Isolation of *Staphylococcus spp.* from Hospital Wastewater and Adjacent Community Household Water: Special Focus on their Antibiotic Resistance.

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Declaration

It is hereby declared that

1. The thesis report submitted is my/our own original work while completing degree at Brac University.

2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and

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3. The report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other

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APPROVAL

The thesis/project titled "Isolation of *Staphylococcus spp.* from Hospital Wastewater and Adjacent Community Household Water: Special Focus on their Antibiotic Resistance" submitted by Kaniz Fatema (ID 18126062) has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on December, 2022

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Abstract

Staphylococcus spp. is a leading cause of human bacterial infections. These infections can damage the skin, soft tissues, bones, circulation, and respiratory system. It has the unusual capacity to rapidly develop resistance to any antibiotic deployed against it. Antibiotic-resistant *S. aureus* strains are rising at an alarming rate, which not only limits treatment options but also makes it impossible to calculate the economic deprivation caused by this superbug. In this research, the antimicrobial resistance patterns of *Staphylococcus spp.* isolated from hospital wastewater and community household water samples were investigated for 15 different antibiotics. The antibiotic resistance pattern of these *Staphylococcus spp.* isolates was determined using the disc diffusion method. *Staphylococcus spp.* was particularly resistant to antibiotics in the penicillin category, such as Penicillin-G, Oxacillin, and Methicillin. 60% isolates of hospital wastewater sample were resistant to both penicillin-G and methicillin & 100% isolates of community household water were resistant to penicillin-G and methicillin; 100% isolates of both hospital and community were resistant towards oxacillin. 40% isolates of hospital wastewater were resistant to Tetracycline. 100% isolates of hospital wastewater showed resistance towards the antibiotic Ceftazidime which belong to the group cephalosporins and 60% isolates of community water were resistant to Ceftazidime. 20% isolates of hospital wastewater and 25% isolates of community water were resistant to the antibiotic Erythromycin of the macrolides group. Since *Staphylococcus spp.* samples were resistant to more than one class of antibiotics, it can be concluded that they exhibited multidrug resistance.

Contents

List of Acronyms

ARB: Antibiotic resistant bacteria

MDR: Multidrug resistant

MSSA: Methicilin Susceptible *Staphylococcus aureus*

MRSA: Methicilin Resistant *Staphylococcus aureus*

DNA: Deoxyribonucleic acid

ug: Micrograms

ul: Microlitres

C: Celsius

bp: Base pairs

PCR: Polymerase chain reaction

rpm: Revolutions per minute

NA: Nutrient agar

MSA: Mannitol Salt Agar

Chapter One

Introduction

1.1 Background of the study

Infectious diseases continue to be a major cause of morbidity and mortality, particularly in developing countries. *Staphylococcus spp.*; especially *Staphylococcus aureus* is associated with a number of skin and soft-tissue conditions, including folliculitis, impetigo, furuncles, carbuncles, hidradenitis suppurativa, and cellulitis (Bamberger and Boyd, 2005). *S. aureus* and *Staphylococcus spp.* infections of the skin and soft tissues aureus often begin as small boils or abscesses, can progress to severe muscle or bone infections, and can migrate to the lungs or heart valves (i.e., endocarditis). Infections caused by *Staphylococcus spp.* are called staph infection. Through toxin production, *Staphylococcus spp.* especially *S. aureus* is also capable of triggering toxic shock syndrome and staphylococcal scalded skin syndrome. The toxic shock syndrome is distinguished by fever, hypotension, a macular rash that desquamates with time, and multiple organ failure. Additionally, the presence of *Staphylococcus spp.* in the circulation can cause endocarditis, sepsis, and metastatic foci of infection. According to Fowler et al. (2003), infective endocarditis affects around 12% of individuals with *S. aureus* bacteremia. Endocarditis is an infection that affects the heart valves and has the potential to cause either heart failure or a stroke. Osteomyelitis is a bone infection that may be induced by *S. aureus* migrating through the bloodstream or by direct contact, such as after trauma (foot puncture or IV drug abuse). Also, as a prevalent infection in nosocomial pneumonias, *Staphylococcus spp.* can cause pneumonia through hematogenous dissemination or aspiration. Pneumonia is an infection that most frequently strikes those who already suffer from an underlying lung condition, particularly those who are dependent on mechanical ventilators. Infections of the joints are almost always caused by *S. aureus*. Even though there is a lack of data about treatment, it is typically treated with drainage in conjunction with an antimicrobial treatment plan that lasts for four weeks. Early infections of *S. aureus* are treated with incision and drainage, frequently accompanied by beta-lactam antibiotics, which are also effective against beta-hemolytic *streptococci.* However, recently *S. aureus* strains have been resistant to beta-lactam antibiotics which are also known as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Methicillin-Resistant *Staphylococcus aureus* (MRSA) became more common when Methicillin-Susceptible *Staphylococcus aureus* (MSSA) started using a gene called mecA, which makes bacteria resistant to methicillin (Mukherjee et al.,2021). Even though drug companies have made a lot of new antibacterial drugs in the past few years, bacteria have become more resistant to these drugs, which is now a worldwide problem. In general, bacteria possess the genetic capacity to transfer and acquire drug resistance. *Staphylococcus aureus* is regarded as one of the leading causes of community and hospital-acquired infections among individuals (Al Saimary, 2012). Antibiotic-resistant bacteria have become more prevalent due to the overuse and poor management of antibiotics by medical professionals, as well as patients' failure to follow recommended antibiotic courses. By 2050, according to a study by the World Health Organization, drug-resistant illnesses may cause 10 million deaths yearly (Hugo and Russell, 1987).

1.2 Literature Review

Lowy (1998) states that *Staphylococcus aureus* and *Staphylococcus spp.* is the source of a wide variety of pyogenic illnesses, despite being a commensal of human skin and nares. It has become the primary cause of hospital and community-acquired infections during the past several decades. *Staphylococcus spp.* has a high infection incidence, and the recent rise in awareness of community-acquired infections has significant clinical and pharmacological consequences for the health care practitioner. According to a number of global studies, *Staphylococcus aureus* and *Staphylococcus spp.* from natural flora appears to be a significant reservoir of antimicrobial resistance genes that can be passed to other microbial pathogens, hence promoting resistance traits among microbial populations (Ugwu et al., 2009). A study performed in Bangladesh on 2014 demonstrated that samples of *Staphylococcus aureus* from hospitals and slaughterhouses have significant levels of antimicrobial resistance. According to their research, the multidrug resistance pattern seen in this research region may have originated in hospitals and expanded to the community (Ahaduzzaman et al.,2014). The globe is now moving into what is being called a "post-antibiotic" era. Antibiotics belonging to the beta-lactam class are ineffective against 88% of *S. aureus* infections that occur in Nigeria. The frequency of MRSA in Pakistan ranges from 42-52%, as stated by Akinkunmi and Lamikanra (2012). Researchers observed that 95% of the adult population in India and Pakistan has bacteria that are resistant to β-lactam antibiotics in a separate study (Reardon, 2014). Despite the limited number of treatment options that are currently available in the United States, research show that MRSA is responsible for roughly 11,000–18,000 annual deaths and 80,000 invasive infections (Morgenstern et al., 2016). According to Calfee et al (2008)., the percentage of methicillin-resistant *S. aureus* strains that are responsible for hospital-associated *S. aureus* infections in the United States has been progressively climbing over the past few years, furthermore, they stated that in hospitals in the year 2004, MRSA was responsible for 63% of all *S. aureus* infections. Vancomycin is the final line of defense against *Staphylococcus spp.,* especially *S. aureus* infections because methicillin failed to prevent the development of MRSA (Methicillin-resistant Staphylococcus aureus) strains, however, recent studies proved that *S. aureus* is also resistant to vancomycin proving as multi drug resistance.

1.3 Objective

This experiment isolated and investigated the prevalence of *Staphylococcus spp.,* especially *Staphylococcus aureus* and its antibiotic resistance in hospital wastewater and community household water next to hospitals in Dhaka, Bangladesh. While similar initiatives have been conducted in other countries, the somewhat unregulated and underdeveloped status of water treatment and disposal in an economically developing nation makes it a good research topic for multidrug-resistant *Staphylococcus spp.* populations. *Staphylococcus spp.* causes dangerous infections in people, such as endocarditis, abscesses with a deep location, and osteomyelitis. A hospital environment is very straightforward to monitor and regulate; therefore, hospitals can reduce the likelihood of nosocomial infections caused by *Staphylococcus spp.* Once

Staphylococcus spp. from hospital wastes reach aquatic reservoirs such as households, lakes and rivers, they can rapidly spread throughout the ecosystem and become extremely difficult to monitor and regulate. There are numerous strains of *Staphylococcus spp.* that are resistant to numerous antibiotics. The two most notable antibiotic resistances attained by *Staphylococcus aureus* are methicillin and vancomycin resistance according to some research. This study will be helpful to find out the antibiotic resistance pattern of hospital wastewater and community water in order to come up with alternative treatments of diseases caused by *Staphylococcus spp*. Antibiotic resistance has become an alarming fact worldwide, hence, doctors, scientists, researchers, microbiologists, clinicians, public health specialists, policymakers, and engineers, as well as anybody else involved in this sector, must be made aware of this contemporary issue and must devise an innovative, effective method to deal with it.

Chapter Two

Methodology

2.1 Sample Collection:

Four wastewater samples were collected from tertiary hospitals in Dhaka, these hospital samples given code names are- DNCHW1, JUDNCH, JUNCH & SEPDSH respectively, alongside five adjacent community household water samples per hospital- DNCWN1, JKYN1, JUDNCW1, JUDNCW2, JUDNCW3 were collected; in total 9 samples were collected. Each sample was collected in a sterilized airtight bottle and they were transferred to the laboratory within 1-2 hours of collection.

2.2 Selective isolation of *Staphylococcus spp.*

1. 100 ml hospital wastewater samples such as DNCHW1, JUDNCH, JUNCH & SEPDSH went through a membrane filtration process to trap the microorganisms. Then the filter paper was inserted into TSB+7.5% NaCl broth. Tryptic Soy Broth (TSB) is a nutrient-rich medium that promotes the growth of a wide range of microorganisms, including typical aerobic and facultative anaerobic bacteria. NaCl was added because *Staphylococcus spp.* are salt-tolerant bacteria, and the majority of its strains grow well in media with a high concentration of sodium chloride.

2. After inserting the filter paper inside the falcon tube of TSB+7.5% broth, the tubes were incubated at 37°C for 24 hours. Growth inside those broth media was indicated by the presence of turbidity after 24 hours.

3. Dilutions of each broth were done by serial dilution with sterile .9% saline buffer at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} dilutions respectively. Raw broth, 10^{-2}, 10^{-4}, & 10^{-6} dilutions were used to isolate colonies by the spread plate method on Himedia Mannitol salt agar. It is both selective and differential medium that can be used to isolate and identify *Staphylococcus spp.* from clinical and non-clinical samples. It promotes the development of a group of bacteria while limiting the growth of other microorganisms.

4. Similarly, samples of community water were subjected to repeated dilution. Each sample was serially diluted with a sterile 0.9% saline buffer to yield 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} dilutions sequentially. Mannitol Salt Agar were used to selectively isolate colonies from the water samples by spread plate technique of inoculation.

5. After 24 hours of incubation at 37 degree C, single colonies of bacteria were selected from the petri dishes based on color and colony morphology. Yellow/pink colonies were selected and inoculated again onto Mannitol Salt Agar by streak plate method to grow discrete colonies. Then

the petri dishes were incubated at 37°C for 24 hours. After observing bacterial growth after an overnight incubation, positive samples were selected based on colony morphology and validated by Gram's staining.

6. Using a sterile needle, a single colony of bacteria was isolated from every sample's petri dishes and then stabbed into T1N1 media. Next, immersion oil was poured to the media to increase its stocking level.

2.3 Antimicrobial susceptibility testing

The phenotypic characterization for antibiotic resistance was performed by the Kirby Bauer Disk diffusion method. Antibiotics selected were- Gentamicin (10 ug), Ceftazidime (30 ug), Ceftriaxone (30 ug), Cefuroxime (30 ug), Erythromycin (15 ug), Oxacilin (5 ug), Cloxacilin (5 ug), Vancomycin (30 ug), Penicillin-G (6 ug), Methicillin (5 ug), Tetracycline (30 ug), Azithromycin (15 ug), Levofloxacin (5 ug), Meropenem (10 ug) & Amikacin (30ug).

Using a sterile inoculating needle, colonies were taken from T1N1 stock of the isolates, and then streaked on nutrient agar were suspended in 9ml of sterile saline in screw cap test tubes. The tubes were vortexed to create a uniform suspension and turbidity was adjusted to 0.5 McFarland standard. Sterile cotton swabs were used to create lawns of the isolates on petri dishes containing MHA, after which antibiotic disks are placed over the surface of the media by sterile forceps. The plates are incubated at 37 degree C for 16 hours, after which the zone-of-inhibition diameters are measured. Results were interpreted as resistant/ intermediate/ sensitive in reference to breakpoints defined by the Clinical and Laboratory Standards Institute.

2.4 Molecular Analysis

DNA of each isolate is extracted by boiling lysis extraction method. The selected isolates were inoculated in 5ml of Luria bertani broth .1000 µl of broth was dispensed into a microcentrifuge tube and centrifuged for 13000 rpm for 6 minutes, after which the supernatant was discarded from the top and only cell pellet remained. Pellet was vortexed with 150 µl TE buffer and further centrifuged at 12000 rpm for 5 minutes. Subsequently the supernatant is discarded and the pellet is washed with 200ul of TRIS EDTA buffer. The samples were heated in a water bath for 15 minutes at 99°C, after which they were immediately cold shocked by placing on ice and transferred to a -20°C fridge compartment for 10 minutes. After the samples are removed from ice, they are centrifuged at 14000 rpm for 5 minutes. Supernatant was collected into labeled microcentrifuge tubes and stored at -20°C until use.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) rose to prominence when Methicillin-Susceptible *Staphylococcus aureus* (MSSA) began adopting a specific gene(methicillin-resistant gene) known as mecA, which is mediated by a genetic element known as *Staphylococcal* cassette chromosome (SCC) and is relocated into the MSSA via conjugation or

transformation (Horizontal gene transfer). This mec gene is responsible for encoding the penicillin-binding protein 2a. This PBP-2a is distinct from other PBPs. This protein's affinity for beta-lactam is lower than that of other PBPs. This decreases the binding of PBPs to beta-lactam antibiotics, producing a highly resistant organism. Recent research has demonstrated that MRSA stains have also developed multidrug resistance. In this work, MRSA is identified using a standard PCR. This assay detects two genes: the nuc gene, which encodes for a *S. aureus*-specific thermostable nuclease, and the mecA gene, which encodes for PBP2a, which confers beta-lactam antibiotic resistance. Strains of *S. aureus* produce thermostable nuclease (thermonuclease [TNase]) extracellularly at a rate comparable to coagulase production. TNase is an endonuclease protein with a molecular mass of 17,000 Da that destroys both DNA and RNA, and its enzymatic activity can tolerate temperatures of 100°C for at least an hour. It has been thoroughly reported that the TNase protein corresponds to a nuc gene. To identify *S. aureus* from the selected isolates, numerous laboratories use an enzymatic test for TNase production.

Table 2.1: Primer information and PCR conditions

Target gene of primer used	mecA	nucA
Sequence of reverse primer	CCACTTCATATCTTGTAACG	AGCCAAGCCTTGACGAACT
Sequence of forward primer	ACCAGATTACAACTTCACCAGG	GCGATTGATGGTGATACGG
Volume of each primer used/ ul	0.5	0.5
volume of NF water used / ul	3.5	3.5
Volume of mastermix	7.5	7.5
Number of isolates / ul	10	10
amplicon size	162bp	279bp
Conditions	An initial temperature of 95°C (1min), denaturation at 95°C (15s), annealing at 45°C (15 s), and elongation at 72°C (30s), with a final extension at 72°C (4min).	An initial temperature of 95°C (1min), denaturation at 95°C (15s), annealing at 45°C (15 s), and elongation at 72°C (30s), with a final extension at 72°C (4min).

2.5 Biochemical Confirmation

The selected isolates were subjected to a series of biochemical tests for the identification of *Staphylococcus spp.* which included the triple sugar iron test for sugar fermentation, motility-indole-urease test, Methyl Red – Voges-Proskauer test, citrate utilization test, catalase

test, oxidase test as well as gram staining. Then, the results were compared with the biochemical result reference chart (Given below) for more accuracy.

Table 2.2: Reference chart for identification by biochemical tests

Organism	Gram Stain	TSI Fermentation				Motility	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase Activity	Oxidase Activity
		Lactose	Dextrose	Sucrose	H_2S								
Staphylococcus spp.	Cocci +	A	A	A	-	-	-	+	±	-	-	+	-

Chapter 3

Results

After 24 hours of incubation pink and yellow colonies were observed. While *Staphylococcus spp.,* especially *Staphylococcus aureus* creates yellow colonies with yellow zones, other coagulase-negative *staphylococci* produce pink or red colonies without changing the color of the medium. The source of fermentable carbohydrates is mannitol, and during fermentation, acid is produced. *Staphylococcus spp.* thrives on this substrate and ferments mannitol to produce yellow colonies. The majority of mannitol-intolerant *Staphylococci* species develop small red colonies and lack mannitol fermentation.

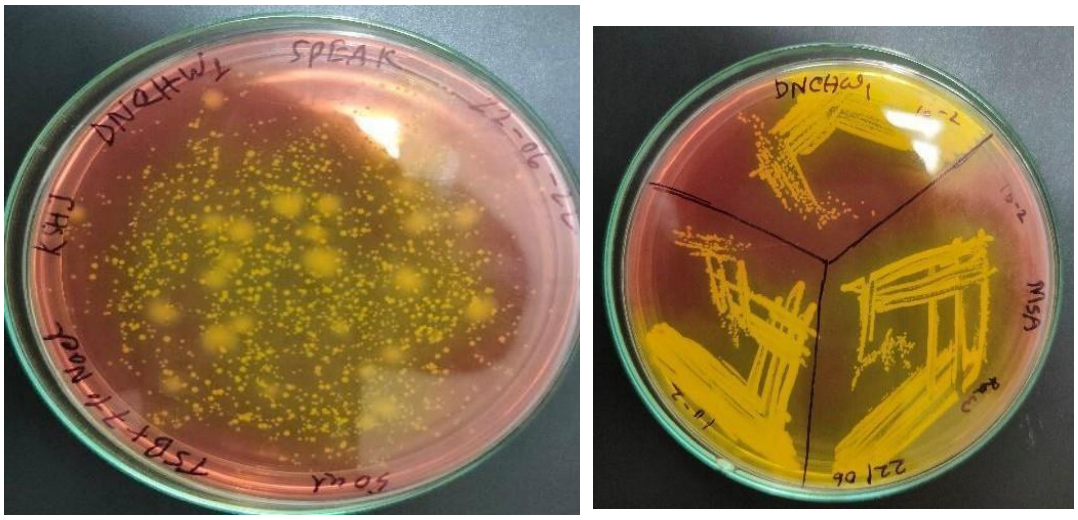

Figure 3.1: Yellow & pink colonies on MSA were observed then streaked on MSA in order to yield discrete colonies.

Medium sized colonies were randomly selected and streaked on MSA in order to yield discrete colonies of *Staphylococcus spp*. Biochemical tests were done in order to confirm *Staphylococcus spp.*

3.1 Biochemical test result

All of the 10 suspected *Staphylococcus spp.* isolates went through biochemical tests in order to confirm that they were indeed *Staphylococcus spp.*

Sample	Gram Stain	TSI Fermentation				Motility	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase Activity	Oxidase Activity
		Lactose	Dextrose	Sucrose	H_2S								
JUDNCW3	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
DNCHW1	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
SEPDSH (5HS)	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
JUDNCH	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
JUDNCW2	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
JUDNCW1	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
JKYN	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
JUNCH	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
DNCWN1	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-
SEPDSH (12)	Cocci +	A	A	A	-	-	-	-	-	-	-	+	-

Table 3.1: Biochemical tests result

For further confirmation, the presence of the nucA gene was confirmed by PCR. *Staphylococcus aureus* identification is a key challenge in medical microbiological diagnostics. The thermonuclease-encoding nuc gene is commonly employed as a particular target for the identification of S. aureus by PCR. One isolate JUDNCW3 was confirmed to have the nucA gene among 10 isolates of suspected *Staphylococcus spp*.

.Figure 3.2: JUDNCW3 nucA positive (270 bp) .

3.2 Antimicrobial assay results

All of the tested 10 isolates exhibited phenotypic antibiotic resistance to 6 of the selected antibiotics, as confirmed by the antibiotic susceptibility assay. Hence, 77.77% of all examined isolates exhibited phenotypic multidrug resistance. 4 of these isolates were sourced from clinical wastewater, while 3 isolates of them were from community sources. Hence, 100% of clinical isolates were multidrug resistant while 60% of environmental isolates were multidrug resistant. Percentage of resistance isolates, segregated by *Staphylococcus spp.*, is outlined in table 3.2.

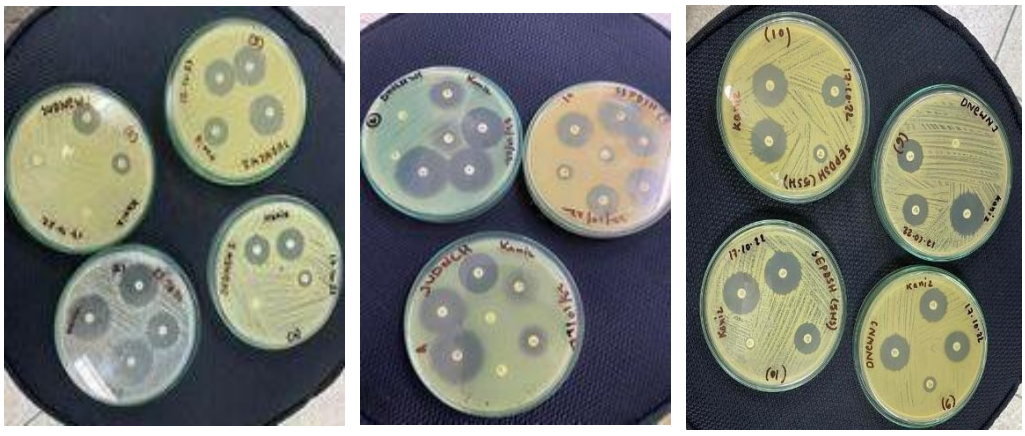

Figure 3.3: Here, the isolates show multidrug resistance of Ceftazidime, Erythromycin, Oxacilin, Methicillin, Penicillin-G & Tetracycline antibiotics.

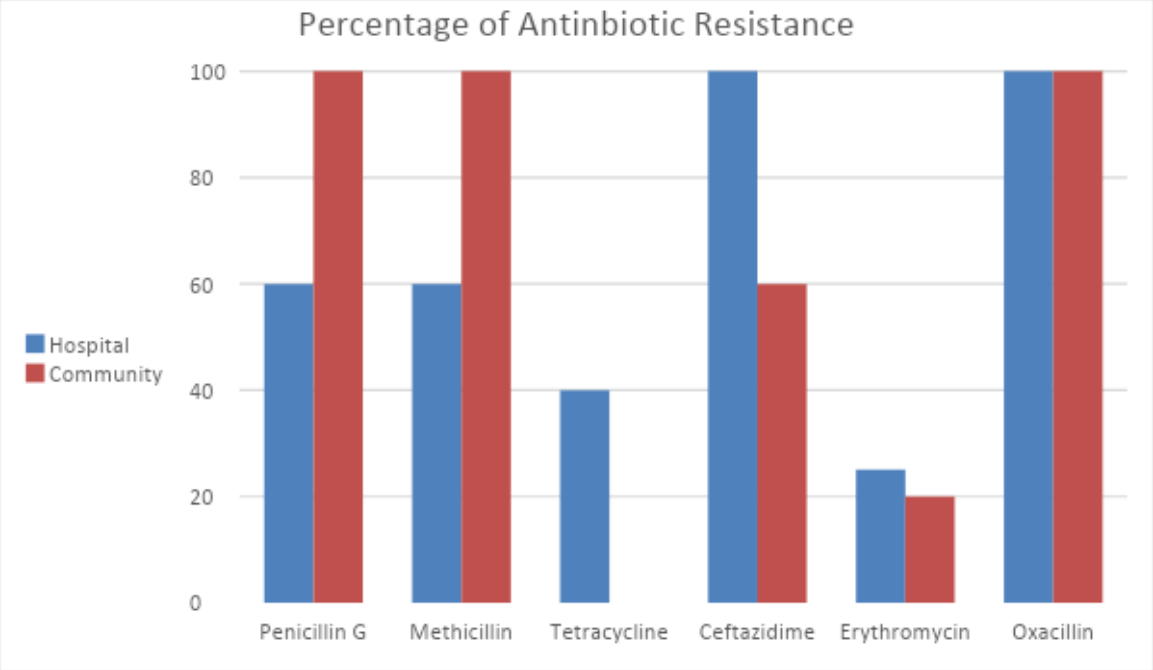

Table 3.2: Percentage of resistant isolates

3.3 Molecular characterization

Only one isolate was mecA positive for *Staphylococcus spp.* gene which is DNCHW1 (162 bp). It indicates one isolate contains methicillin-resistant *Staphylococcus aureus* strain. The development of altered penicillin-binding proteins PBP2a or PBP2′ with reduced affinity for β-lactam antibiotics is the fundamental mechanism of methicillin resistance in *S. aureus*.

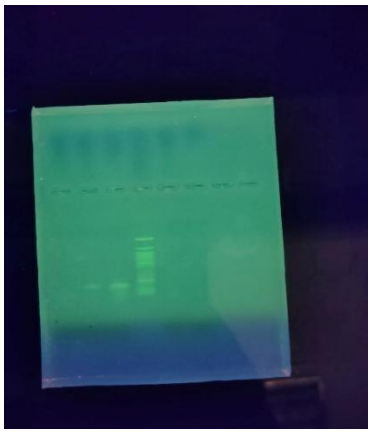

Figure 3.3: DNCHW1 mecA positive

Chapter 4

Discussion:

The aim of this research is to find out the multi-drug resistance pattern of *Staphylococcus spp.* isolates. *Staphylococcus spp*. showed most resistance to antibiotics of the penicillin group, such as, Penicillin-G, Oxacillin, Methicillin. 60% isolates of hospital wastewater sample were resistant to both penicillin-G and methicillin & 100% isolates of community household water were resistant to penicillin-G and methicillin; 100% isolates of both hospital and community were resistant towards oxacillin. 40% isolates of hospital wastewater were resistant to Tetracycline. 100% isolates of hospital wastewater showed resistance towards the antibiotic Ceftazidime which belong to the group cephalosporins and 60% isolates of community water were resistant to Ceftazidime. 20% isolates of hospital wastewater and 25% isolates of community water were resistant to the antibiotic Erythromycin of the macrolides group. Therefore, it can be concluded that the *Staphylococcus spp.* isolates were multi-drug resistant since they were resistant to more than one group of antibiotics. Bukhari et al., (2011) found that *Staphylococcus aureus* resistance to oxacillin, ampicillin, and penicillin was 100%, and cephalothin resistance was 92.4% and these findings match the data of my findings since I also found the resistance pattern of beta-lactam antibiotics. Qureshi found that 97.8% of the bacteria were resistant to gentamicin, which contradicts the findings of my research because all of the bacteria were susceptible to the gentamicin. A recent study of antibiotic resistance pattern reveals that 15.1% of MRSA isolates were resistant to vancomycin, while intermediate levels of resistance were observed with clindamycin (54.5%), gentamicin (45.5%), and tetracycline (48%) and high levels of resistance with ciprofloxacin (72.7%) and erythromycin (90%) (Hanif & Hassan, 2019), however, this contradicts with my study because in my study gentamycin and vancomycin were susceptible to all of the *Staphylococcus spp.* isolates. In addition, an Indian investigation revealed that vancomycin was the sole antibiotic with 100% uniform sensitivity (Rajaduraipandi et al., 2006), which is similar to the results of my study because the isolates were 100% sensitive to vancomycin. This result is also consistent with the findings of Shah et al. (2016), Hizbullah et al. (2015), Ullah et al. (2016), Bukhari (2004) and Hafeez et al. (2004). Every single one of these studies has found no evidence of resistance to vancomycin of *Staphylococcus spp*.

According to this study, the overall trend of antibiotic resistance is on the rise, and the duration of resistance development is concerning. Vancomycin, gentamicin, azithromycin, levofloxacin, and meropenem are the antibiotics that can currently be used to treat *Staphylococcus spp.* infections, as these drugs shown 100% susceptibility compared to other antibiotics evaluated in this study.

Despite my best efforts, I was unable to conduct the study without certain limitations. In this investigation, only 10 suspected *Staphylococcus spp.* isolates were taken. Furthermore, specific primers were not available in the lab to confirm the presence of *S. aureus*. Also, pathogenicity was not tested such as coagulase test.

Chapter 5

Conclusion

To sum up, the general trend of antibiotic resistance is rising, and the time span over which resistance develops is quite concerning according to the findings of this research. Existing medications for the treatment of *Staphylococcus spp.* infections are limited, necessitating the development of a new arsenal of antibiotics to combat this lethal superbug. For the future of medicine and antibiotic therapy, the frequency of MDR *Staphylococcus spp.* in hospital wastewater and environmental reservoirs, as demonstrated in the current study and in other studies conducted throughout the decades, is particularly concerning. This indicates an urgent need for antibiotic management. Lakes and rivers in which significant microbiological loads of MDR bacteria are identified should be recognized as hazardous and banned from human exposure and activities that put humans at risk of exposure, such as bathing, fishing, and boating. When feasible, health practitioners should ensure that antimicrobial medications are provided with caution and only when necessary, with preventive use regulated according to the known susceptibility of the infecting strain.

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