattern of total isolates

4.7 Antimicrobial resistance pattern in hospital wastewater isolates

Only 2 isolates or 6.06% of the isolates had been gained from hospital wastewater. Both of them were resistant to Aztreonam, Cefixime, Erythromycin, Clindamycin and Ciprofloxacin

and susceptible to Amoxyclav, Tetracycline, Imipenem, Amikacin, Gentamicin, Vancomycin and Chloramphenicol. But one of them was resistant to Ceftriaxone and one of them showed intermediate zone. Again, one of them was resistant to Cefepime but showed susceptibility to it.

Antibiotics	**Resistant**	**Intermediate**	**Susceptible**
Aztreonam	100%	0%	0%
Cefixime	100%	0%	0%
Erythromycin	100%	0%	0%
Ceftriaxone	50%	50%	0%
Clindamycin	100%	0%	0%
Ciprofloxacin	100%	0%	0%
Cefepime	50%	0%	50%
Tetracycline	0%	0%	100%
Amoxyclav	0%	0%	100%
Amikacin	0%	0%	100%
Gentamicin	0%	0%	100%
Imipenem	0%	0%	100%
Vancomycin	0%	0%	100%
Chloramphenicol	0%	0%	100%

Table 5: Antimicrobial resistance pattern in isolates of Hospital effluents

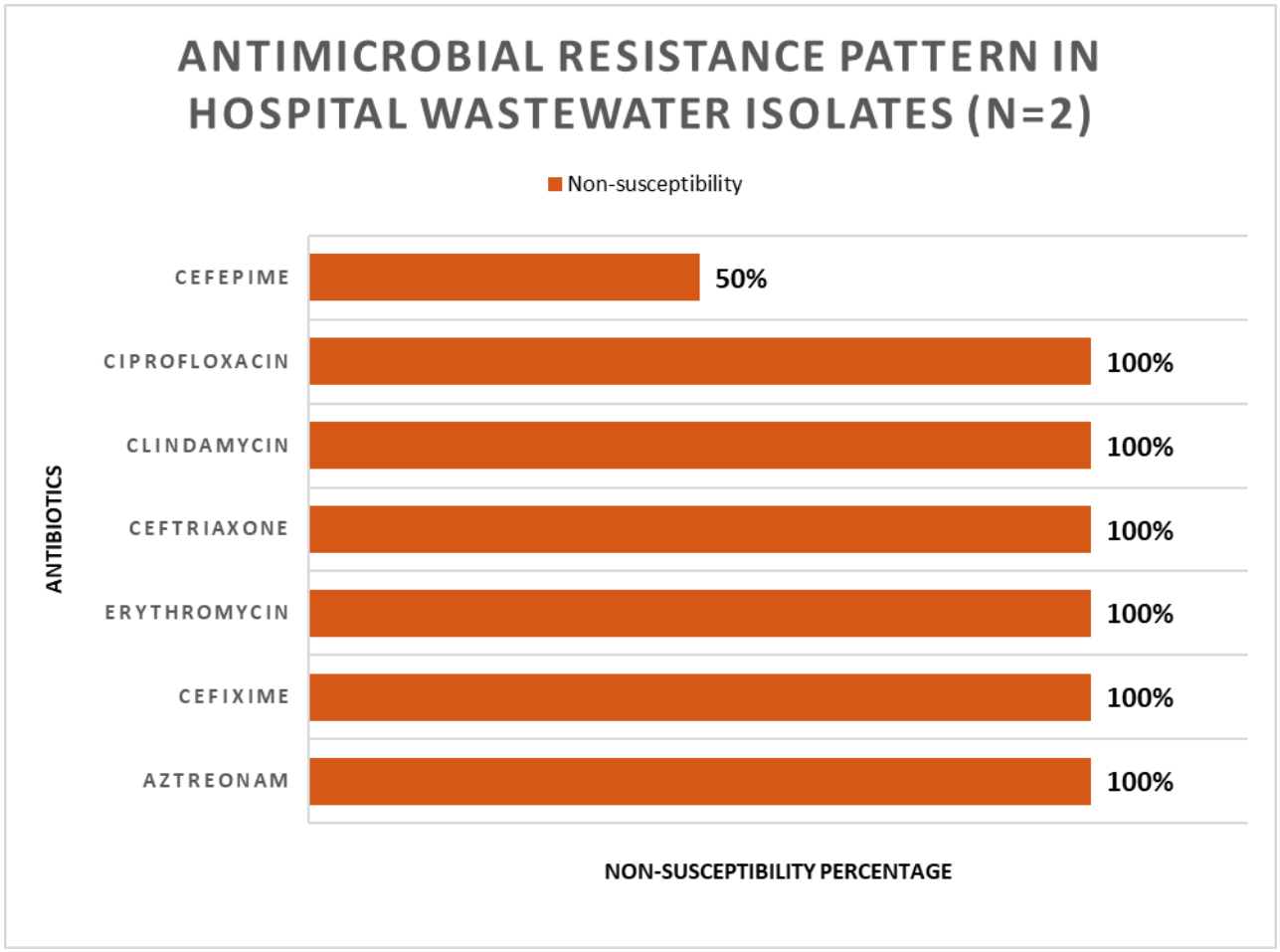

Figure 9: Antimicrobial resistance pattern in hospital wastewater isolates

4.8 Antimicrobial resistance pattern in isolates of Hospital adjacent communities

31 isolates or 93.94% of the isolates had been gained from hospital adjacent communities. All of them (100%) were resistant to Aztreonam and Cefixime and showed greater susceptibility (100%) to Amoxyclav, Imipenem, Gentamicin, Vancomycin, Chloramphenicol and Amikacin. Furthermore, 16.13% , 29.03% , 48.39% , 19.35% and 87.09% of the isolates were resistant to Tetracycline, Clindamycin, Ceftriaxone, Ciprofloxacin & Cefipime and Erythromycin, respectively. 6.45% , 12.90% , 32.26% , 9.68% and 16.13% isolates showed intermediate zone against Tetracycline, Clindamycin, Ceftriaxone, Ciprofloxacin and Cefipime, respectively. Also, 77.42% ,58.06% , 19.35% , 70.97% , 12.90% and 64.52% isolates had

shown sensitivity against Tetracycline, Clindamycin, Ceftriaxone, Ciprofloxacin Erythromycin and Cefepime ,respectively.

Antibiotics	Resistant	Intermediate	Susceptible
Aztreonam	**100%**	**0%**	**0%**
Cefixime	**100%**	**0%**	**0%**
Erythromycin	**87.09%**	**0%**	**12.90%**
Ceftriaxone	**48.39%**	**32.26%**	**19.35%**
Clindamycin	**29.03%**	**12.90%**	**58.06%**
Ciprofloxacin	**19.35%**	**9.68%**	**70.97%**
Cefepime	**19.35%**	**16.13%**	**64.52%**
Tetracycline	**16.13%**	**6.45%**	**72.42%**
Amoxyclav	**0%**	**0%**	**100%**
Amikacin	**0%**	**0%**	**100%**
Gentamicin	**0%**	**0%**	**100%**
Imipenem	**0%**	**0%**	**100%**
Vancomycin	**0%**	**0%**	**100%**
Chloramphenicol	**0%**	**0%**	**100%**

Table 6: Antimicrobial resistance pattern in isolates of Hospital adjacent communities

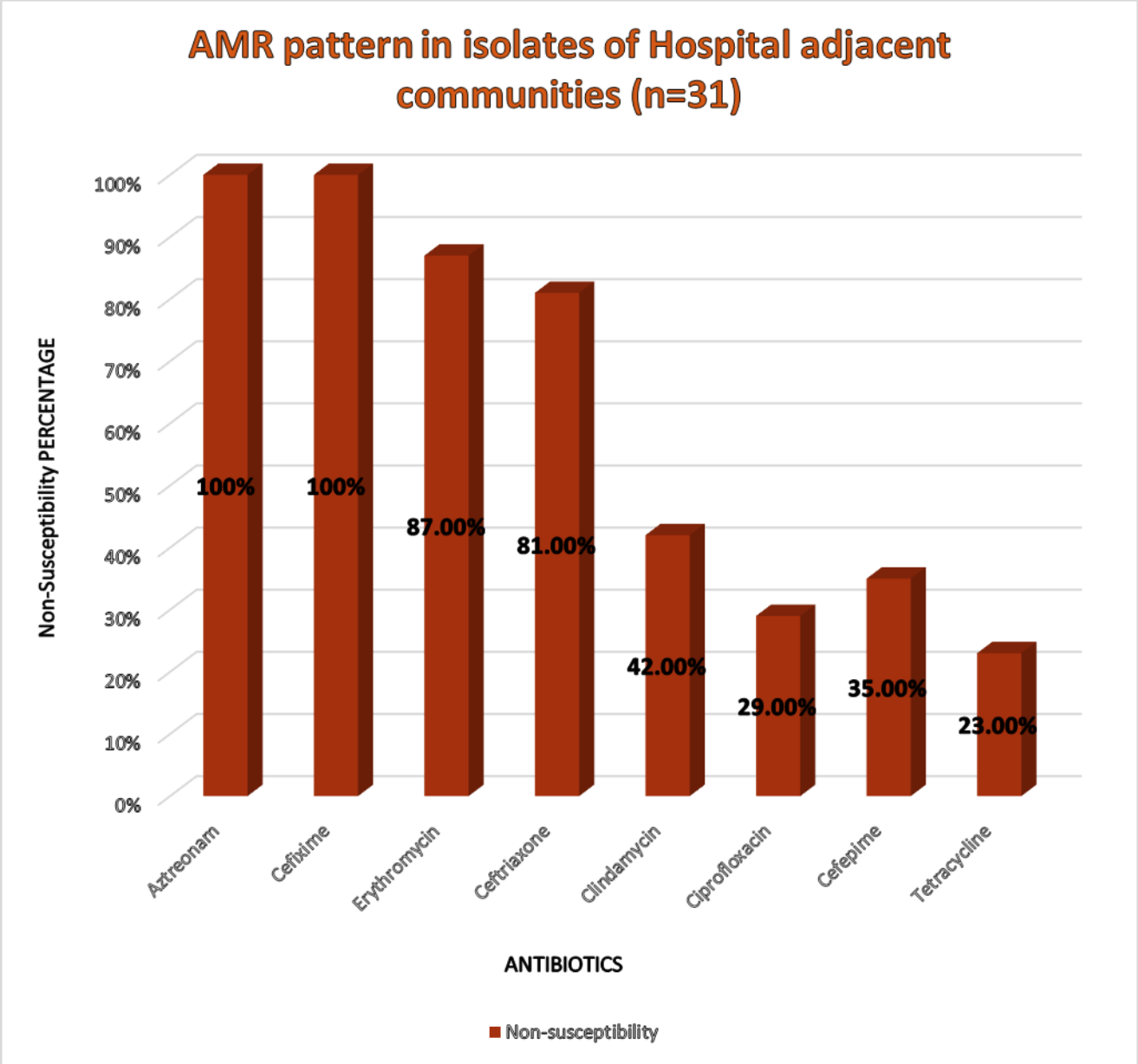

Figure 10: AMR pattern in isolates of Hospital adjacent communities

4.9 Comparison of the AMR patterns between the isolates from hospital effluents and the isolates from the communities adjacent to the hospital

Two different types of isolates were obtained throughout the study period because the study was carried out in the hospital and the areas nearby. A total of 33 positive isolates, 2 from hospital wastewater and 31 from community water had been obtained. It was explored that

isolates from the hospital were 100% resistant to Aztreonam, Cefixime, Erythromycin, Clindamycin, and Ciprofloxacin. But isolates from the community water sample were 100% resistant to Aztreonam and Cefixime and 16.13%, 29.03%, 48.39%, 19.35%, and 87.09% of the isolates were resistant to Tetracycline, Clindamycin, Ceftriaxone, Ciprofloxacin & Cefipime and Erythromycin, respectively. From the observation, we noticed that the two hospital isolates were resistant to 5 antibiotics. And most of the isolates from community samples were resistant to 5 or 6 antibiotics. The isolates from hospital effluents were thought to be more pathogenic than isolates from communities because it was first thought that the patients or visitors to that hospital were the most likely source of the isolates from wastewater. But from our research, it can be observed that there were no significant differences between the antibiotic resistance patterns of hospital samples and community samples. Surprisingly, 3 of the isolates from community samples had shown resistance against 7 antibiotics. So, these community strains were more pathogenic. Again, it can be concluded that isolates from both hospital and community samples were mostly susceptible to Amoxyclav, Imipenem, Amikacin, Gentamicin, Vancomycin, and Chloramphenicol. The data indicating the comparative study of AMR pattern between the isolates of hospital effluents and surrounding communities are described and illustrated in the following figure.

Antibiotics	**Hospital Resistant**	**Community Resistant**	**Hospital Intermediate**	**Community Intermediate**	**Hospital Sensitive**	**Community Sensitive**
Aztreonam	**100%**	**100%**	**0%**	**0%**	**0%**	**0%**
Cefixime	**100%**	**100%**	**0%**	**0%**	**0%**	**0%**
Erythromycin	**100%**	**87.09%**	**0%**	**0%**	**0%**	**12.90%**
Ceftriaxone	**50%**	**48.39%**	**50%**	**32.26%**	**0%**	**19.35%**
Clindamycin	**100%**	**29.03%**	**0%**	**12.90%**	**0%**	**58.06%**

Ciprofloxacin	**100%**	**19.35%**	**0%**	**9.68%**	**0%**	**70.97%**
Cefepime	**50%**	**19.35%**	**0%**	**16.13%**	**50%**	**64.52%**
Tetracycline	**0%**	**16.13%**	**0%**	**6.45%**	**100%**	**72.42%**
Amoxyclav	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**
Amikacin	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**
Gentamicin	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**
Imipenem	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**
Vancomycin	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**
Chloramphenicol	**0%**	**0%**	**0%**	**0%**	**100%**	**100%**

Table 7: Comparison of the AMR patterns between the isolates from hospital effluents and the isolates from the communities adjacent to the hospital

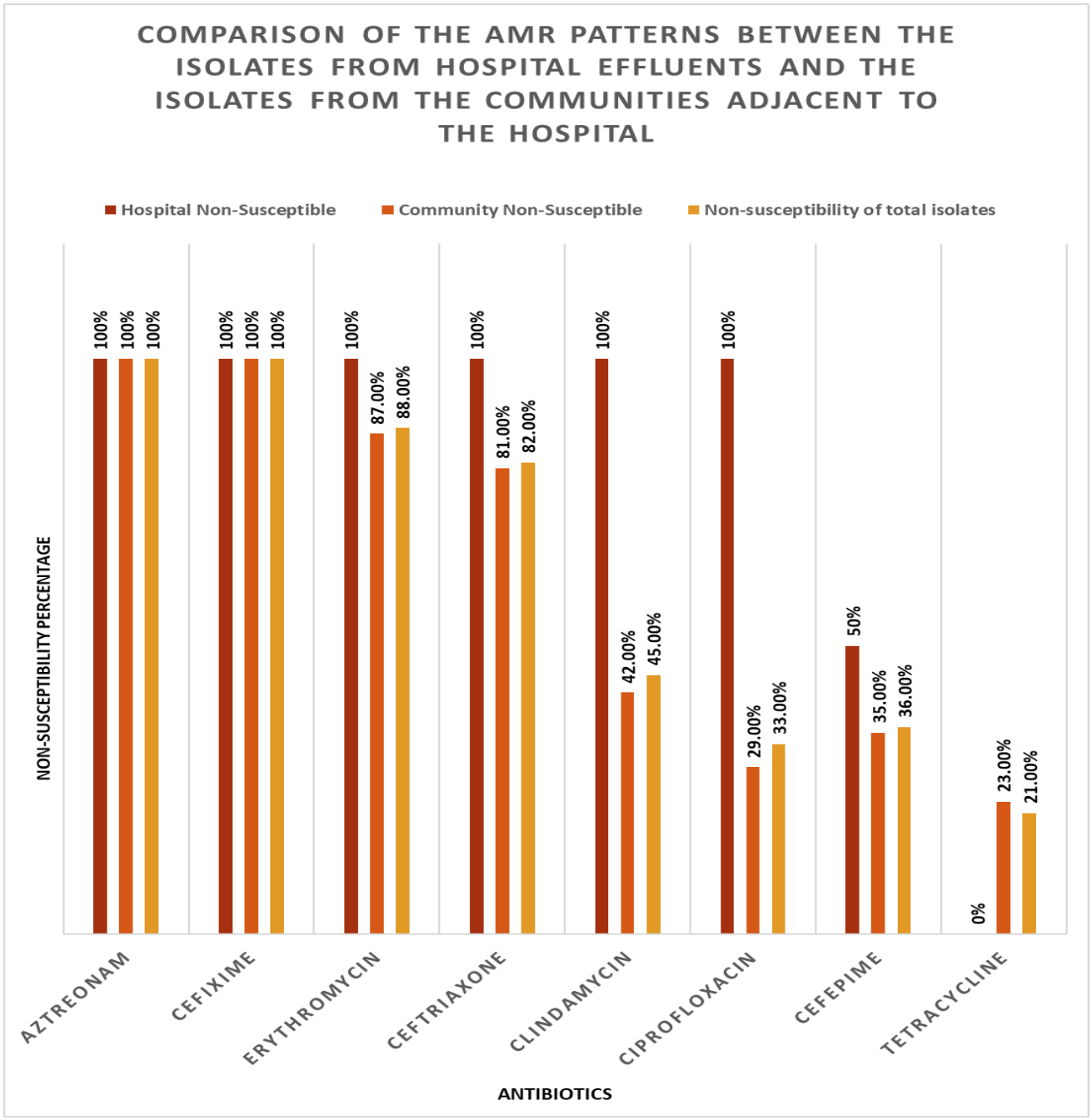

Figure 11: Comparison of the AMR patterns between the isolates from hospital effluents and the isolates from the communities adjacent to the hospital

Chapter 5

Discussion

5.1 Result analysis-based discussion

Nosocomial infections are the infections which are obtained during the process of receiving healthcare. But the infection was not present while taking admission. This is also referred as healthcare-associated infections (HAI). HAIs can be obtained during different areas of healthcare delivery, such as in hospitals, long-term care facilities, and ambulatory settings, and can also appear after discharge. Occupational infections can also be obtained that may affect staffs. It is the most common adverse event in healthcare system that threatens the patient's safety. Significant morbidity, mortality, and financial burden on patients, families, and healthcare systems can be caused by HAIs. Another complications that can be seen by HAIs are the emergence of multi-drug resistant organisms (Sikora & Zahra, 2023). *Staphylococcus aureus* is one of the most significant and harmful bacteria resulting from HAIs. *Staphylococcus aureus* is an opportunistic, gram positive pathogen. From a long time, it has been recognized as one of the most important bacteria that causes disease in humans. The leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis is *Staphylococcus aureus*. Though most infections caused by *Staphylococcus aureus* is not serious, but several infections like bloodstream infections, pneumonia, or bone and joint infections can be caused by it (Minnesota Department of Health Fact Sheet Revised February, 2010). It is one of the most leading causes of nosocomial infections and is known for it's ability to develop resistance against antibiotics. A study showed that methicillin resistant *Staphylococcus aureus* (MRSA) is a problem as a nosocomial pathogen in our hospitals. The prevalence of MRSA is increasing in a rapid way. Community associated MRSA is creating a great problem in the world. It has been shown that ARGs could spread

from hospitals to the outside environment, and as a result, community water isolates have been discovered to be antibiotic-resistant. A study was conducted in hospitals of Chattogram from October 2018-December 2018. From 100 samples, 66 (23 from hospital drain water) *S. aureus* were confirmed. They showed 80% resistance against oxacillin, Gentamicin & Cefotaxime and 25% susceptibility to chloramphenicol & cefotaxime. They also showed more than 15% susceptibility to Gentamicin, tetracycline & Ciprofloxacin (Islam *et al.,* 2018).

Our study was mostly conducted in the Dhaka North municipal corporation's hospital areas and adjacent communities. Our research was conducted in, and the adjacent communities of DNCC Dedicated Covid-19 Hospital, Mohakhali, Dhaka-1212, National Institute of Cancer Research & Hospital (NICRH), and Dhaka Shishu (Children) Hospital, Shaymoli-1207 around them. The Staphylococcus aureus strains that obtained from these two locations were consequently labelled as "community water isolates" and "hospital effluents isolates." The findings showed that 48.53% of the S. Aureus isolates came from the sample size. By use of PCR-based molecular detection, all the isolates were verified and sent for additional examination.

The results of our research revealed that different antibiotics used in hospitals and released into the environment through hospital effluents may have transmitted resistant bacteria and increased the prevalence of ARGs in the environment. As a result, human diseases have grown more resistant, which makes it more challenging to cure infections, raises the burden of sickness on society, and eventually raises mortality rates.

Like hospital associated MRSA, Community associated MRSA is also susceptible to multiple classes of antibiotics (Sharma et al., 2013). From our study, it can be observed that the isolates showed strong susceptibility to Amoxyclav, Imipenem, Amikacin, Gentamicin

Vancomycin and Chloramphenicol. The current mainstay of therapy for serious infections caused by MRSA is now Vancomycin and other glycopeptide antibiotics. MRSA is a strain of *Staphylococcus aureus* that has obtained resistance against β-lactam antibiotics including penicillins and cephalosporins. These versatile and significant nosocomial pathogens often cause postsurgical wound infections. This leads to increased mortality, morbidity rate. It has been reported by WHO that 64% of MRSA-infected patients are more likely die than non-MRSA-infected patients. The frequency of community-acquired MRSA and hospital-acquired MRSA infections have increased rapidly. They contribute to the failure experimental therapy. Due to the emergence of multidrug resistance (MDR), the treatment for staph infections become more difficult (Gurung et al., 2020).

From our research, it has been found that all of the positive isolates are resistant to Aztreonam and Cefixime that belong to Beta-Lactamase group and cephalosporin group respectively. Besides, 87.87% of the isolates were resistant to Erythromycin that belongs to macrolide group and 48.48% of the isolates were resistant to Ceftriaxone belongs to cephalosporins. As we have discussed that MRSA strains are resistant to penicillin and cephalosporin group, so it is a great alarming indication that the strains can increase mortality and morbidity rate gradually.

5.2 Limitations of our study

Due to the study's focus on the long-term study of the particular location, the study's sampling sites were limited to three hospital areas. The actual situation might be revealed by an extensive investigation of both city corporations in Dhaka. Additionally, the study was primarily concerned with the microbiological examination of hospital effluent that might have contained pathogens that were released into the environment. A lot of heavy metals, chemicals, and other hazardous substances may have been released into the environment by

hospitals, yet physical and chemical examinations went undetected. Additionally, molecular characterizations of ABRs and ARGs were not possible because specific antibiotic-resistance gene primers were not available. Also, due to resource unavailability, oxacillin could not be used for observing antibiotic resistance pattern. Further research will be done to characterize the ABRs and ARGs and to observe MRSA patterns.

Chapter 6

Conclusions

6.1 Recommendations and conclusions

In other parts of the world, hazardous waste management has gotten a lot of attention recently, and studies on the microbial communities in hospital wastewater have increased significantly. However, these analysis for tracking the prevalence of diseases were not carried out recently in Bangladesh. Pathogenic microorganisms in HWW have a long history of affecting human health, and antibiotic-resistant organisms have been rapidly increasing in number. The problem of antibiotic resistance has been seen throughout the world. Antibiotics have been utilized and released in a variety of settings, and they have spread resistant bacteria and therefore, the prevalence of ARGs in the environment have been increased. As a result, human diseases have become more resistant, increasing the difficulty of treating infections and the mortality rate.

The result of our research has shown that the emergence of ARGs in the strains of *Staphylococcus aureus* in the community setting has increased significantly. These ARBs and ARGs were thought to be transmitted from the hospital settings by the hospital's untreated effluents. But in our study, we have got more positive isolates from community samples. Only 2 isolates have been obtained from hospital wastewater sample. The reason could be the sample collection site. If the sample from hospital was collected from soil or environment, then, there was a greater possibility to find more *Staphylococcus aureus*. But it can be presumed that the multi drug resistant isolates found from community water samples might be transmitted from the hospital samples. In our research, it has been reported that 100% of the isolates are resistant to Aztreonam and Cefixime. 87.87% of the isolates were resistant to Erythromycin. 48.48% of the isolates were resistant to Ceftriaxone. 33.33%, 24.24%,

21.21%, 15.15% of the isolates were resistant to Clindamycin, Ciprofloxacin, Cefepime & Tetracycline, respectively. It is

difficult to treat multi drug resistant organisms. 3 of the isolates from community samples were found more pathogenic because of their resistance pattern against 7 antibiotics. Easily they can cause disease to immunocompromised patients. Also, the persons who have wounds or skin damage or mucosal can easily be penetrated by *Staphylococcus aureus*. They can come contact with hospital patients and it will be transmitted in the hospital wastewater, soil, environment or instruments. Again, the organisms will then be spread in community water samples and the cycle will be continued.

However, further monitoring of the presence and spread of antibiotic resistance in the environment is necessary due to the extensive effects it has on human health. Additionally, metagenomics technologies should be introduced for advance research on the analysis of the overall microbial profile and acquire a better knowledge of the microbial abundance observed in HWW. Cooperation between the scientific community and public authorities is required for the further planning and implementation of strategies, policies, and experimental techniques to reduce the use of antibiotics, identify microbial communities from wastewater, and map resistance mechanisms. To conclude, personal awareness like not sharing personal items, avoiding contact with other people's wounds, keeping cuts and scrapes clean and covered with bandages until they heal and cleaning hands properly can prevent staph infections.

Chapter 7

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