The prevalence of *Staphylococcus* in cooked chicken collected from local restaurants in Dhaka city

By

Tahmina Dola

A thesis submitted to the **Program of Biotechnology** in the **Department of Mathematics and Natural Sciences (MNS)** in partial fulfillment of the requirements for the degree of **Bachelor of Science in Biotechnology**

B. Sc. In Biotechnology

Department of Mathematics and Natural Sciences (MNS)

BRAC University

Declaration

It is hereby declared that-

1. The thesis submitted is our original work while completing the degree at BRAC University.

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

3. The thesis does not contain material that has been accepted or submitted for any other degree or diploma at a university or other institution.

4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tahmina Dola

19136015

Approval

The thesis/project titled "**The prevalence of *Staphylococcus* spp. and *Staphylococcus aureus* in animal-originated food (cooked chicken) collected from local restaurants of Dhaka city and the antibiotic susceptibility pattern of *Staphylococcus aureus*"** submitted by Tahmina Dola (19136015) of Spring-2019 has been accepted as satisfactory partial fulfillment of the Bachelor of Science in Biotechnology degree requirements on 26 June, 2025.

Examining Committee

Supervisor:

(Member)

Kashmery Khan

Senior lecturer
Department of Mathematics and Natural Sciences

BRAC University

Program Coordinator:

(Member)

Munima Haque, PhD

Associate Professor
Department of Mathematics and Natural Sciences

BRAC University

Departmental Head:

(Chair)

Md. Firoze H. Haque, PhD

Associate Professor and Chairperson
Department of Mathematics and Natural Sciences

BRAC University

Aim

The aim of this project is to find out the presence of *Staphylococcus* spp. and *Staphylococcus aureus* in the cooked (processed) meat collected from restaurants. Consuming contaminated food with pathogenic bacteria is one of the major health risks that we are facing presently. But this is highly neglected by the health care sectors. So, our objective is to isolate and characterize the disease-causing bacteria. Moreover, as multiple data have proven the existence of multi-drug resistant (MDR) bacteria, we also aim to carry out antibiotic susceptibility tests (AST) on the isolated bacteria.

Acknowledgment

I would like to begin by expressing our heartfelt gratitude to Almighty Allah for granting me the strength and courage to successfully complete our thesis. I would also like to thank all those who have supported and guided us throughout this journey.

First and foremost, I am deeply thankful to my thesis supervisor, Kashmery Khan, for her unwavering support and invaluable guidance. Her expertise, patience, and dedication were instrumental in shaping this research. I also extend sincere appreciation to our Teaching Assistants, who took the time to teach us the essential laboratory techniques and procedures needed for our thesis and help us with any query or doubt harboring our minds. Their consistent presence and constant encouragement throughout the entirety of our thesis kept us going strong during the toughest times. I owe profound gratitude to our respected Lab officers, Md. Mahmudul Hasan and Shamima Akhter Chowdhury for their help in solving various technical difficulties, constant assistance, and insightful feedback, which significantly enhanced the quality of this work. I extend our genuine thanks to the lab attendants, Ashik E Khuda, Tanzila Alam, and Nadira Yeasmin for always helping us by providing the lab equipment and setups we require and always looking out for us.

Furthermore, I want to acknowledge the contributions of my friends who offered their assistance and encouragement when we needed it the most. Your bits of advice and feedback were invaluable.

Last but not least, I am grateful to my family members for their unending love, encouragement, and understanding during the long hours and numerous revisions required for this project. Their constant support has been an enormous source of strength.

To sum it up, I am deeply appreciative of the opportunity to pursue this academic journey, and I am grateful for the support of all those who have played a part, no matter how big or small, in the completion of this thesis.

Thank you all.

Acronyms

SFP	Staphylococcal Food Poisoning
MRSA	Methicillin-Resistant *Staphylococcus aureus*
AST	Antibiotic Susceptibility Test
PCR	Polymerase Chain Reaction
MSA	Mannitol Salt Agar
TSI	Triple Sugar Iron
MR	Methyl Red
VP	Voges-Proskauer

Keywords

- *Staphylococcus aureus*
- Cooked chicken
- Food contamination
- Antibiotic resistance
- Enterotoxin
- Ready-to-eat food
- Dhaka restaurants
- Public health
- Gram-positive cocci
- Cross-contamination

Abstract

Food poisoning remains a significant public health concern, affecting thousands of individuals each year. Among the primary bacterial agents implicated, *Staphylococcus aureus* is recognized as a leading cause, raising concerns about its potential to contribute to widespread outbreaks. It is commonly assumed that traditional cooking practices, such as prolonged heat exposure and the use of antimicrobial spices are sufficient to eliminate pathogenic bacteria from food. However, the frequent occurrence of foodborne illness among consumers in Dhaka city suggests otherwise. In this study, densely populated areas of Dhaka were selected for the investigation of *Staphylococcus* contamination in cooked meat samples sourced from local restaurants. Biochemical testing revealed that *Staphylococcus* spp. was detected in 81% of the samples, with one specific area showing a 100% contamination rate. Notably, 28% of these isolates exhibited high halotolerance. Molecular analysis further confirmed the presence of the *nucA* gene, a marker specific to *Staphylococcus aureus*, among the isolates. Additional assessments, including antibiotic susceptibility testing and hemolytic activity profiling (alpha and beta hemolysis), yielded noteworthy findings. Future research will focus on characterizing the enterotoxigenic profiles of these *Staphylococcus* strains. The results of this study underscore the urgent need for enhanced food safety measures and public health interventions in Dhaka, particularly in high-density urban settings.

Table of Contents

List of Table

List of figures:

Chapter 1

1.1 Introduction

People worldwide are habituated to consuming ready-to-eat (RTE) foods available in restaurants because of their availability, convenience, simplicity of handling, etc. However, there remains a concern about the food safety of these cooked foods. The microbial hygiene in such places may underlie the quality of the consumed food items and the probability of microbial contamination, thus, food-borne illnesses are becoming one of the major health issues globally (Sarker et al., 2013; Akhtar et al., 2014). Reports from recent years have stated that food-borne diseases arose due to consuming contaminated non-homemade foods (Happy et al., 2018). It is quite common to have pathogenic bacteria in raw food items, especially in raw meat items (Paudyal et al., 2017). Still, the problem arises when the cooked meals are getting contaminated and we are directly consuming them (Hoque et al., 2015). Numerous studies have been conducted for bacterial detection in raw food items and some were specific for raw meat. Still, very few are available for cooked foods and more specifically cooked meat items (Safarpoor et al., 2017; Shahraz et al., 2012; Lakhanpal et al., 2019; Hoveida et al., 2019; Mahadi et al., 2014; Haghi et al., 2021; Soltan-Dallal et al., 2010; Comi et al., 2005; Ranucci et al., 2004; Heo et al., 2020; Chung et al., 2021; Rhee and Woo, 2010; San Moon et al., 2007; Oh et al., 2007; Krupa et al., 2014; Podkowik et al., 2012; Rodríguez-Lázaro et al., 2017; Gousia et al., 2011).

Usually, foods that are already cooked or processed are not treated thermally or pasteurized anymore (Khater-Dalia et al., 2013), thus making RTE foods one of the major routes for bacteria to enter our body if the food is already contaminated. *Staphylococcus aureus*, commonly known as *S. aureus*, is a type of bacteria well-known for its virulence and ability to resist antimicrobial treatment. It commonly colonizes different parts of the human body, such as the nose, throat, armpits, and groin of healthy individuals, etc. The rate of colonization in populations is approximately 25%, which varies based on geography, age, and other risk factors (Lindsay, 2019). Foods contaminated with *Staphylococcus* spp. enterotoxigenic strains have become a major issue. It is also considerably underreporting due to the short duration of clinical symptoms and lack of

medical care. These bacteria themselves are a commensal and opportunistic pathogen. Its pathogenicity and antimicrobial resistance have made it more threatening (Freitas et al., 2023).

Staphylococcus aureus (*S. aureus*) is a bacterium that causes foodborne illnesses. These illnesses are typically contracted through the consumption of contaminated food items such as meat, fish, milk, and eggs. Symptoms of such illnesses include minor boil infections and other related symptoms like- food poisoning, gastrointestinal diseases, vomiting, nausea, etc. (Argudín et al., 2010). Enterotoxins produced by pathogenic *Staphylococcus* strains are responsible for foodborne disease (FBD) outbreaks. Enterotoxins are produced in suitable conditions like- temperature, pH, and water activity and these are resistant to various stressful situations, such as - freezing, drying, heat treatment, and low pH (Freitas et al., 2023).

The primary requirement for staphylococcal food poisoning is that the food must be contaminated by an enterotoxigenic strain of *Staphylococcus* spp. (Freitas et al., 2023). These harmful bacteria can be transmitted through cooked foods due to various factors, such as poor food handling, contamination during food preparation and serving, exposure to air, dust, and insects, and cross-contamination from raw food items (Sarker et al., 2013; Hoque et al., 2015; Hoveida et al., 2019). It is concerning that pathogenic bacteria are developing resistance to multiple drugs and heat, making them more resilient and posing a global health threat (Chajęcka-Wierzchowska et al., 2014). Unfortunately, these issues are often overlooked by the health sector, leading to outbreaks of epidemics and diseases (Happy et al., 2018). Therefore, it is crucial to enhance local surveillance of food quality and safety to protect public health.

It is essential to determine the prevalence of bacteria to identify the most common species for treatment. In Bangladesh, a country with a growing population, there is an increasing demand for protein-based food. Traditionally, food is cooked for extended periods, which tends to destroy most bacteria present in raw ingredients due to exposure to high temperatures and the addition of spices, which have antimicrobial properties. However, despite these measures, pathogenic bacteria can still be present in ready-to-eat (RTE) foods due to contamination. Meat items, in particular, are cooked for longer periods, making them susceptible to cross-contamination if pathogenic bacteria are present. Unfortunately, restaurant employees are not adequately trained in maintaining a hygienic environment, handling food, and serving it hygienically. Given the large population, maintaining a hygienic environment from cooking to serving is challenging, potentially leading to

contamination and subsequent foodborne illnesses. Therefore, it is crucial to prioritize the detection and analysis of pathogenic bacteria in cooked food for public health reasons. Our research aims to identify pathogenic bacteria in cooked meat obtained from restaurants. We are focusing on cooked meat to highlight that even thoroughly cooked or processed meat can serve as a source of infectious bacteria. The widespread antibiotic resistance of bacteria has rendered existing treatments less effective, underscoring the need to explore new treatment options.

1.2 Ecology and Pathogenicity of *Staphylococcus* spp.

The *Staphylococcus* genus belongs to the Micrococcaceae family. These bacteria are gram-positive, non-motile, non-spore-forming, and aerobic to facultative anaerobe. There are more than 20 recognized species in this genus, distributed in various habitats. Some colonize the skin surface, cutaneous glands, and human or animal mucosal membranes. In animals, such as birds and mammals, the skin and mucous membranes, especially those in the nasopharyngeal region, are considered the primary reservoirs for *Staphylococcus aureus* (SA). Reports have shown that healthy individuals carry the organism, indicating that this microorganism has a high potential to exist, transmit, and contaminate animal food products (Mashouf et al., 2015; Ho et al., 2014).

Staphylococcus aureus is a symbiotic and opportunistic organism. It causes a variety of illnesses due to its toxic-mediated pathogenicity, invasiveness, and antimicrobial resistance (AMR). Food-borne diseases related to *S. aureus* can result from consuming contaminated foods, leading to nausea, vomiting, dizziness, hypothermia, stomach cramps, weakness, and diarrhea lasting 1-6 hours (Odetokun et al., 2023). While a few bacterial cells ingested by a healthy person can be neutralized by stomach acid, immunocompromised individuals are at greater risk. *S. aureus* can cause infections and even enter the bloodstream, potentially leading to fatal outcomes (Pesavento et al., 2007).

S. aureus is a pathogen (Archer et al., 1998) that is commonly found in community and hospital-acquired infections. It is frequently isolated from infectious bloodstream, lower respiratory tract, skin, and soft tissue. This bacterium is responsible for causing a range of diseases in both animals and humans, from minor skin lesions to life-threatening septicemia. It has also developed resistance to antibiotics, with the number of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital-acquired infections significantly increasing in recent years. The ubiquitous nature of *S.*

aureus, carried by almost 30% of healthy individuals, especially in the anterior nares, creates a reservoir for infection. To address the challenges posed by *S. aureus*, various initiatives are being undertaken to raise awareness, reduce contamination rates, and develop new prophylaxis and therapies (García-Lara and Anozie, 1990).

1.3 Prevalence of *Staphylococcus* spp. and *Staphylococcus aureus* in food products

Numerous reports have highlighted the widespread presence of *Staphylococcus* species and *Staphylococcus aureus* in both cooked and raw food samples. However, the analysis of cooked meat is very few (Mashouf et al., 2015; Sokari and Anozei, 1990; San Moon et al., 2007; Pesavento et al., 2007; Guven et al., 2010). These studies aimed to identify, isolate, and characterize the bacteria in animal-derived foods such as poultry, beef, dairy, pork, etc. Since these bacteria are commonly found on the skin and mucous membranes (García-Lara et al., 2005), contamination during food processing and serving is not uncommon (Sarker et al., 2013; Safarpoor et al., 2017; Hoveida et al., 2019).There are several reasons for such contamination, such as poor handling of utensils and food ingredients, lack of hygiene during food preparation and serving, contaminated air, dust and insects, and cross-contamination from raw food items (Sarker et al., 2013; Safarpoor et al., 2017; Hoveida et al., 2019).

Animal-originated food is prone to microbial contamination (Thomas et al., 2008; Dehkoedi et al., 2019; Fijalkowski et al., 2016; Chajęcka-Wierzchowska et al., 2014; Jofré et al., 2008; Argudin et al., 2010), and experiments were done in many countries. For example- in Hongkong, they worked on pork for the detection of *Staphylococcus aureus* contamination (Young et al., 2014); in India, they also investigated the incidence and antimicrobial susceptibility of *S. aureus* by using Momo, sandwiches, and soup as the samples as all these have meat (Lakhanpal et al., 2019)*;* moreover, in Italy, detection of *S. aureus* as well as detection of enterotoxin was also analyzed from animal originated food (Pepe et al., 2016; Gallina et al., 2013). Other countries like Iran, Korea, Poland, Spain, and Turkey, also analyzed animal-originated food (raw and cooked) for the prevalence check, presence of enterotoxin-producing strain, and antibiotic susceptibility test (Oh et al., 2007; Dehkordi et al., 2019; Fijałkowski et al., 2016; Argudin et al., 2010; Jofre et al., 2008; Hoveida et al., 2019; Rodríguez-Lázaro et al., 2017; Yildirim et al., 2017).

S. aureus is recognized as a common cause of infection in both humans and animals and it is among the four most common causes of infection. The main reason for the infection is the presence of enterotoxins produced by these bacteria. *Staphylococcal* enterotoxins (SEs) are the most important virulence factor in food poisoning. In Korea, food poisoning outbreaks took place primarily because of meat products (Oh et al., 2007).

Moreover, studies revealed that the strains of *S. aureus* can adapt to stressful environments (Ma et al., 2019) as a result, it can sustain cooked food or survive during food processing. Although during the food processing and cooking period, the heat and the spices that we use in the food have the possibility to kill *S. aureus* cells, thermo-stable *staphylococcal* enterotoxin genes retain their natural biological activity and create a major public health concern (Pexara et al., 2010).

1.4 Antibiotic resistance of *Staphylococcus* spp. and *Staphylococcus aureus*

The emergence of bacterial antibiotic resistance has become a global issue and a major health problem (Hassan et al., 2021). If these microbes develop resistance to three or more distinct antibiotic classes, they may turn into multidrug-resistant (MDR) organisms. Inappropriate use or misuse of antibiotics and residue of antimicrobial agents remaining in agriculture, and poultry is one of the key drivers of the emergence of antimicrobial resistance (Hassan et al., 2021). Moreover, the horizontal gene transfer method is also responsible for the resistance mechanism as the genes that act against the antibiotics, can be transferred from one bacterium to another (Chajęcka-Wierzchowska et al., 2014). Genes expressing antibiotic resistance in *S. aureus* can be in chromosomic or plasmid DNA. The plasmid DNA that contains antibiotic-resistance genes can easily migrate from one bacterium to another (Pesavento et al., 2007).

The first evidence of *S. aureus* resistance to Penicillin was reported in 1941. This resistance is a plasmid, which spreads quickly to several other strains. MRSA (Methicillin-resistant *Staphylococcus aureus*) has emerged as a risk factor in the general population, especially in immunocompromised individuals. The prevalence of MRSA in humans depends on the site of infection, region, and whether it is a nosocomial infection. In 2001, a community-acquired MRSA incident was reported, where a family ingested pork meat that was later revealed to be contaminated with MRSA (Pesavento et al., 2007).

The bacterium *S. aureus* has become resistant to antibiotics, making it challenging to treat using conventional methods. Studies have found that *S. aureus* is Multi-Drug Resistant (MDR) and shows resistance to antibiotics such as penicillin, ampicillin, tetracycline, clindamycin, and erythromycin. For instance, a report from India indicated that 81.3% of isolates were multi-drug resistant, with high resistance levels observed against methicillin (93.7%), clindamycin (68.8%), erythromycin (56.3%), and vancomycin (43.8%) (Lakhanpal et al., 2019). Similarly, a report from Iran showed that out of 98 isolates examined, the most frequent resistance was observed against erythromycin, tetracycline, clindamycin, and gentamicin (Mashouf et al., 2015).

It's important to remember that food can unfortunately introduce harmful microorganisms to the general population, potentially leading to the transfer of antibiotic-resistant bacteria to consumers' intestinal tracts (Klein, 1999). This can result in the exchange of antibiotic-resistant genes between non-pathogenic bacteria, pathogenic bacteria, and opportunistic bacteria, which is concerning.

1.5 *Staphylococcus aureus* pathogenicity

S. aureus is capable of producing one or more toxins called enterotoxins known as *Staphylococcal* enterotoxins (SEs). These enterotoxins are potent gastrointestinal exotoxins that are synthesized by *S. aureus* during the logarithmic phase of growth. SEs are one of the major causes of food poisoning that occurs after the ingestion of foods that are contaminated with SA (food like meat or dairy). The symptoms such as - vomiting, nausea, and diarrhea are rapid onset. Though the illness is self-limiting, occasionally it can be severe enough to get hospitalized (Argudin et al., 2010).

S. aureus does not typically compete well with the natural microorganisms found in raw food. Its presence is mainly due to contamination, resulting from improper food handling and storage conditions. Foods commonly associated with staphylococcal intoxication include meat, meat products, eggs, and dairy products. SEs are active in high nano-gram to low microgram quantities. They are classified into classic enterotoxins (SEA–SEE) and new enterotoxins (SEG–SEQ and SER–SEU) based on their biological and serological characteristics. Among these twenty types of SEs, A to E is highly isolated from food poisoning outbreaks in most cases (90%). Studies performed in the USA and UK revealed that SEA and SEB have been isolated in 69% of food

poisoning cases, while new enterotoxins were caused in only 5% of cases. SEs are usually considered significant virulence factors that belong to a large family of pyrogenic toxin superantigens (PTSAgs). To detect SEs from isolated strains and food, phenotypic methods have been introduced along with immunological methods (agglutination and biological and chromatographic assays). However, two of the major limitations of these methods are- they are of low accuracy and lack of comprehensiveness [San Moon et al., 2007; Mashouf et al., 2015; Sokari and Anozie, 1990; Normanno et al., 2007].

Chapter 2

2.1 The Rationale of the Thesis

During the review of the study, it became visible that *Staphylococcus* spp. and especially, *Staphylococcus aureus* are one of the commonly found pathogens in meat products and cause adverse effects on human health. This led to a research gap regarding the identification of these bacteria, especially found in chicken curry found in restaurants.

With the growing civilization, people are getting more and more habituated to restaurant foods. Among all the protein sources, the most common food is chicken because of the price elasticity. As a result, it is the most consumed protein in South Asian regions, including Bangladesh. A report from Selvanathan, E. A. et al, from 2000 to 2017 showed that the consumption of meat has increased dramatically, especially, chicken consumption has increased the most (Figure 1) (Selvanathan et al., 2020).

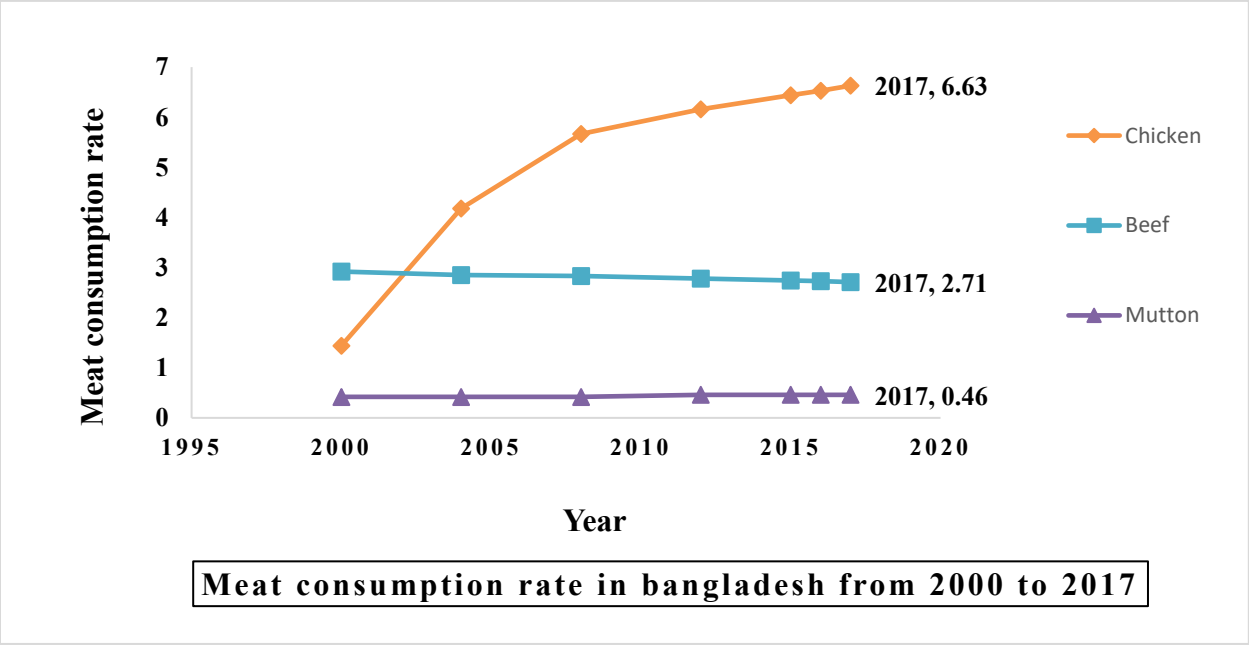


Figure 1: Meat consumption rate in Bangladesh from 2000 to 2017.

Additionally, there are great quantities of reports conducted on raw meat products but among them, very few samples were cooked (Lakhanpal et al., 2019; Hoveida et al., 2019; Dehkordi et al., 2019;

Chajęcka-Wierzchowska et al., 2014; Argudin et al., 2010; Oh et al., 2007; Yildirim et al., 2017; Goja et al., 2013; Organji et al., 2018; Igbinosa et al., 2016). As already mentioned, cooked meat was not considered for the bacterial analysis in most of the studies, investigations are yet to be conducted on chicken curry likewise. Keeping all these in consideration, this study was performed. Not only the bacterial identification but also the antibiotic test for the *Staphylococcus aureus* strains were also carried out. Although the chicken is cooked at high temperatures and with spices, making it less likely to be contaminated with bacteria, some studies were carried out on similar dishes, having the presence of *Staphylococcus aureus.* This recommends the contamination possibility in the chicken curry. Moreover, proper hygiene is not maintained in most of the restaurants and the polluted environment is also making the atmosphere around the restaurant vulnerable to contamination. Our study, of paramount importance, aims to isolate and identify *Staphylococcus* spp. and *Staphylococcus aureus* from cooked chicken (chicken curry). The samples were collected from 3 residential areas and 60 different restaurants.

2.2 Objectives of this Study

- Evaluate the food quality of the restaurants.
- The overview of the antibiotic resistance pattern of the isolated colonies.
- Assess the potential health risk posed by the presence of the isolates.
- Raise awareness among the general public and restaurant workers about the importance of food quality and hygiene.

Chapter 3

3. Materials and methods

3.1 Sample collection site and processing

The study was conducted from June 2022 to June 2023. Samples were collected from three different locations. The locations are - Mirpur, Mohammadpur, and Mohakhali (Figure 3). From each area, 20 samples were collected from 20 different low-ranged restaurants. Moreover, 10 homemade chicken curries (from Mirpur) were also taken as control for this study. All the samples for this study were collected aseptically using sterile instruments. 25 grams of each sample was homogenized using mortal pastel (Happy et al., 2018) and was transferred to 100 ml peptone broth (0.85%) (Sarker et al., 2018; Popelka et al., 2005; Barman et al., 2018) and was in the incubator for 24 hours at 37° C. For microbiological assay, the suspensions were subjected to serial dilutions up to 10^{-5}. Figure 2 depicts the general workflow of the experiment. On the other hand, Figure 3 points out the three areas that were selected for the sample collection.

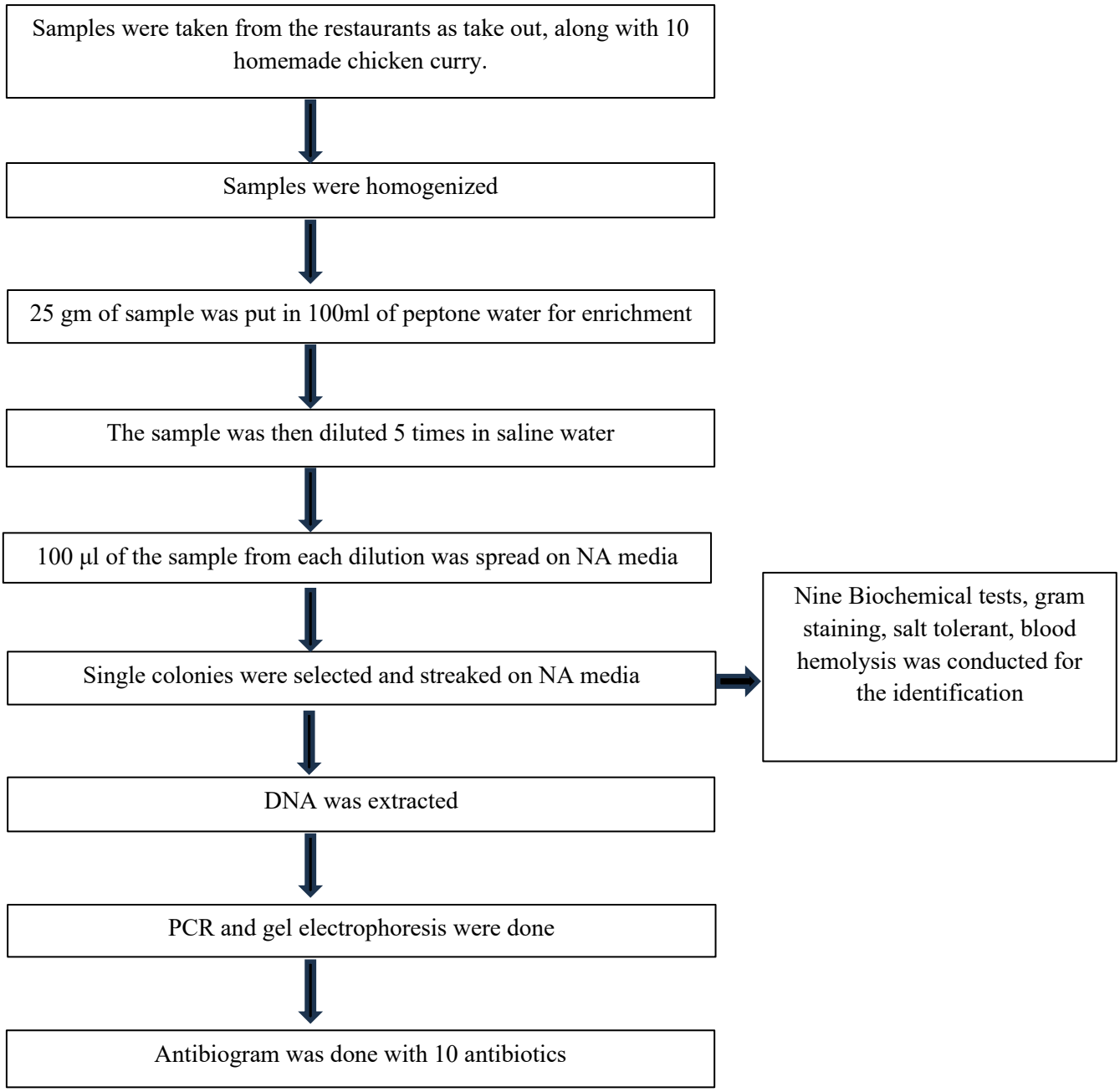


Figure 2: General workflow of the entire experiment.

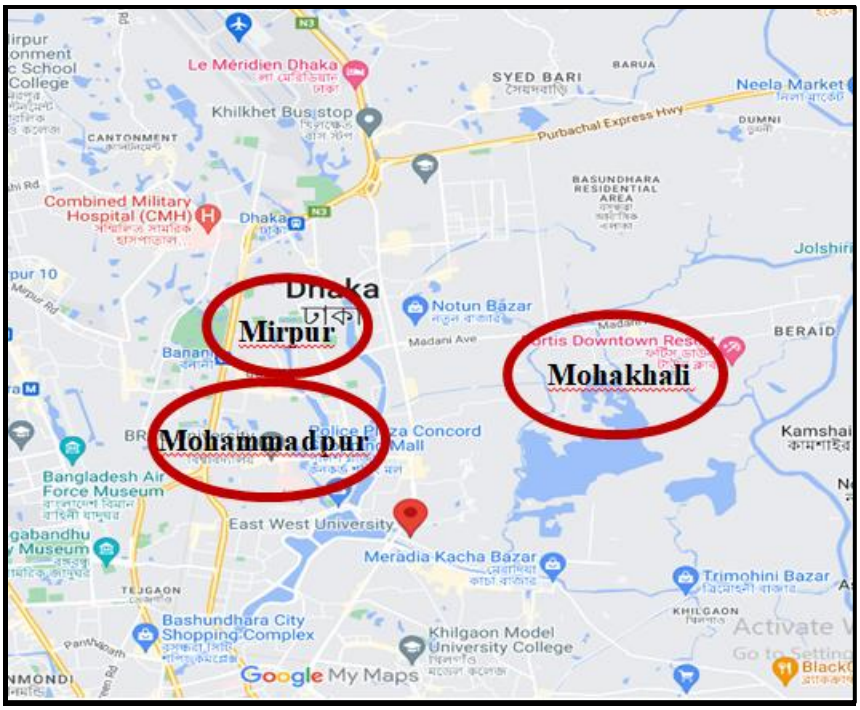


Figure 3: Three locations from where 60 samples were collected for the prevalence check of *Staphylococcus* spp. and *Staphylococcus aureus*.

3.2 Microbiological examination of collected samples

From each diluted tube, 100 µl was transferred into Nutrient agar (NA) plates (Figure 4). All NA plates were incubated for 24 hours at 37°C (Nishat et al., 2013; Microbiological Hazard Analysis and Exposure Assessment, 2018; Adjrah et al., 2013). All the plates were examined and morphologically distinct colonies were selected and tested on MSA media (Figure 5) (Happy et al., 2018). Strains that gave yellow and pink color in MSA media were chosen for biochemical tests and molecular analysis.

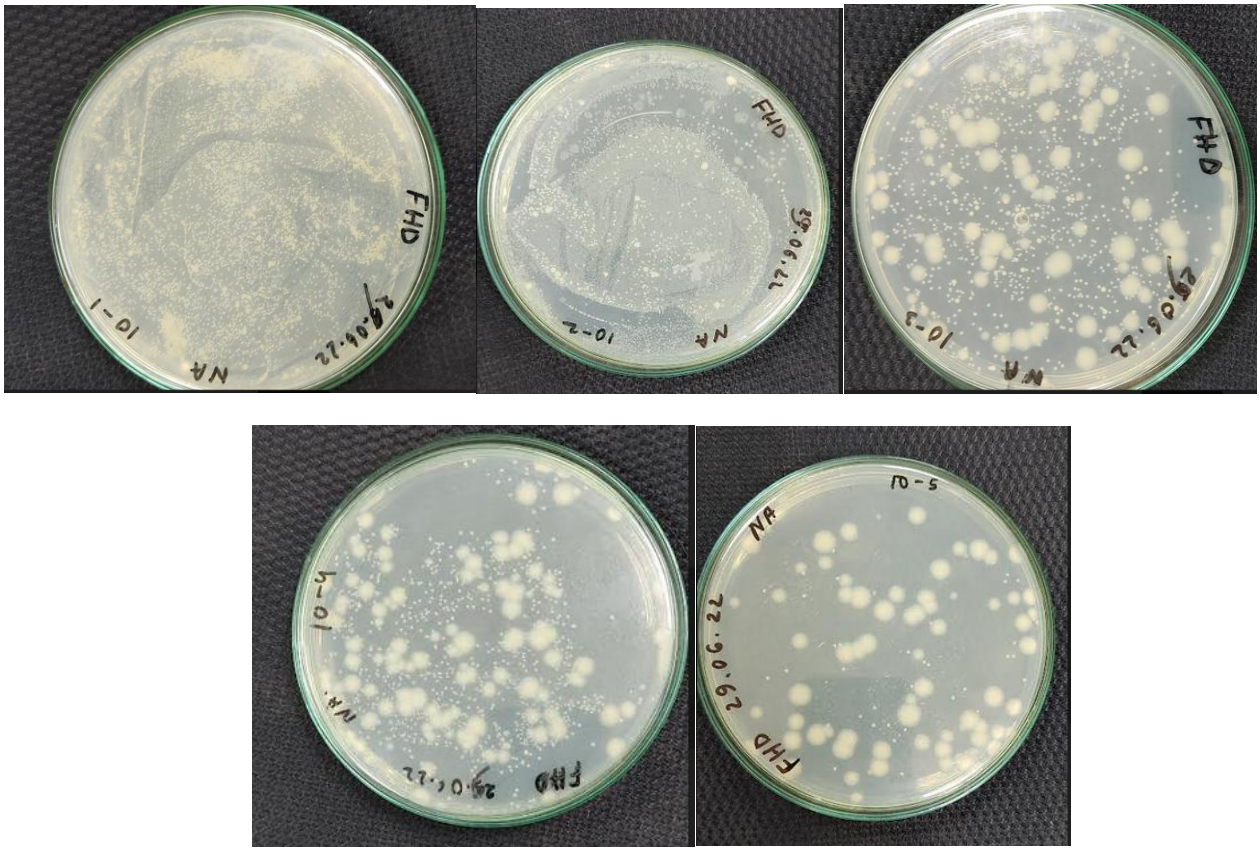


Figure 4: Spread plate method on NA media of 5 times serial dilution solution.

3.2.1. Nutrient Agar (NA) Media

Nutrient Agar is a nutrient medium used for the cultivation of microbes that supports the growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow various types of bacteria and fungi and it also contains many nutrients needed for bacterial growth. It is mostly used for the isolation and purification of cultures.

3.2.2 MSA (Mannitol Salt Agar) Media

MSA is used for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. If the strain is *S. aureus,* the media will give yellow colonies with yellow zones. In the case of Staphylococci other than *S. aureus (*e.g. *Staphylococcus epidermidis),* it will give colorless or red colonies with red zones.

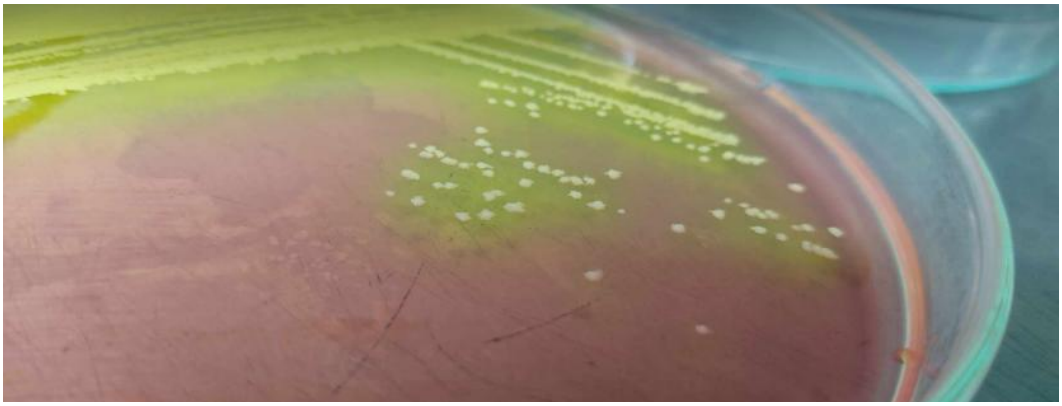


Figure 5: Streak plate method on MSA Media.

3.3 Phenotypic characterization of isolates

Biochemical tests were done by following the standard protocol (Aneja, 2003). The tests included-

a.

Gram staining (Krupa et al., 2014).

b.

1. catalase test,
2. oxidase test,
3. triple sugar iron (TSI),
4. motility indole urease test,
5. indole test,
6. Methyl Red Test,
7. Voges Proskauer Test, and
8. Simmons Citrate Agar tests.

Figure 6 demonstrates the gram staining and biochemical test results.

3.3.1 Gram staining:

Gram staining technique is particularly significant in the microbiology field. The test commonly utilizes crystal violet or methylene blue as the principal dye. When observed under a microscope, gram-positive organisms maintain a purple appearance. Microscopic examination reveals that the microorganisms that do not absorb the main stain have a red coloration, indicating that they are Gram-negative organisms.

The initial stage in gram staining entails the application of crystal violet on a slide to stain. Later, for dye fixation, iodine was used to create a crystal violet-iodine complex, which impedes the removal of the excess dye on the slide. Then, a decolorizer, typically ethanol, is employed to eliminate the dye. The fundamental idea of gram staining is around the bacterial cell wall's capacity to retain the crystal violet dye while exposed to a solvent. As gram-positive bacteria have thicker

peptidoglycan layers than gram-negative bacteria, the crystal violet dye with iodine remains in the cell wall.

The last stage of the gram-staining process involves the application of a primary fuchsin stain that impart a pink hue for the decolorization of gram-negative bacteria, thereby, facilitating their identification. This stain is also commonly known as a counterstain. While safranin is used as a counterstain in some laboratories, basic fuchsin is admitted to stain gram-negative organisms more intensely than safranin. Moreover, *Haemophilus* spp., *Legionella* spp., and some other anaerobic bacteria exhibit little staining affinity with safranin (Aneja, 2003).

3.3.2 Catalase test

This test demonstrates the presence of catalase enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate bacteria that produce an enzyme called catalase, such as *staphylococci*, from non-catalase-producing bacteria such as *streptococci*.

3.3.3 Oxidase test

The oxidase test detects the presence of a cytochrome oxidase system that catalyzes the transport of electrons between electron donors (in the bacteria) and a redox dye- tetramethyl-*p*-phenylene-diamine. The dye is reduced to a deep purple color. As *Staphylococcus* spp. is oxidase negative, it will not change the color into purple.

3.3.4 The Triple Sugar Iron (TSI) test

The Triple Sugar Iron (TSI) test tests a microorganism's ability to ferment sugars and produce hydrogen sulfide. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. *Staphylococcus* spp. can ferment all three sugars (dextrose, lactose, and sucrose) making the whole media yellow. It also forms gas, which can be seen with the bubble formation and the crack in the media.

3.3.5 Motility indole urease (MIU) test

MIU test is useful for the identification of gram-negative bacilli bacteria. Three tests in a single tube help to differentiate the organisms based on motility, urease, and indole production. *Staphylococcus* spp. is non-motile, indole negative, urease positive.

3.3.6 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane into indole. Indole production test is important in the detection of Enterobacteria. When indole is combined with Kovac's Reagent, the solution turns from yellow to cherry red**.** As amyl alcohol is water-insoluble, the red coloration will form in an oily layer at the top of the broth. *Staphylococcus* spp. is indole negative.

3.3.7 Methyl red (MR) test

The methyl red (MR) test detects the production of acid by glucose fermentation and the maintenance of conditions such that the pH of an old inoculated broth is sustained below a value of about 4.5, as shown by a change in the color with the addition of methyl red indicator. *Staphylococcus* spp. can change the color and turn the media red color.

3.3.8 Voges Proskauer (VP) test

The Voges-Proskauer (VP) test determines if an organism produces Acetyl-methyl Carbinol from glucose fermentation. If present, Acetyl-Methyl Carbinol is converted to diacetyl in the presence of strong alkali (40% KOH), ∝- naphthol, and atmospheric oxygen. *Staphylococcus* spp. shows positive results in the VP test.

3.3.9 Simmons citrate agar

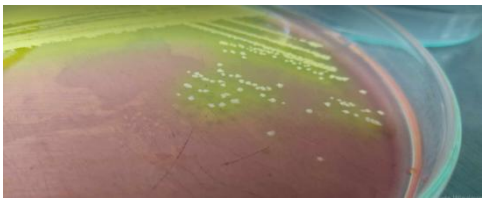
Simmons Citrate Agar is used for the differentiation of Enterobacteriaceae based on the utilization of citrate as the only source of carbon. It is used to test an organism's ability to utilize citrate as a source of energy. If the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which will further increase alkalinity. The change in pH turns the indicator (bromothymol blue) in the medium from green to blue above pH 7.6. *Staphylococcus* spp. can utilize the carbon and change the media color.

3.3.10 Blood agar

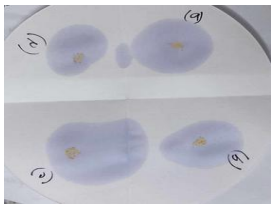
Generally, Blood Agar is used to culture bacteria or microbes that do not grow easily as it is an enriched media. Such bacteria are defined as "fastidious bacteria" as they require a special, enriched nutritional environment compared to routine bacteria. It is also used to identify and differentiate hemolytic bacteria like *Staphylococcus aureus, Streptococcus pneumoniae,* and *Klebsiella pneumoniae.*

Table 1: Types of bacterial hemolysis in Blood Agar and the names of the bacteria

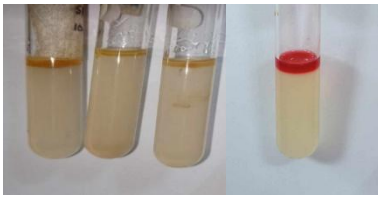
Media	Bacteria	Type of hemolysis
Blood Agar	*Staphylococcus aureus*	Beta hemolysis (Complete hemolytic)
	Streptococcus pneumoniae	Alpha hemolysis (Partial hemolytic)
	Klebsiella pneumoniae	Gamma hemolysis (Non hemolytic)



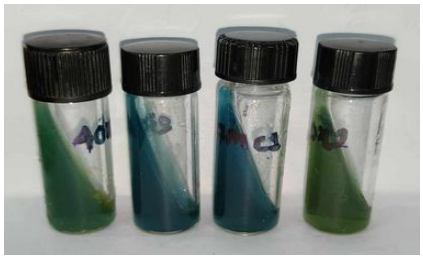
A. *Staphylococcus aureus* in MSA plate.



B. Oxidase test



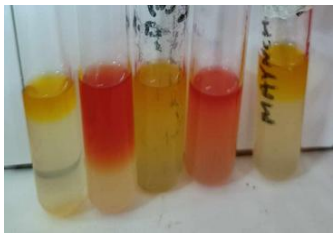
C. Indole test



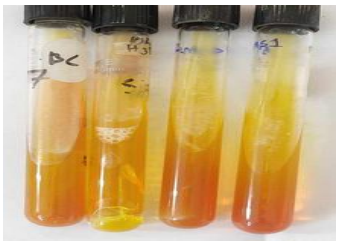
D. Citrate test

E. Catalase test

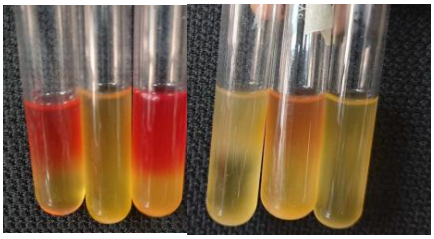
F. MIU test



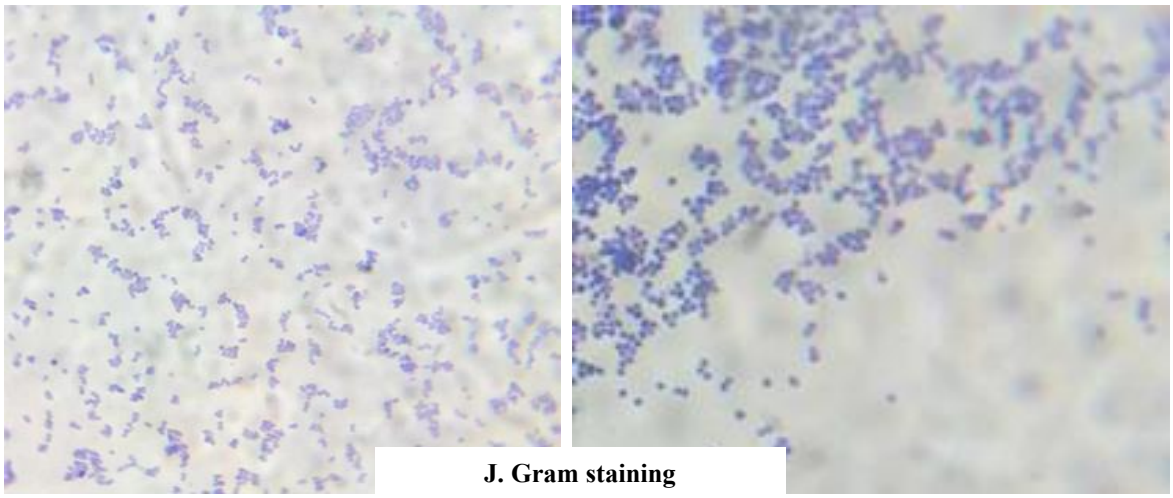
G. Methyl red test



H. TSI test



I. VP test



J. Gram staining

Figure 6: Biochemical tests and gram staining for the detection of *Staphylococcus* spp. and *Staphylococcus aureus*. A. *Staphylococcus aureus* in MSA plate. B. Oxidase test. C. Indole test. D. citrate test. E. Catalase test. F. MIU test. G. Methyl red test. H. TSI test. I. VP test. J. gram staining (positive result).

3.4. Molecular analysis of *Staphylococcus* spp. and *Staphylococcus aureus*

3.4.1. Genomic DNA extraction

Selected isolates were grown in 2 ml Luria-Bertani (LB) broth overnight at 37°C (Haghi et al., 2021). The cells were pelleted by centrifugation for 10 minutes at 10,000 rpm (Mashouf et al., 2015). Then 0.15ml of Tris EDTA (TE) buffer was added (Sarker et al., 2013). Pellets were mixed with vortex, heated at 100° C for 10 minutes, the heated solution was put into ice for 10 minutes, and centrifuged again at 12000rpm for 3 minutes. Supernatants were collected and stored at -20° C until use (Canizalez-Roman et al., 2013).

3.4.2. Detection of *Staphylococcus spp.* and *Staphylococcus aureus* by PCR

Subsequently, to confirm the presumed isolates of *Staphylococcus* spp. and *Staphylococcus aureus*, a PCR assay, targeting the *tuf* gene and *nucA* gene was performed **(Table 2)**. The reaction mixture of the PCR assay was 20 µl and was prepared as follows: 10 µl of 1x Taq premix Mastermix, 5 µl of double distilled water, 1 µl of forward and reverse primer, and 3 µl of DNA sample.

3.4.2.1 Detection of *Staphylococcus* spp. by PCR

The DNA of the sample, positive control (*Staphylococcus* spp. ATCC), and negative control were amplified by the initial denaturation step for 3 minutes at 94°C followed by 40 cycles of 94°C for 60 seconds, 55°C for 30 seconds, 72°C for 1 minute and a final extension step at 72°C for 10 minutes (**Table 3**). After the amplification reaction, 6 µl of each PCR product was visualized under a UV trans illuminator (Mashouf et al., 2015).

3.4.2.2 Detection of *Staphylococcus aureus* by PCR

The DNA of the sample, positive control (*Staphylococcus aureus* ATCC), and negative control were amplified by the initial denaturation step for 5 minutes at 94°C followed by 35 cycles of 94°C for 60 seconds, 50°C for 60 seconds, 72°C for 30 seconds and a final extension step at 72°C for 3

minutes (**Table 4**). After the amplification reaction, 6 µl of each PCR product was visualized under a UV trans illuminator (Mashouf et al., 2015).

Table 2: Primers used for molecular analysis (nucA for *Staphylococcus aureus*, TStag for *Staphylococcus* spp.)

Primer	Primer sequence (5'-3')	Target Gene	PCR product	Annealing temperature	Reference
nucA	F-5'-GCGATTGATGGTGATACGGTT-3′ R-5'-AGCCAAGCCTTGACGAACTAAAGC-3′	*nucA*	270	50°C	(Mashouf et al., 2015).
TStag	F-5′-GGCCGTGTTGAACGTGGTCAAATCA-3′ R-5′-TIACCATTTCAGTACCTTCTGGTAA-3′	*tuf*	370	55°C	(Morot-Bizot et al., 2004).

Table 3: PCR Thermal Cycle Parameters for *tuf* gene

For *Staphylococcus* spp., conventional PCR was conducted targeting the *tuf* gene, utilizing the following PCR conditions (Morot-Bizot et al., 2004).

Step	Temperature	Time	Cycle
Initial Denaturation	94°C	3 min	1x cycle
Denaturation	95°C	1 min	40x cycle
Annealing	55°C	30 second	
Extension	72°C	30 second	
Final Extension	72°C	3 min	1x cycle

Table 4: PCR Thermal Cycle Parameters for *nucA* gene

For *Staphylococcus aureus*, conventional PCR was conducted targeting the *nucA* gene, utilizing the following PCR conditions (Mashouf et al., 2015).

Step	Temperature	Time	Cycle
Initial Denaturation	94°C	5 min	1x cycle
Denaturation	94°C	1 min	35x cycle
Annealing	50°C	1 min	
Extension	72°C	1 min	
Final Extension	72°C	10 min	1x cycle

3.5 Gel Electrophoresis:

After PCR, the PCR amplicons were subjected to agarose gel electrophoresis using a 2% agarose gel in 1x TBE buffer (pH 8.0-8.5). 0.003% (wt/vol) ethidium bromide (Et-Br) was added to the agarose gel before it was poured into the gel casting tray, for DNA staining. A total of 5µL of the PCR amplicons and ladder, (CLV-CSL-MDNA-1KB PLUS and Abclonal 100 bp ladder) were loaded in wells.

Gel electrophoresis was conducted at 110 volts for 40 minutes. After electrophoresis, the agarose gel was visualized and photographed on a UV transilluminator to observe DNA bands.

The bands of 370 bp, were the bands of *Staphylococcus* spp., and the bands of 270 bp, were the bands of *Staphylococcus aureus*.

3.6 Antibiotic susceptibility test

For all confirmed *Staphylococcus aureus.,* an antibiogram was carried out by standard Kirby-Bauer disk agar diffusion (DAD) on Mueller Hinton agar using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) document M100-S20 (Sarker et al., 2013; Mashouf et al., 2015; Normanno et al., 2007; Haghi et al., 2021; Wayne, 2010). The following antibiotic discs were used in this study: methicillin (MET, 5mcg), cefipime (CPM, 30mcg), doxycycline (Do, 30mcg), gentamicin (GEN, 120mcg), amoxicillin (AMC, 30mcg), erythromycin (E, 15mcg), vancomycin (VA, 30mcg), penicillin (P, 10 units), tetracycline (TE, 30mcg) (Safarpoor et al., 2017; Shahraz et al., 2012; Mashouf et al., 2015; Lakhanpal et al. 2019; Normanno et al., 2007; Ranucci et al., 2004) **(Table 5)**. All the antibiotics belonged to HiMedia. The results were recorded after 24 h of incubation at 37°C and interpreted according to the manufacturer's instructions. One to two colonies were picked from the young culture and were suspended in 10 mL of sterile normal saline. The turbidity of the suspension was standardized to

0.5 on the McFarland scale and it was streaked over Mueller Hinton agar (MHA) plates with cotton swabs. The antibiotic discs were applied on the MHA plates and the plates were incubated overnight at 37°C (Gousia et al., 2011). The inhibition zones were measured to the nearest millimeter using a ruler and were characterized as sensitive, intermediate resistant, and resistant.

Table 5: Zone diameter interpretative standards as per CLSI 2020 (3rd Edition)

Interpretative categories and zone diameter					
Antibiotics	**Symbol**	**Concentration**	**Sensitive (S)**	**Intermediate (I)**	**Resistant (R)**
Methicillin	MET	5 mcg	≥14	10-13	≤9
Cefepime	CPT	30 mcg	≥18	15-17	≤14
Doxycycline	DO	30 mcg	≥16	13-15	≤12
Gentamicin	GEN	120 mcg	≥15	13-14	≤12
Amoxicillin	AMX	30 mcg	≥18	14-17	≤13
Tetracycline	TE	30 mcg	≥19	15-18	≤14
Erythromycin	E	15 mcg	≥23	14-22	≤13
Vancomycin	VAN	30 mcg	≥17	15-16	≤14
Penicillin	PEN	10 units	≥28	20-27	≤19

Chapter 4

4.1 Observation and Result (for Samples Collected from Restaurants):

After the spread plate method, selected colonies were transferred to MSA media. The colonies that gave off yellow and pink colonies, were selected for biochemical tests and molecular analysis **(Table 6)**. After the initial identification from the MSA media, the strains were again cultured in NA media and distinct colonies were obtained for 8 biochemical tests and gram staining. **Table 7** contains the results of *Staphylococcus* spp. strains.

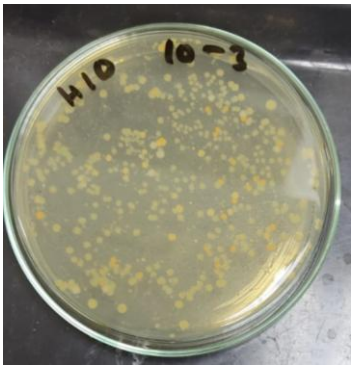
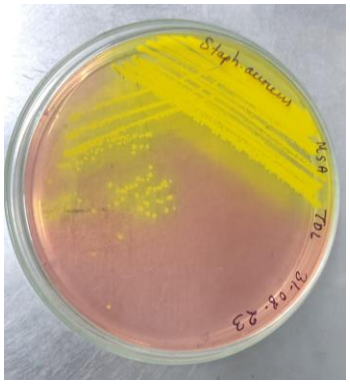
<table>
<tr><th>Media</th><th>Total number of isolates</th><th>Observation</th><th>Picture</th><th>Hypothesis</th><th>Prevalence of positive presumptive</th></tr>
<tr><td rowspan="2">NA</td><td rowspan="3">178</td><td>White and yellow colonies</td><td>  </td><td rowspan="3">Staphylococcus spp.</td><td rowspan="3">After testing on MSA media, only 178 isolates were tested for biochemical tests and molecular analysis.</td></tr>
<tr><td rowspan="2">Yellow colonies</td><td rowspan="2">  </td></tr>
<tr><td>MSA</td></tr>
</table>

Table 6: observational result for NA and MSA media.

Table 7: Biochemical tests and their results

Biochemical Test Result of *Staphylococcus* spp. *and Staphylococcus aureus*:			
Test Name	**Result**	**Color**	**Interpretation**
Catalase Test	Positive (+) (Gas formation)	N/A	The isolates produced gas.
Oxidase Tests	Negative (-)	Colorless	None of the isolates turned into purple color.
TSI			
Sugar Fermentation	Positive (+)	Y/Y (Slant yellow, butt yellow)	The isolates turned the media completely yellow without any black color at the bottom and gas formation.
H2S Production	Negative (-)	Colorless	
Gas Formation	Negative (-)	N/A	
MIU			
Motility	Negative (-)	N/A	The isolates showed only pink color without any motility.
Indole	Negative (-)	Colorless	
Urease	Positive (+)	Pink	
Indole Test	Negative (-)	Colorless	No color was formed
MR Test	Positive (+)	Red	The media turned red with the addition of indicator.
VP Test	Positive (+)	Red	The media turned red with the addition of indicator.
Simmon's Citrate Test	Positive (+)	Blue	The whole media turned blue.
Gram Staining			
Shape	Cocci		The isolates showed cocci shape and purple color, indicating gram positive bacteria.
Color	Purple		

4.1.1 Gram staining:

The presumptive isolates of *Staphylococcus* spp. underwent the gram staining procedure. The staining characteristics were consistent and retained the crystal violet stain, resulting in a purple coloration visible under the microscope **(Figure 7)**. This feature confirms the gram-positive nature of the isolates, helping with the characterization and identification of the bacteria.

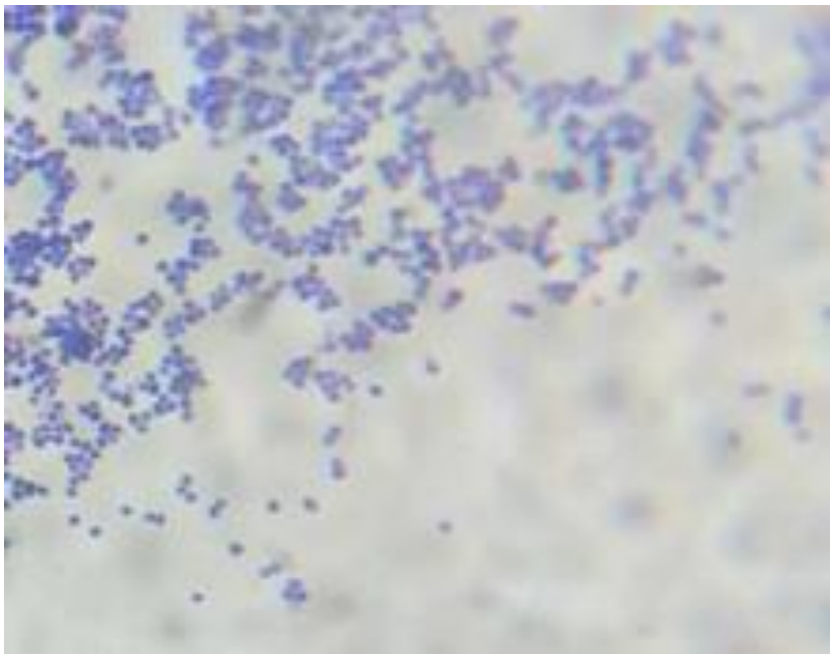


Figure 7: Positive result of gram staining.

4.1.2 Biochemical identification using triple sugar iron test (TSI)

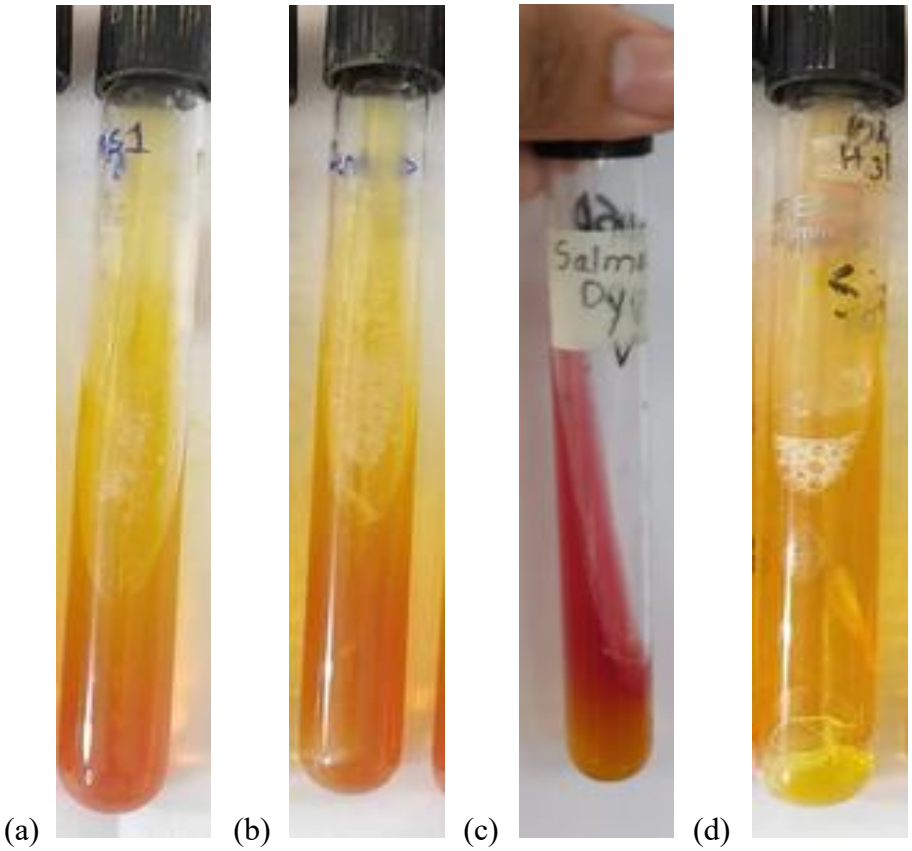
This test determines the bacteria's ability to glucose, lactose, and sucrose fermentation, gas production, and hydrogen sulfide production. If any organism were able to ferment dextrose only, the slant color would have changed to yellow, and the butt color would have remained the same, which means it remains alkaline. If the organism were not able to ferment none of the sugars, the slant, and the butt both would remain alkaline, and the color would have remained red.

All the isolates were inoculated in TSI media and incubated for 24 hours. After incubation, none of the isolates showed the production of hydrogen sulfide (black precipitate) and gas like carbon dioxide or hydrogen. However, the media turned yellow throughout the test tube, indicating that all three of the sugars were fermented. The butt and the slant turned yellow due to the acid production. Due to the presence of the indicator phenol red in the media, the color changes from red to yellow.

Figure 8 is the representation of the TSI test result. Here, we can see one positive control and one negative control. We also have one positive result of the isolates (yellow slant/yellow butt) and one negative result of the isolates (yellow slant/yellow butt, gas formation).

In **Figure 9**, we can see that in the TSI test, 58% of the presumptive *Staphylococcus* spp. were seen to give positive results (Yellow slant/ Yellow butt). The rest of the isolates were seen to have negative results (Red slant/Yellow butt, red slant/Red butt, Gas formation).

The distribution of 58% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 10**. The bar chart shows that the prevalence was highest in Mohakhali (25%), followed by Mohammadpur (22%). Mirpur had the lowest number of positive isolates (11%).



(a) (b) (c) (d)

Figure 8: TSI test result.

(a) Positive control of *Staphylococcus* spp. (b) The positive result of A/A for the sample to be hypothesized *Staphylococcus* spp. (c) Negative control (d) Negative result.

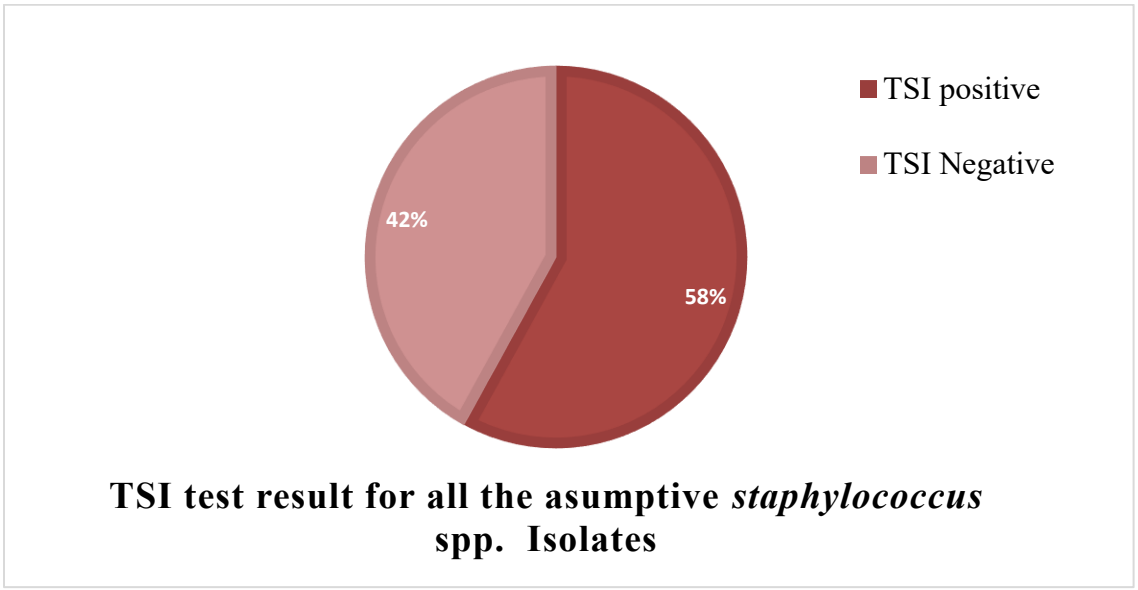


Figure 9: Observational result of Triple sugar iron test.

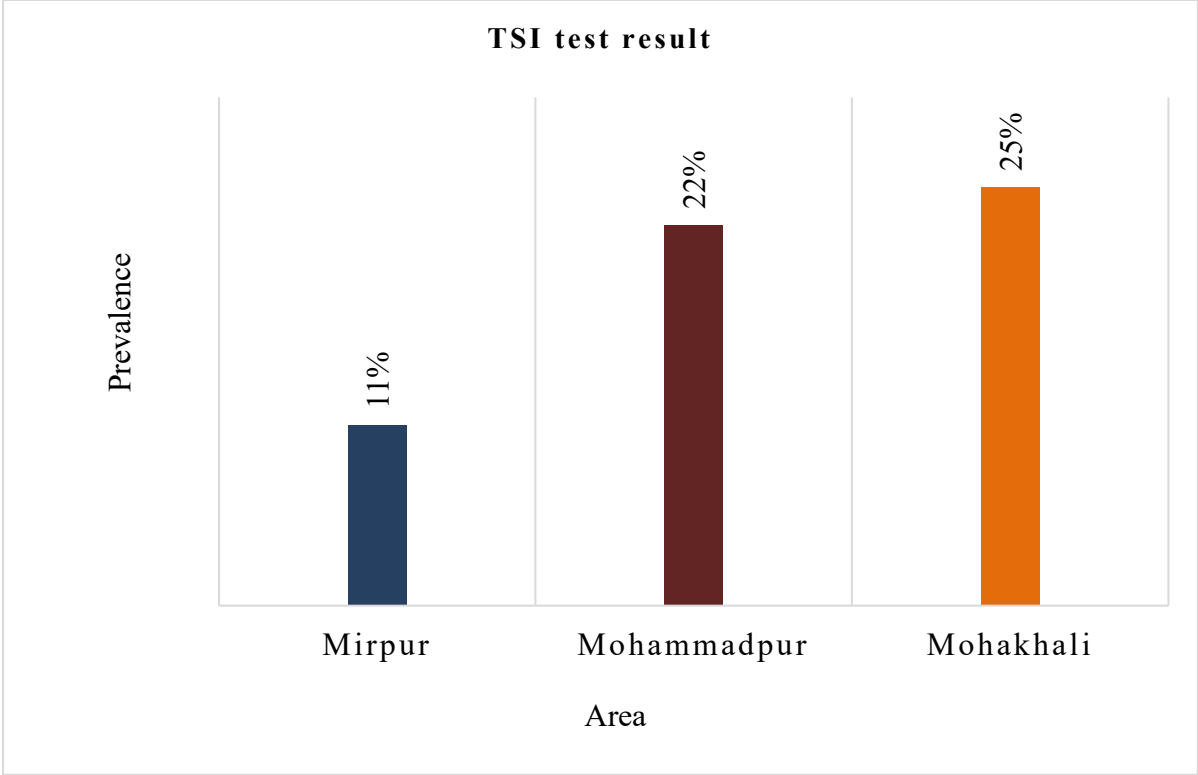


Figure 10: Distribution of pre-assumptive *Staphylococcus* spp. based on TSI results among the collected samples.

4.1.3 Biochemical Identification Using Citrate Utilization Test

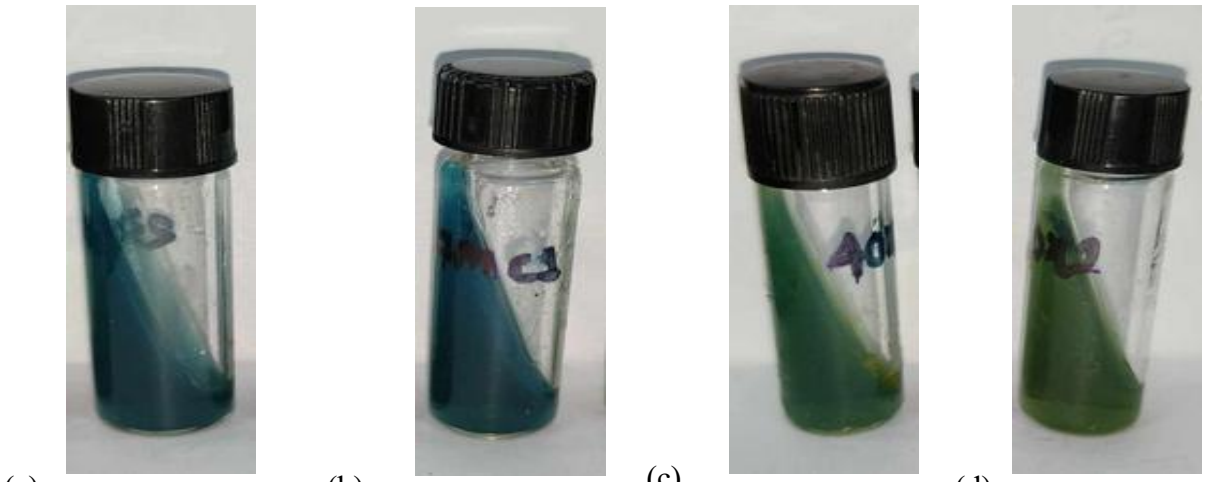
The Citrate Utilization Test characterizes microorganisms using citrate as the main carbon source. The citrate permease enzyme breaks down citrate into pyruvate which enters the organism's metabolic cycle to produce energy. The pH change helps detect organisms using the indicator bromothymol blue. The test is positive if the tube changes color upon the bacterial growth. The test is negative if the color remains green and no bacterial growth is observed.

Firstly, the isolates are inoculated into Simmon's citrate agar media in a vial and then incubated for 24 hours. *Staphylococcus* spp. can use citrate as its carbon source; hence, citrate is fermented and the color transforms into blue from green.

Figure 11 depicts the results of the citrate test. We have one positive control, one negative control, and two of the results of the isolates. One is citrate positive (probable *Staphylococcus* spp.) and one is citrate negative.

Figure 12 demonstrates that in the citrate test, 39% of the presumptive *Staphylococcus* spp. were seen to give positive results (the color turned blue from green). The rest of the isolates were seen to have negative results (61% of them remained green).

The distribution of 39% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 13**. The bar chart shows that the prevalence was highest in Mohakhali (36%), followed by Mohammadpur (33%). Mirpur had the lowest number of positive isolates (12%).



(a) (b) (c) (d)

Figure 11: Citrate test result.

(a) Positive control of *Staphylococcus* spp. (b) positive result of the sample to be hypothesized *Staphylococcus* spp. (c) Negative control (d) Negative result

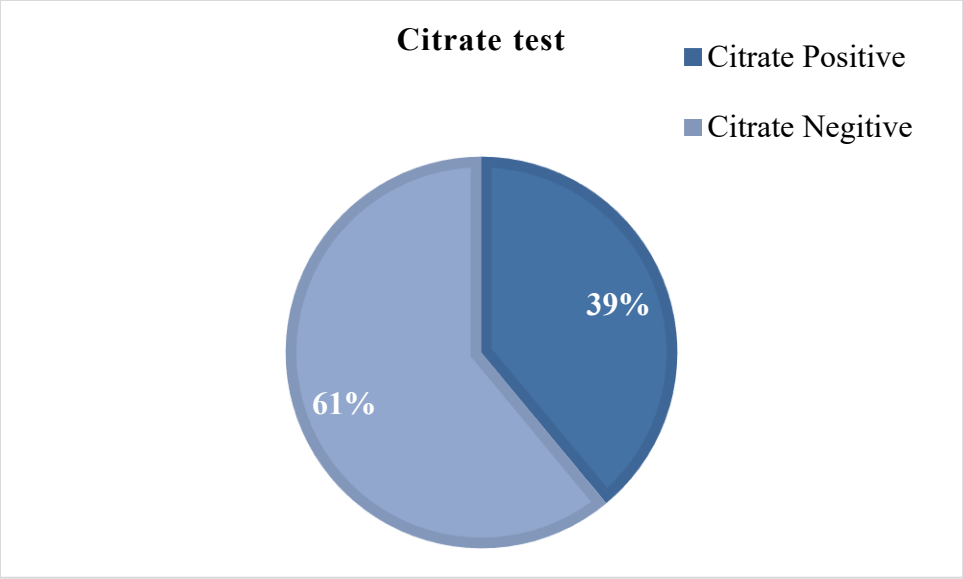


Figure 12: Observational result of Citrate test.

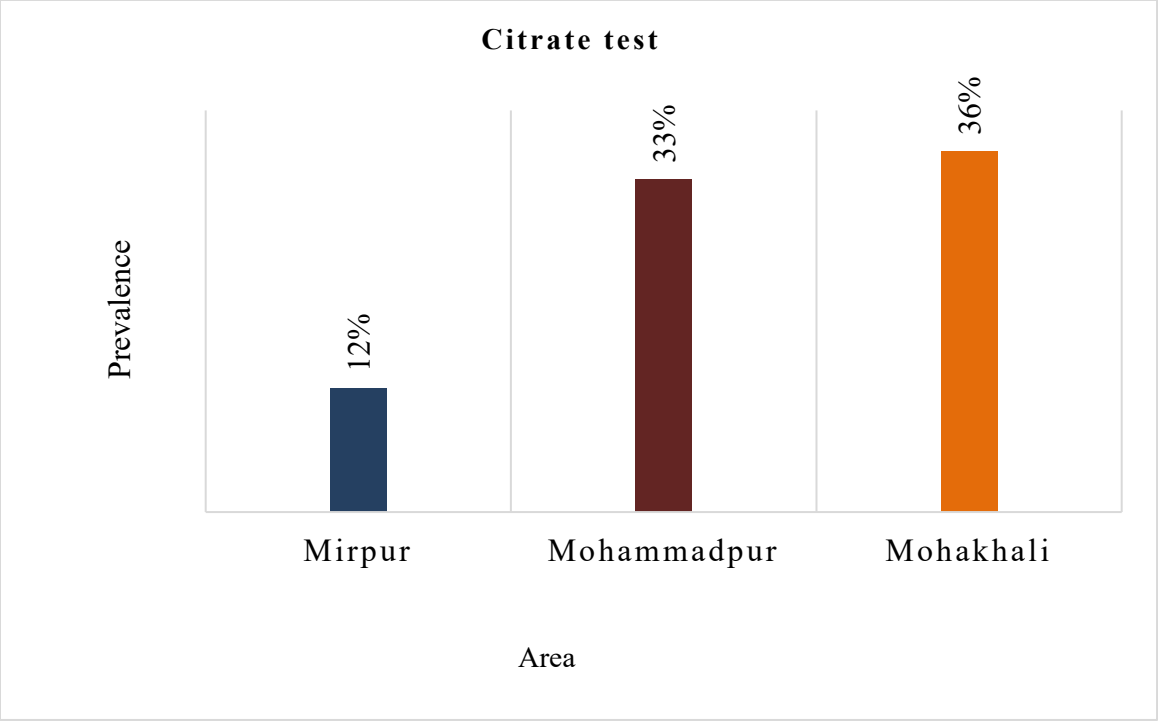


Figure 13: Distribution of pre-assumptive *Staphylococcus* spp. based on Citrate results among the collected samples.

4.1.4. Biochemical Identification Using Catalase Test

It demonstrates the presence of catalase enzyme. This enzyme catalyzes the release of oxygen from hydrogen peroxide. It is used to differentiate bacteria from whether it can catalyze hydrogen peroxide or not. If any oxygen bubble is formed after the mixing of hydrogen peroxide and bacteria then the test will be positive.

Staphylococcus spp. can produce catalase enzyme. All hypothesized *Staphylococcus* spp. isolates were tested for the precedence of catalase enzymes by adding 2-3 drops of hydrogen peroxide on a glass slide where smears of the samples were already present. Within a few seconds, bubbles were observed on glass slides.

Figure 14 depicts the results of the catalase test. We have one positive control, one negative control, and one of the samples that has a positive result for the catalase test (bubble formation).

Figure 15 demonstrates that in the catalase test, 53% of the presumptive *Staphylococcus* spp. were seen to give positive results (the color turned blue from green). The rest of the isolates were seen to have negative results (47% of them remained green).

The distribution of 53% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 16**. The bar chart implies that the prevalence was highest in Mohammadpur (34%), followed by Mirpur (30%). Mohakhali had the lowest number of positive isolates (27%).

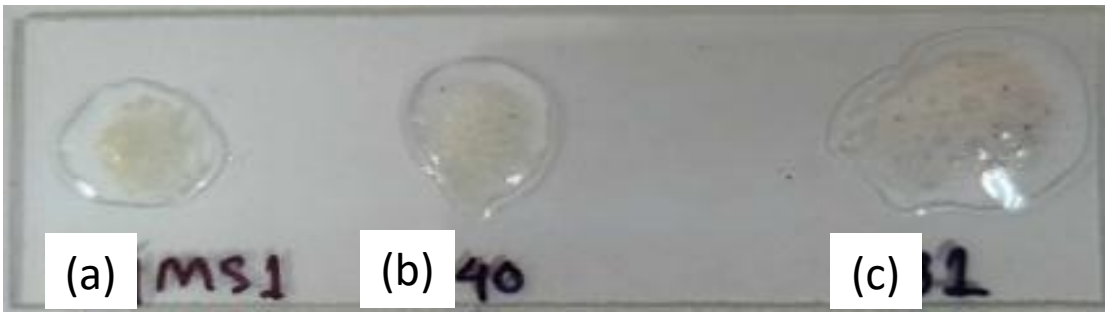


Figure 14: Catalase test result.

(a) Positive control of *Staphylococcus* spp. (b) and (c) positive result of the sample to be hypothesized *Staphylococcus* spp.

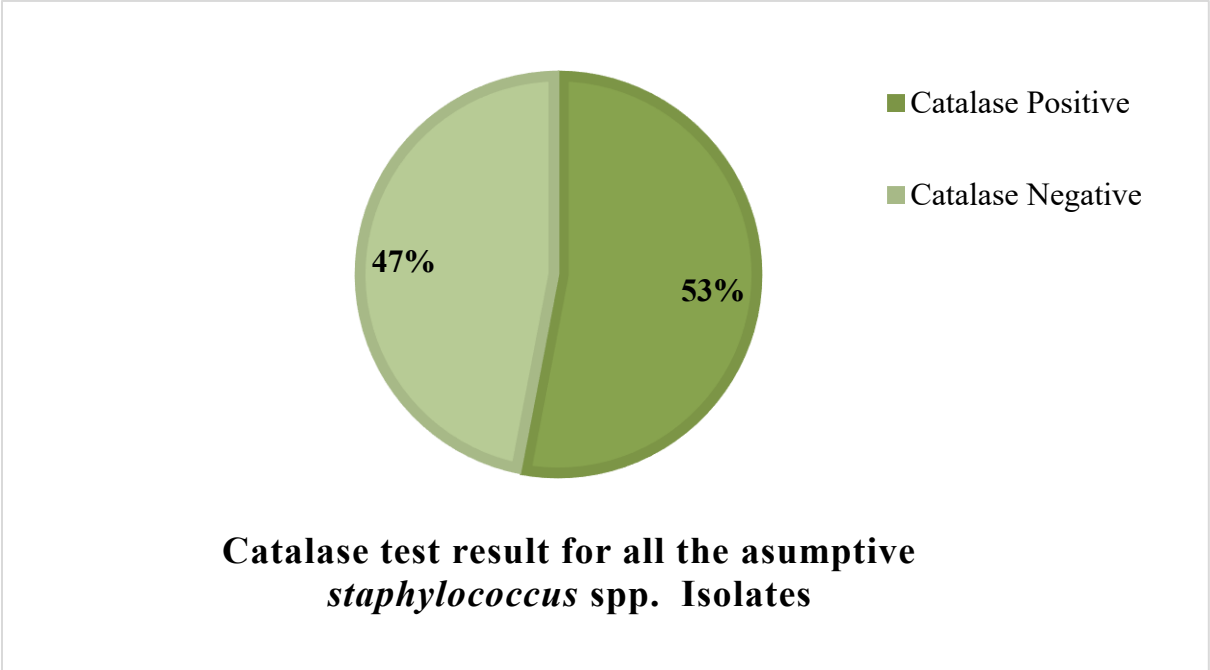


Figure 15: Observational result of Catalase test.

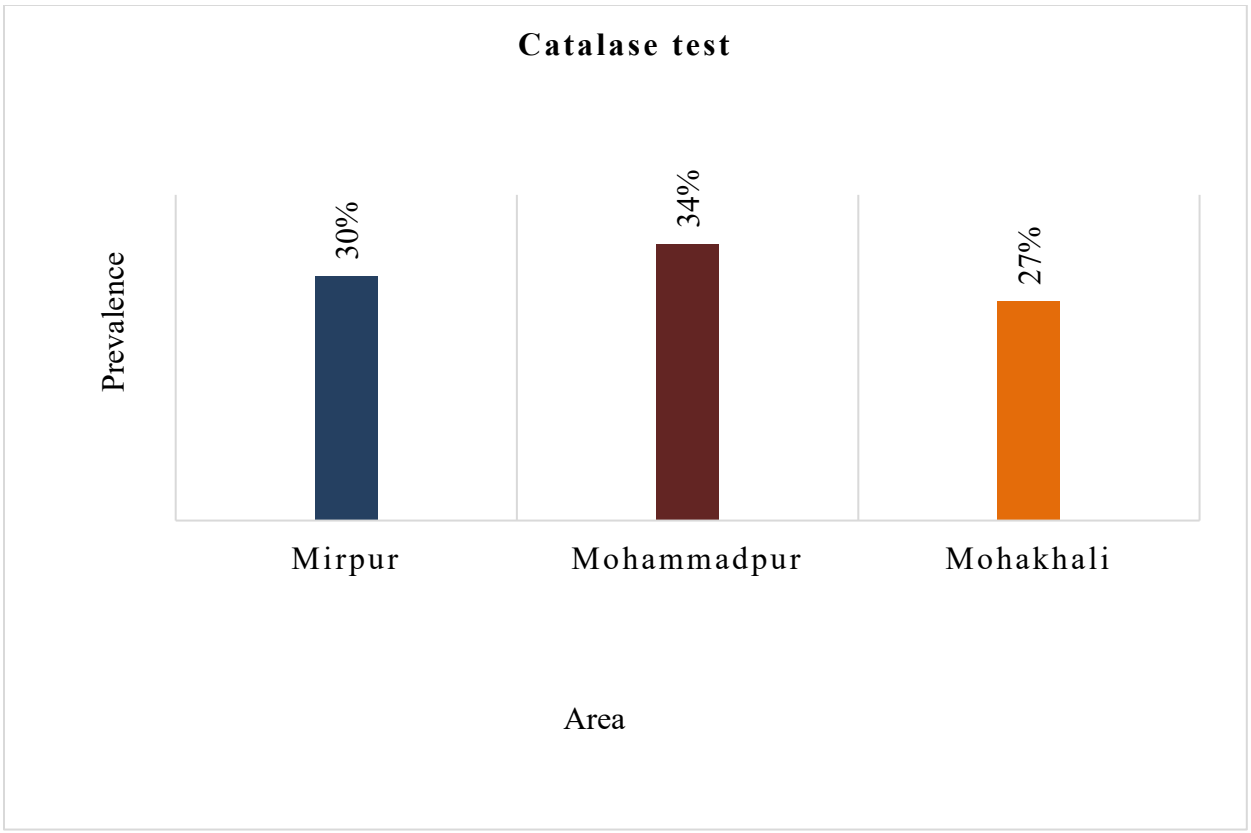


Figure 16: Distribution of pre-assumptive *Staphylococcus* spp. based on Catalase test results among the collected samples.

4.1.5 Biochemical Identification Using Oxidase Test

With this test, bacteria that contain cytochrome can be identified. Bacteria that contain cytochrome produce an intracellular enzyme named oxidase. This enzyme particularly catalyzes cytochrome c oxidation. Mainly gram-negative bacteria can produce this enzyme. If within a few seconds, a purple color appears, after the addition of Kovac's oxidase reagent, with no color formation, the result is positive otherwise the test result is negative.

For this experiment, one loopful of isolates was smeared on what's man filter paper. Then a few drops of Kovacs oxidase reagent were put on the smeared strain.

Figure 17 illustrates the results of the oxidase test. We have one negative control, and 3 pre-assumed *Staphylococcus* spp. isolates that showed negative results by not turning into a purple color after the reaction with Kovac's oxidase reagent.

Figure 18 depicts that in the catalase test, 39% of the presumptive *Staphylococcus* spp. were seen to give positive results (the color turned purple). The rest of the isolates were seen to have negative results (61% of them remained colorless as per **Figure 17**).

The distribution of 39% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 19**. This bar chart demonstrates that the prevalence was highest in Mohammadpur (34%), followed by Mohakhali (30%). In Mirpur, the prevalence was lowest. It was only 25%.

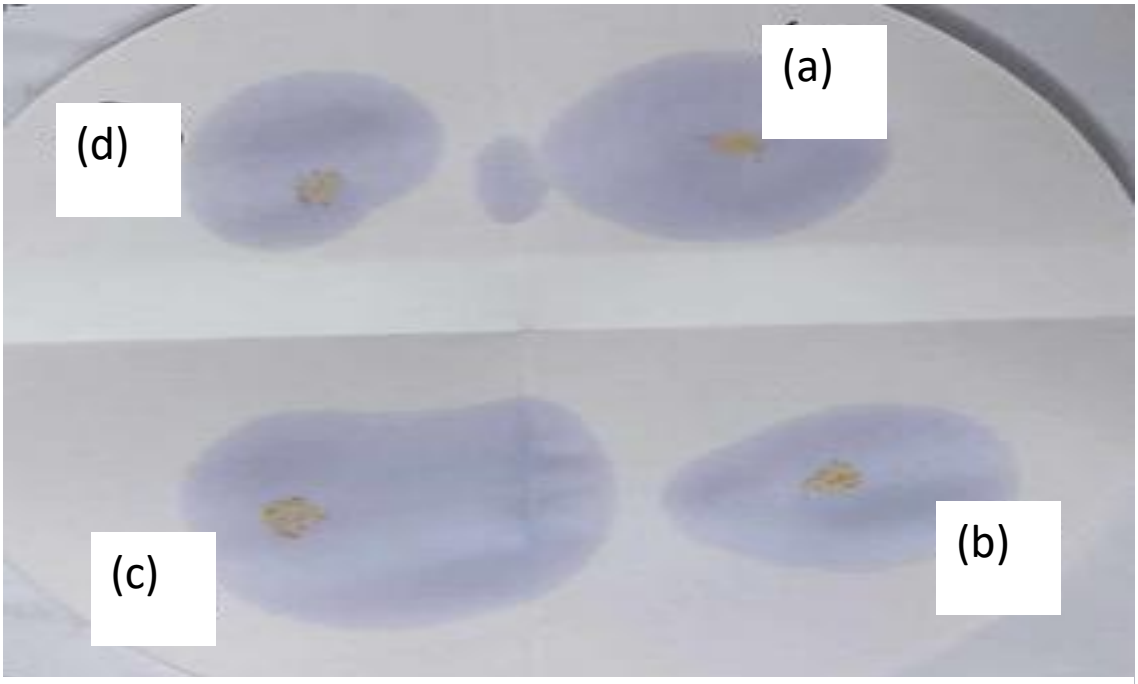


Figure 17: Oxidase test result.

(a) Negative control (*E. coli)* (b), (c) and (d) negative result of the samples, hypothesized to be *Staphylococcus* spp.

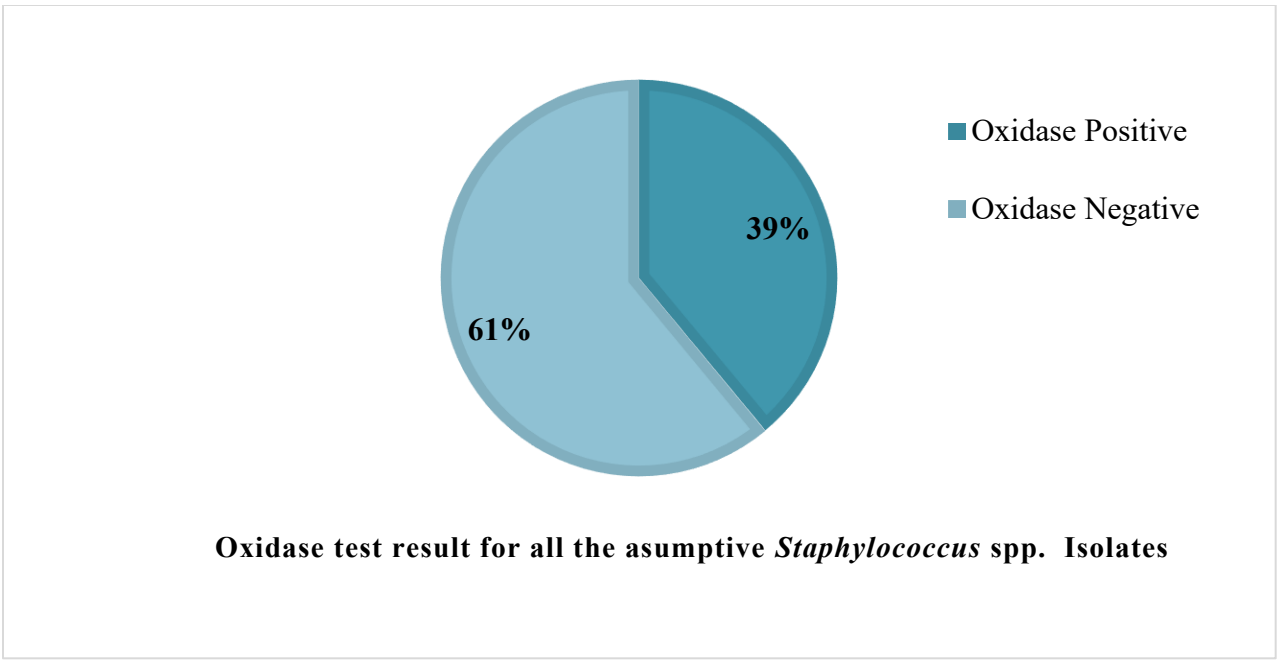


Figure 18: Observational result of Oxidase test.

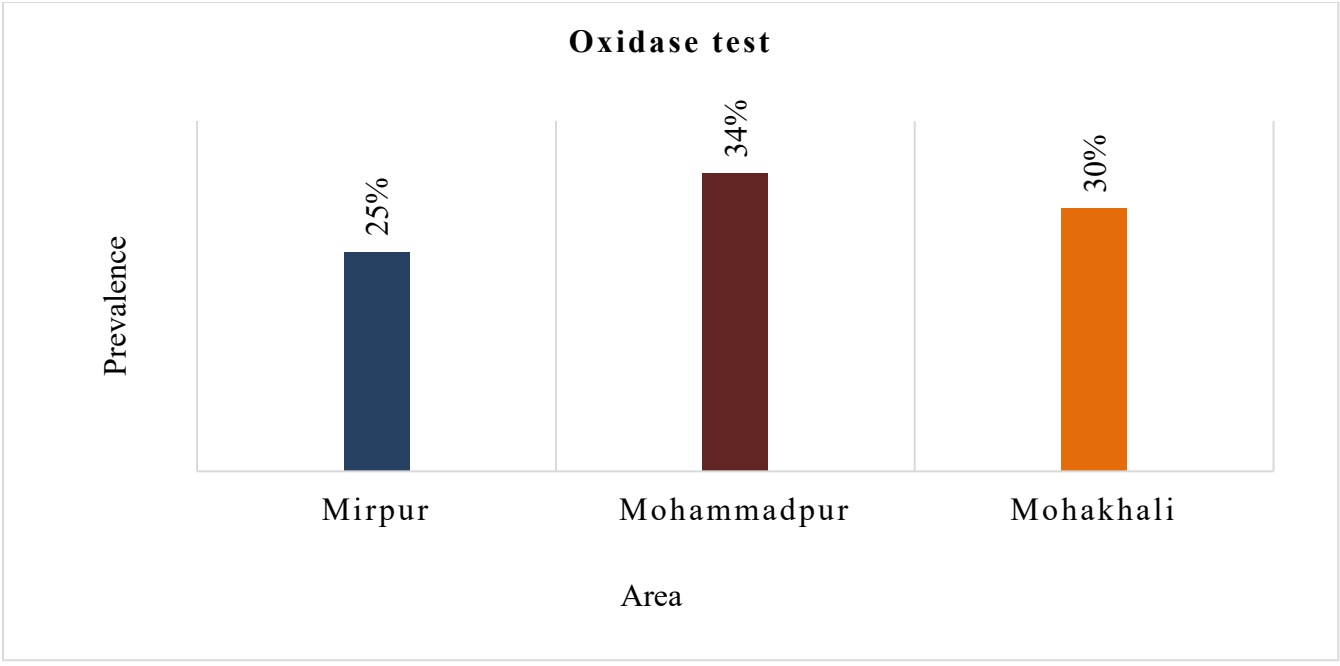


Figure 19: Distribution of pre-assumptive *Staphylococcus* spp. based on Oxidase test results among the collected samples.

4.1.6. Biochemical Identification Using Indole Test

This test displays the ability of a bacteria to decompose the tryptophane which is an amino acid into indole. This characterization is important for the identification of enterobacteria.

Firstly, the media had to be prepared. Then one loopful was inoculated in the solution and incubated for 24 hours. Lastly, Kovacs reagent was added to the solution to see if there was any color change. With a visible red color, the test was positive otherwise the result was negative.

Figure 20 illustrates the results of the indole test. We have one positive control, one negative control, and two of the results of the isolates. Both of them appeared to be negative.

Figure 21 depicts that the conducted indole test showed positive results for 47% of the cases, of the pre-assumed *Staphylococcus* spp. (the color turned yellow). The rest of the isolates were seen to have negative results (53% of them showed a red ring on top of the solution).

The distribution of 47% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 22**. The bar chart demonstrates that the prevalence was highest in Mohakhali (22%), followed by Mohammadpur (18%). In Mirpur, the prevalence was lowest. It was only 7%.

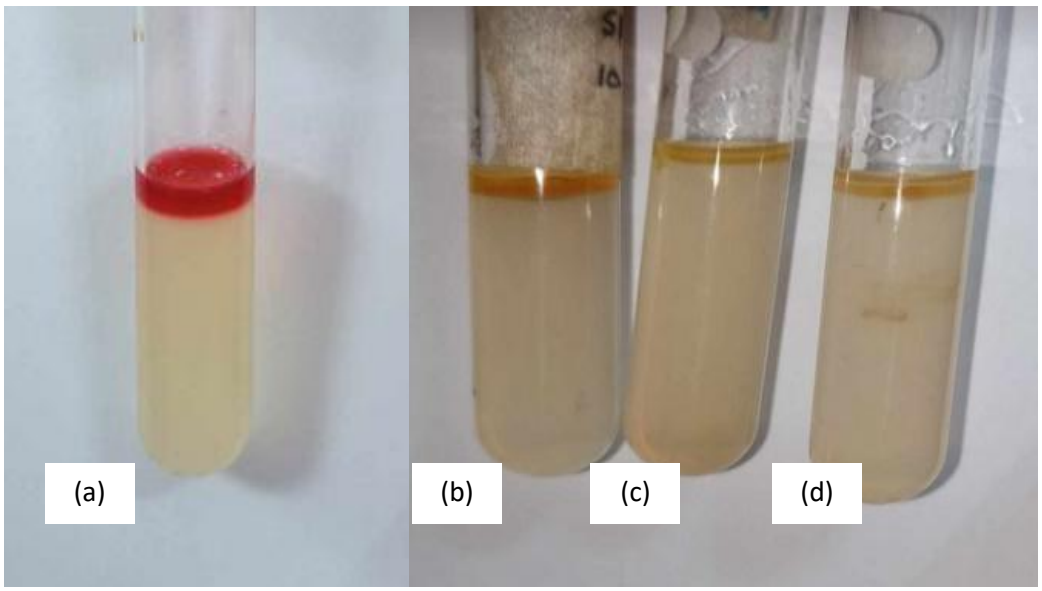


Figure 20: Indole test result.

(a) Positive control (*E. coli)*, (b) Negative control, (c) and (d) Negative result of the pre-assumed *Staphylococcus* spp.

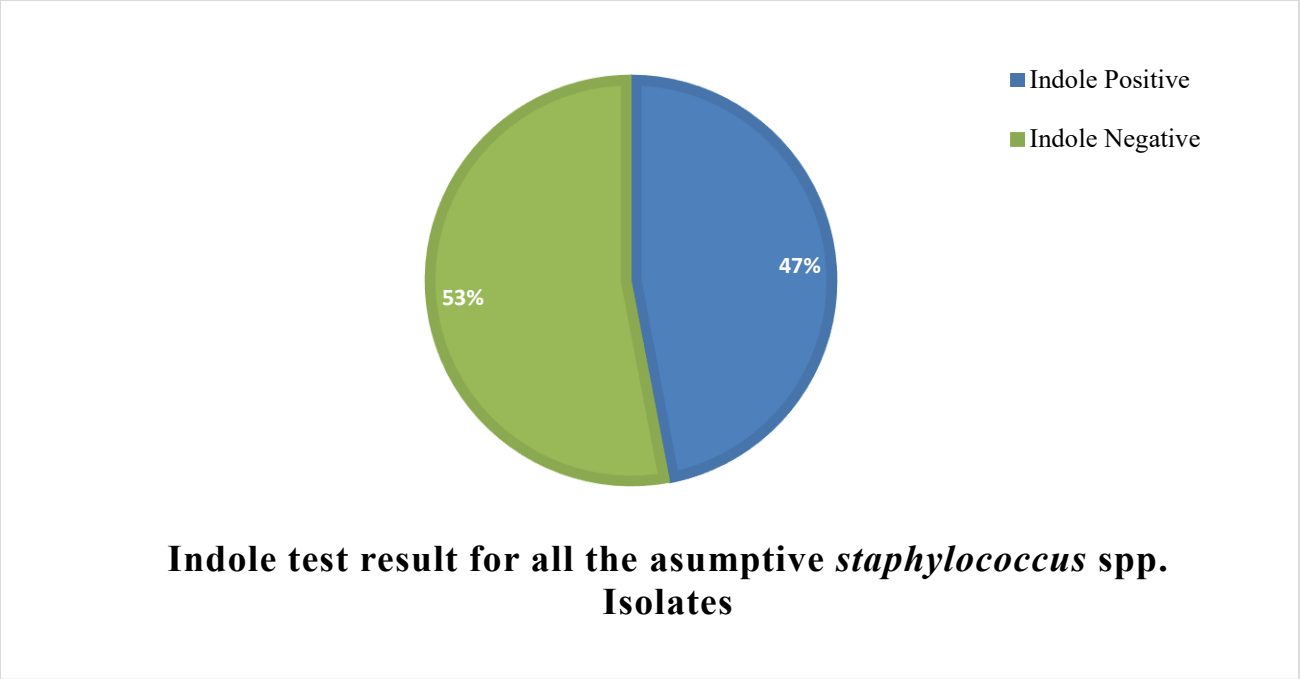


Figure 21: Observational result of Indole test.

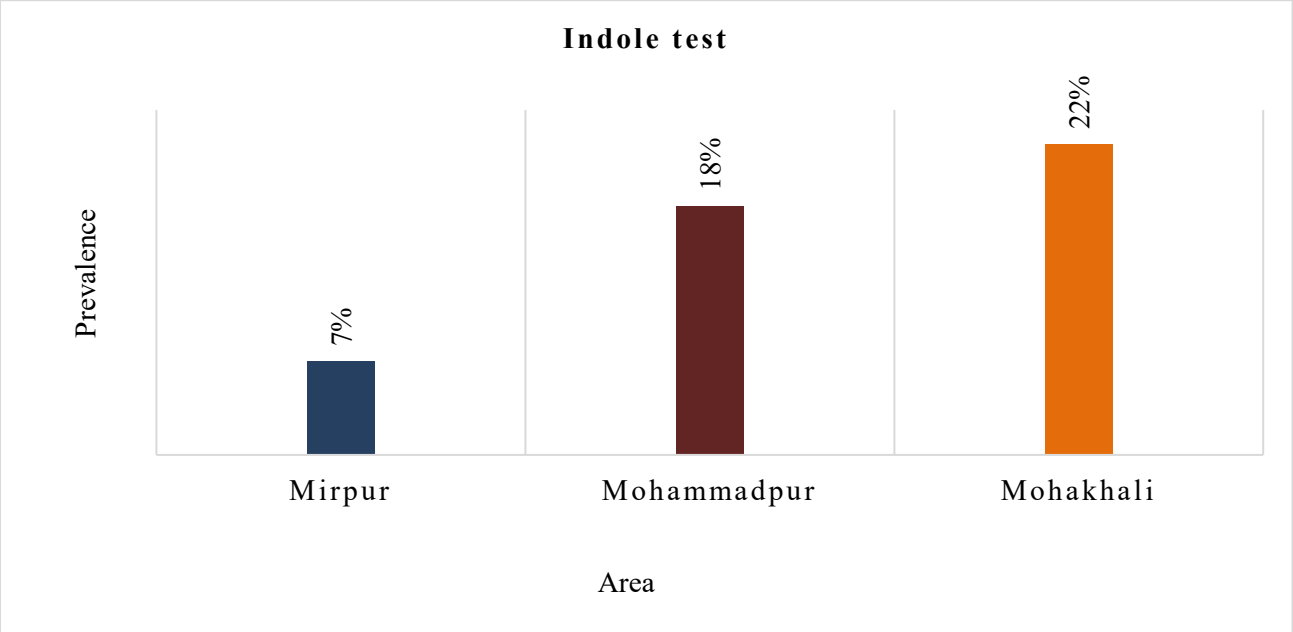


Figure 22: Distribution of pre-assumptive *Staphylococcus* spp. based on Indole test results among the collected samples.

4.1.7. Biochemical Identification Using Methyl Red Test

It detects the production of acid (lactic acid, acidic acid, or formic acid) during the fermentation of glucose in the media. After acid is formed, the pH in the solution lowers to the level that after adding an indicator, it turns red.

Media is prepared and bacterial strain is inoculated in the solution. After incubation for 24 hours, if the bacteria can ferment glucose, then the solution will turn acidic and the indicator methyl red will turn the solution red.

Figure 23 illustrates the results of the methyl red test. We have one positive control, one negative control, and two of the results of the isolates. One is positive (red color test tube (d)) and the other one is negative (yellow color test tube (c)).

Figure 24 depicts that the conducted indole test showed positive results for 87% of the cases, of the pre-assumed *Staphylococcus* spp. (the color turned red). The rest of the isolates were seen to have negative results (13% of them showed yellow color).

The distribution of 87% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 25**. The bar chart demonstrates that the prevalence was highest in Mohakhali (36%), followed by Mohammadpur (31%). In Mirpur, the prevalence was lowest. It was 27%.

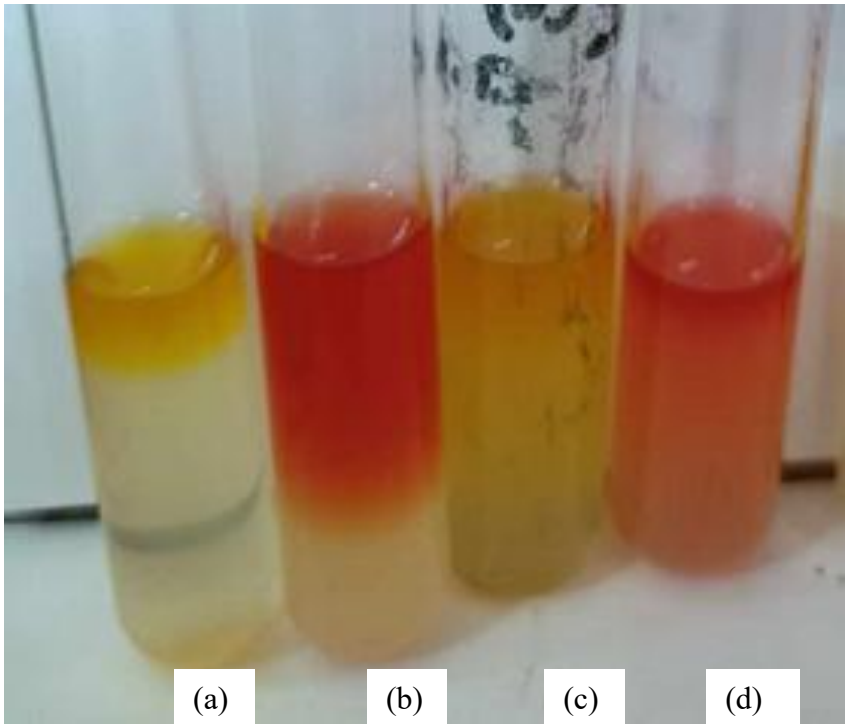


Figure 23: Methyl Red test result.

(a) Negative control of *Staphylococcus* spp. (b) Positive control of *Staphylococcus* spp. (c) Negative result, (d) positive result.

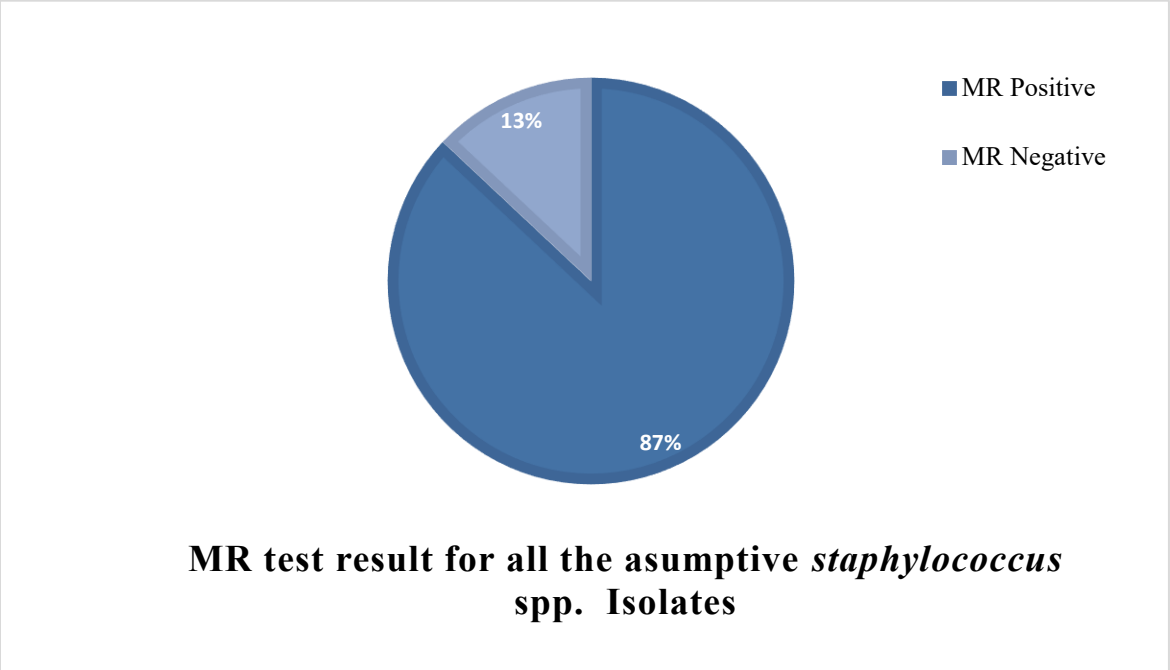


Figure 24: Observational result of Methyl Red test.

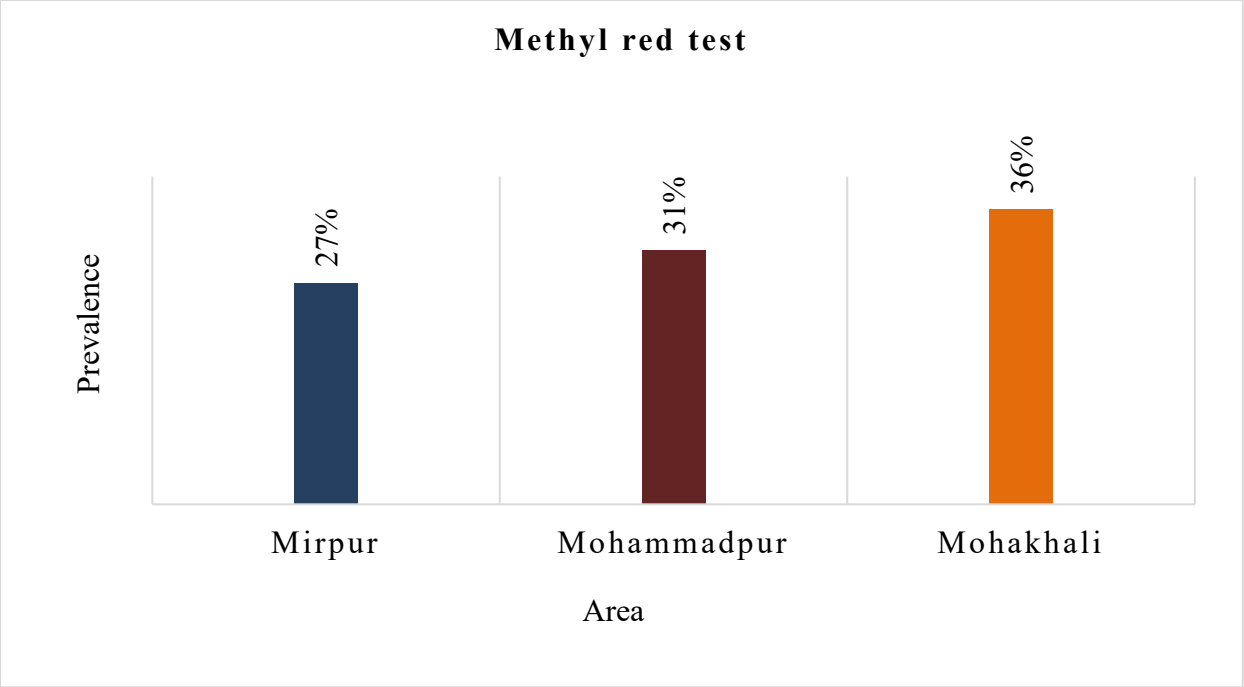


Figure 25: Distribution of pre-assumptive *Staphylococcus* spp. based on Methyl Red test results among the collected samples.

4.1.8. Biochemical Identification Using VP Test

It is a part of the IMViC test series and is suitable for the identification of gram-negative bacteria. It determines if an organism can produce acetylmethyl carbinol from glucose after fermentation. If it is present, **acetylmethyl carbinol** further converts into **diacetyl** in the presence of **∝- naphthol**, alkali (**40% KOH**), and oxygen. The **diacetyl** and **quinidine-containing** compounds found in the **peptones** of the broth then condense to form a **pinkish-red polymer**.

Media is prepared and bacterial strain is inoculated in the solution. After incubation for 24 hours, two types of reagents are required to test the result- 5% Alpha-naphthol Solution (Barritt's Reagent A) and 40% KOH or NaOH solution (Barritt's Reagent B).

Figure 26 illustrates the results of the VP test. We have one positive control, one negative control, and one result of the isolates (red color test tube (c)).

Figure 27 depicts that the conducted indole test showed positive results for 45% of the cases, of the pre-assumed *Staphylococcus* spp. (the color turned red). The rest of the isolates were seen to have negative results (55% of them showed yellow color).

The distribution of 45% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 28**. The bar chart demonstrates that the prevalence was highest in Mohakhali (32%), followed by Mohammadpur (29%). In Mirpur, the prevalence was lowest. It was 25%.

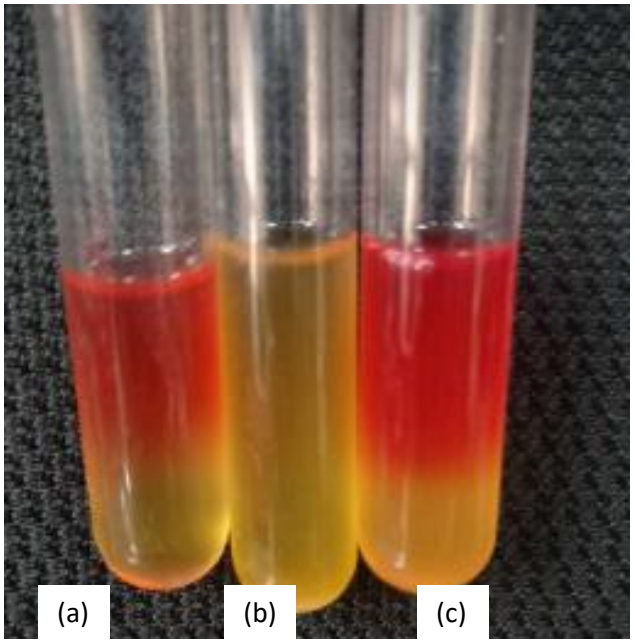


Figure 26: VP test result.

(a) Positive control of *Staphylococcus* spp. (b) Negative control of *Staphylococcus* spp. (c) Positive result.

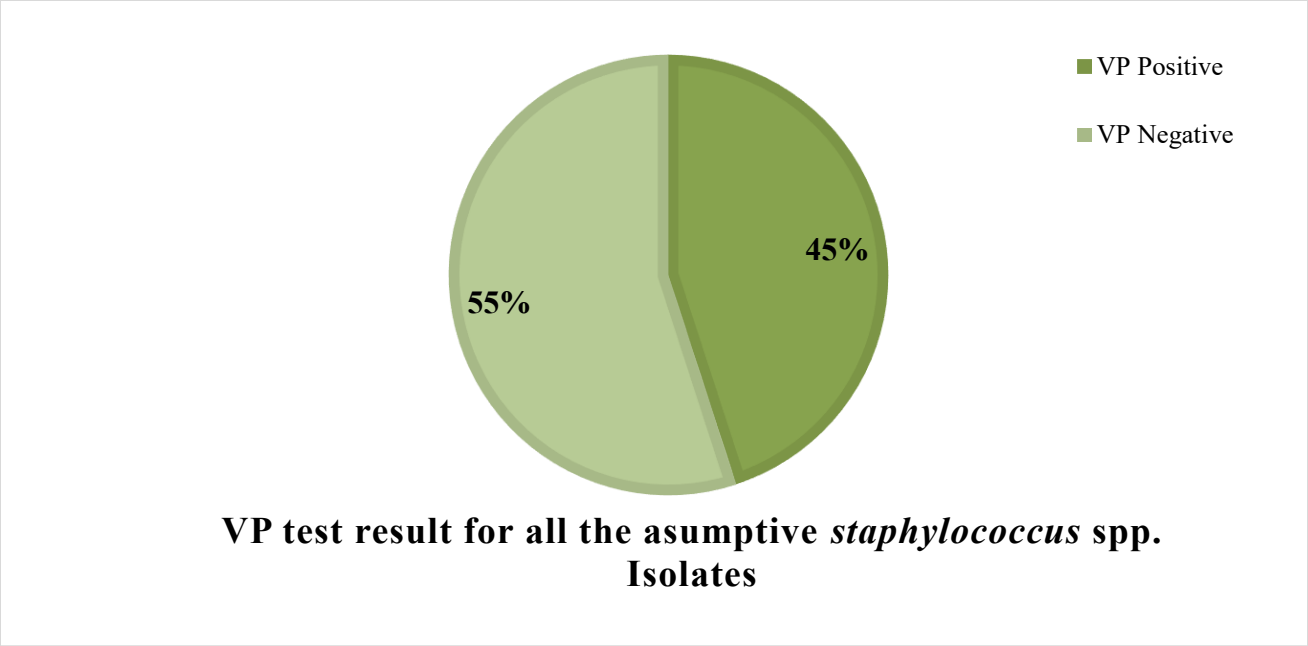


Figure 27: Observational result of VP test.

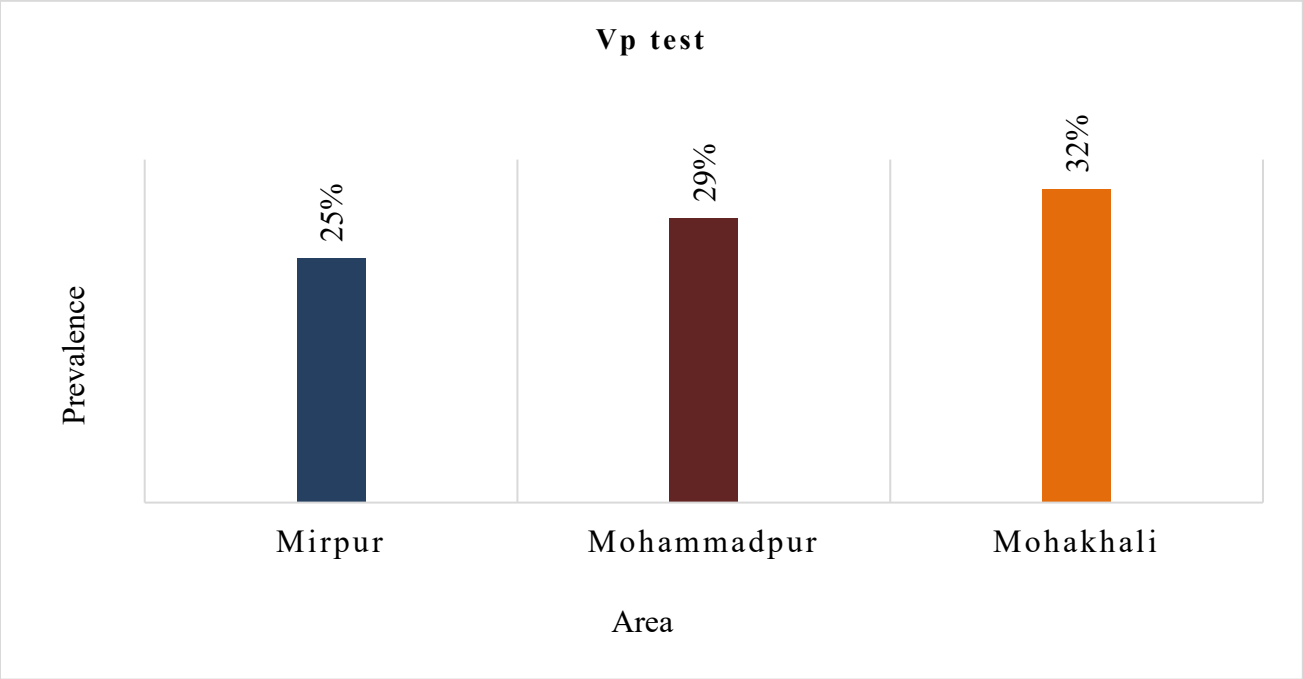


Figure 28: Distribution of pre-assumptive *Staphylococcus* spp. based on VP test results among the collected samples.

4.1.9. Biochemical Identification Using MIU (Motility Indole Urease test) Test

4.1.9.1 Motility test: Motility is the ability of an organism to move by itself through a propeller-like flagella which is unique to bacteria or by special fibrils. This feature has been recognized as an important taxonomic tool of organisms.

4.1.9.2. Indole test: As mentioned previously, this test displays the ability of a bacteria to decompose tryptophane which is an amino acid into indole. This characterization is important for the identification of enterobacteria.

4.1.9.3. Urease test: It is a biochemical test that identifies bacteria that break down urea and turn it into ammonia and carbon dioxide. The formation of ammonia increases the pH of the media and a color change is observed (pink color).

For this experiment, MIU media was poured into vials and with a needle, bacterial colonies were stabbed in a straight line. After 24 hours of incubation, the result was observed. No motility was seen. Only pink color was observed as the bacteria utilized only urea. Here, Figure 28 demonstrates the results of the MIU test.

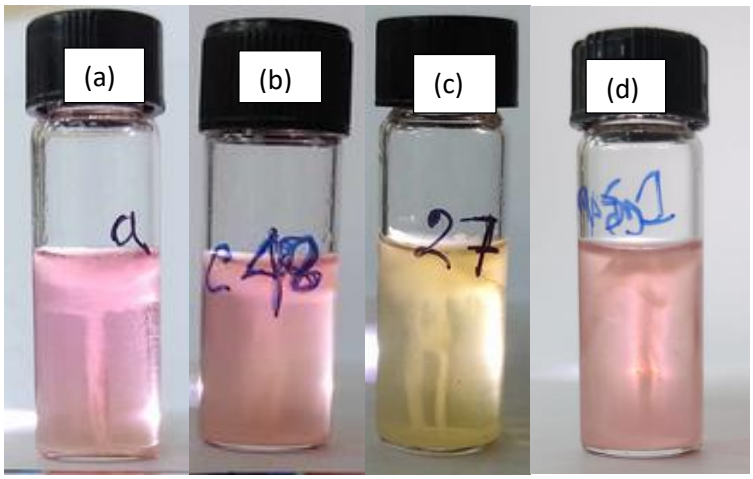


Figure 29: Motility Indole Urease test result

(a) Positive control of *Staphylococcus* spp. (non-motile, urease positive, indole negative), (b) negative result of some of the isolates (motile, urease positive, indole negative), (c) negative result of some of the isolates (non-motile, indole negative, urease negative), (d) positive results of some of the isolates (non-motile, urease positive, indole negative),

Figure 29 depicts that the conducted Motility test (in MIU) showed a positive result for 73% of the cases, of the pre-assumed *Staphylococcus* spp. (non-motile). The rest of the isolates were seen to have negative results (27% of them showed yellow color).

The distribution of 73% of the isolates found positive for *Staphylococcus* spp. is demonstrated in **Figure 30**. The bar chart demonstrates that the prevalence was highest in Mohammadpur (58%), followed by Mohakhali (49%). In Mirpur, the prevalence was lowest. It was about 24%.

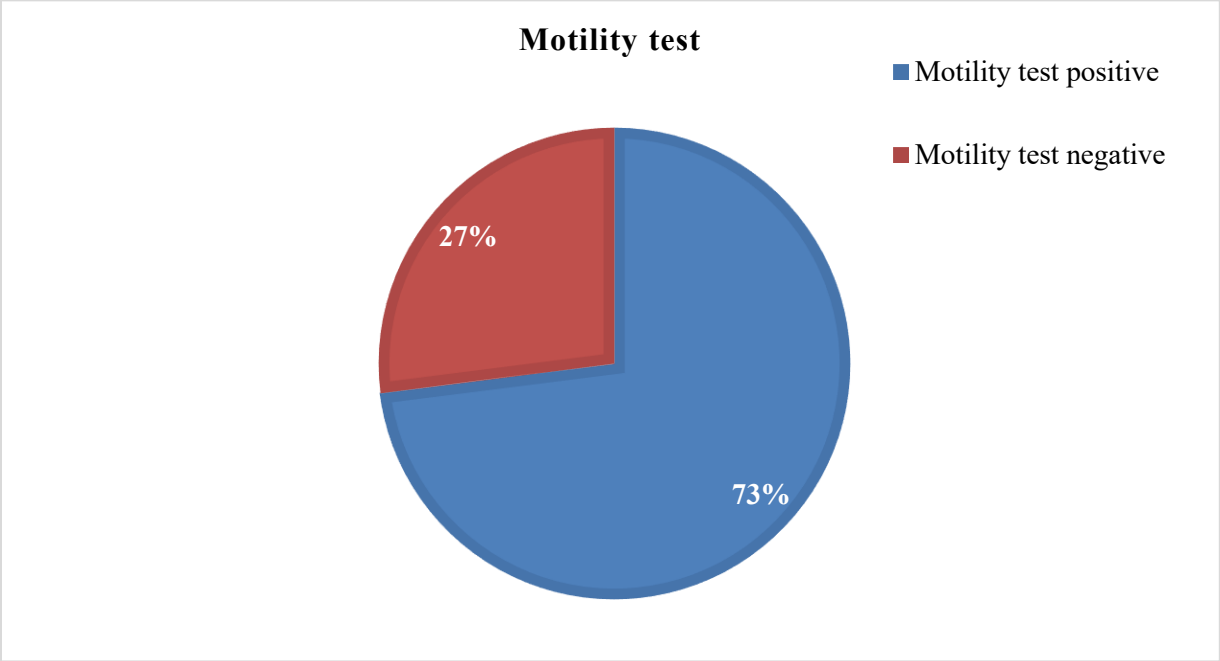


Figure 30: Observational result of Motility test.

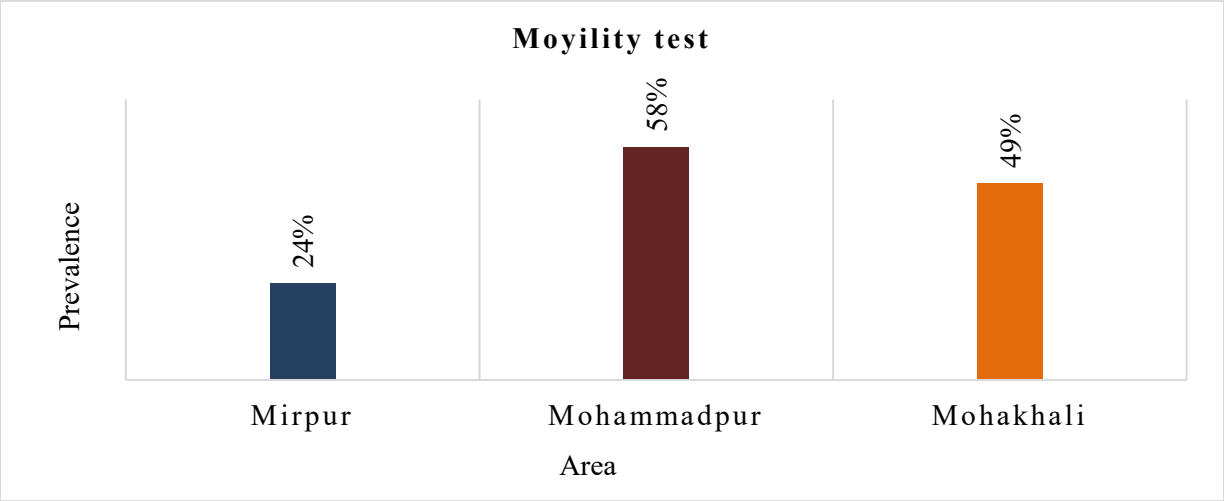


Figure 31: Distribution of pre-assumptive *Staphylococcus* spp. based on Motility test results among the collected samples.

In **Figure 32**, we can see that the conducted Urease test (in MIU) showed a positive result for 79% of the cases, of the pre-assumed *Staphylococcus* spp. (media turned pink from yellow). The rest of the isolates were seen to have negative results (21% of them remained yellow color).

The distribution of 79% of the isolates found presumptively positive for *Staphylococcus* spp. is demonstrated in **Figure 33**. The bar chart demonstrates that the prevalence was highest in Mohammadpur (59%), followed by Mohakhali (53%). In Mirpur, the prevalence was lowest. It was about 29%.

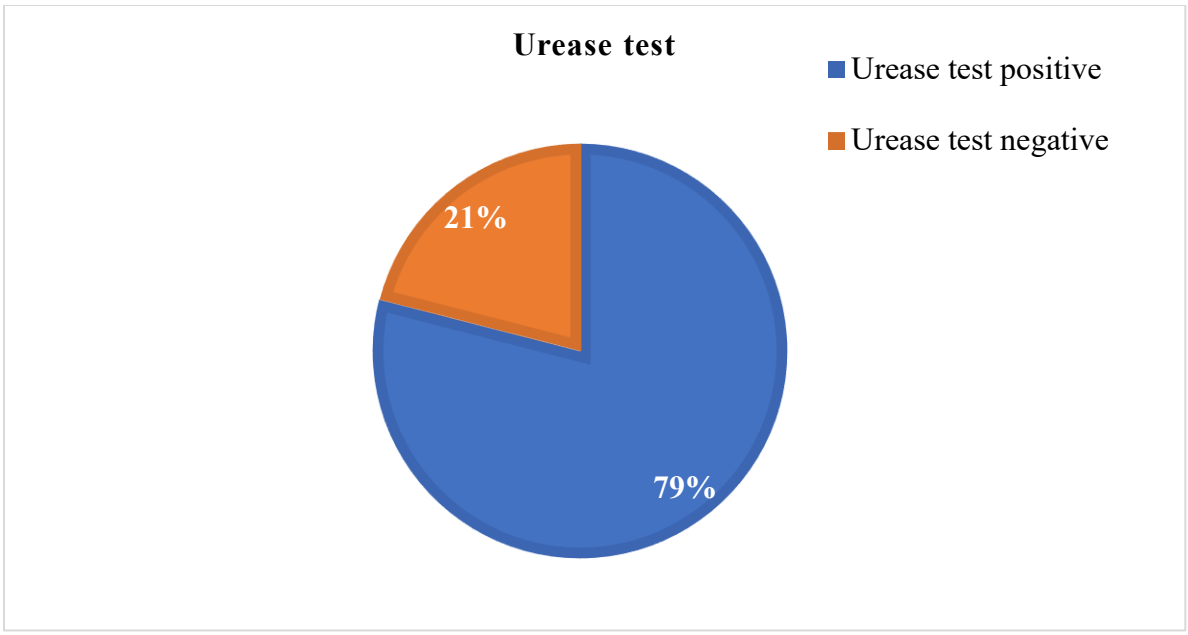


Figure 32: Observational result of Urease test.

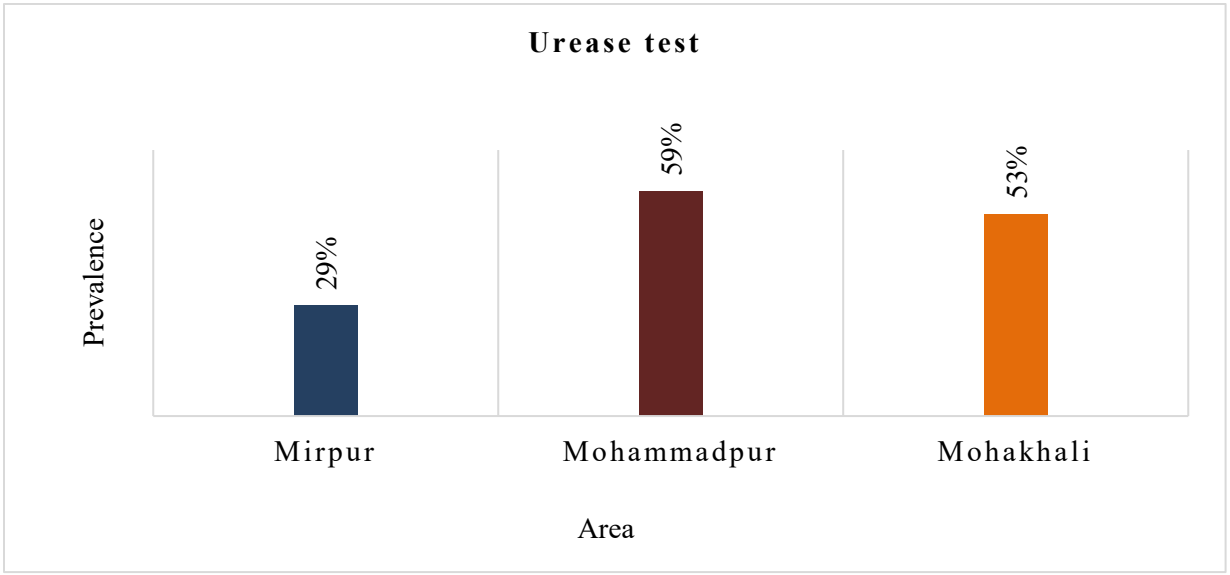


Figure 33: Distribution of pre-assumptive *Staphylococcus* spp. based on Urease test results among the collected samples.

In biochemical tests, considering all the results, a graphical representation is shown in **Figure 34**. In the MIU test, 79% of the isolates showed positive results in Urease and 73% in the motility test. The indole test was performed separately, and it was 47%. For the MR-VP test, 87% of the isolates had positive results for MR, and 45% of the isolates showed positive results for the VP test. The TSI test showed a 58% positive result. Oxidase indicated 39% and catalase 53% positive results. Lastly, the citrate test was 82% positive for all the isolates.

Figure 35 demonstrates the gram-staining results. 82% of the isolates were gram-positive and 18% of them appeared to be negative. Moreover, in **Figure 36** the prevalence in three of the areas are presented according to the chemical analysis. In the bar chart, it is seen that Mohammadpur had the highest prevalence (38%) and Mirpur had the lowest prevalence (23%). On the other hand, Mohakhali was seen to have 34%.

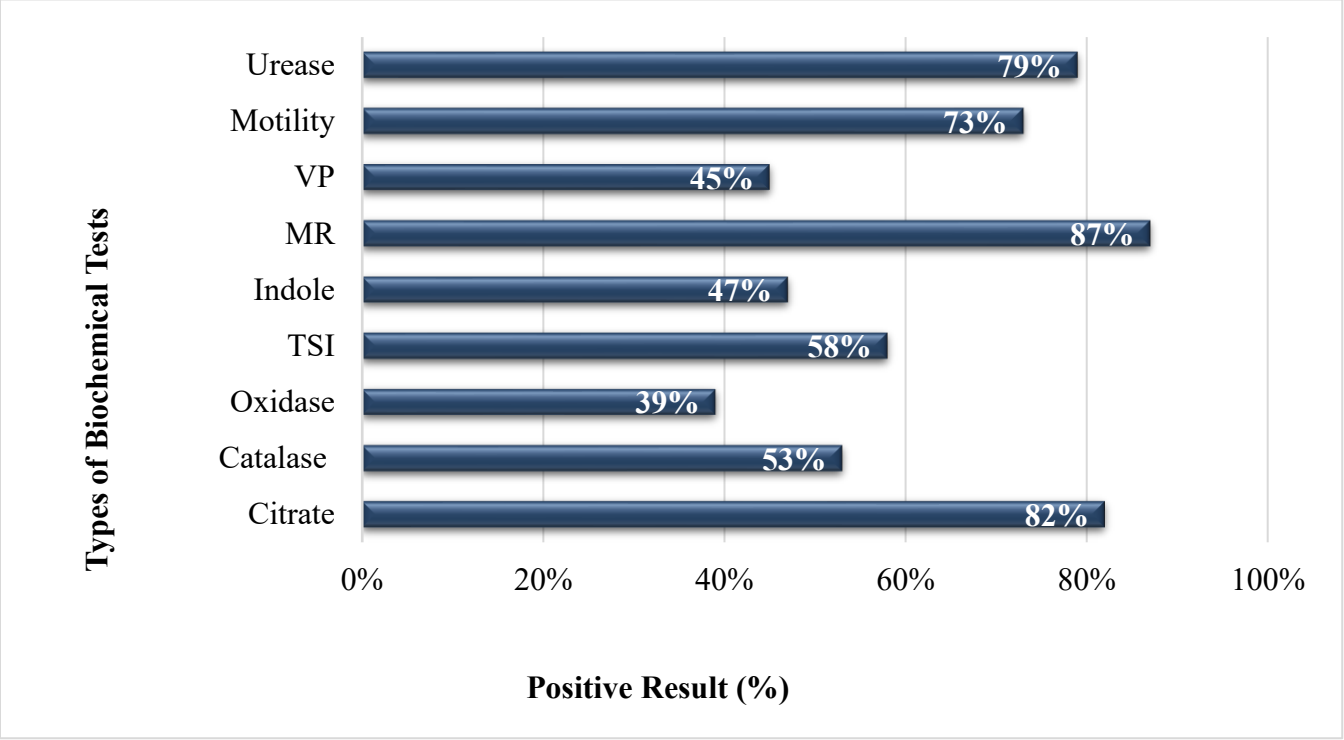


Figure 34: Test results of the biochemical tests for 178 isolates.

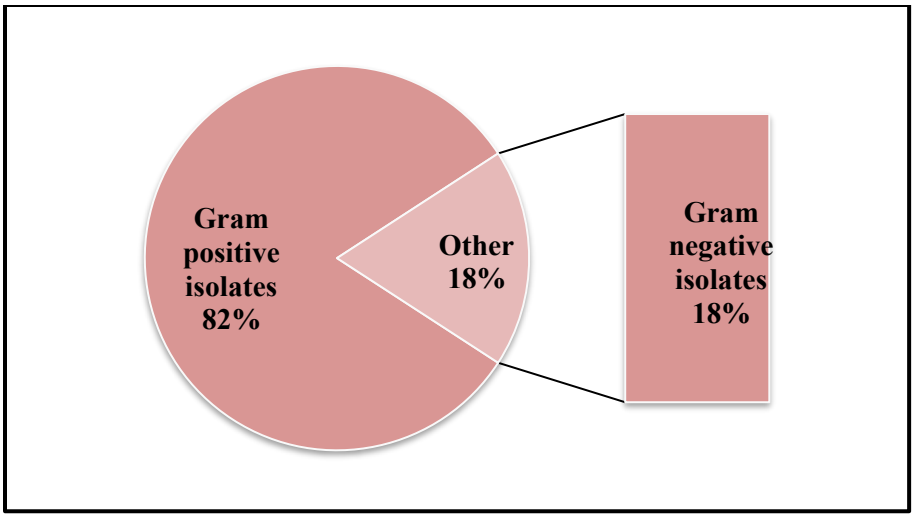


Figure 35: Gram staining result of 178 isolates showing 82% are gram-positive.

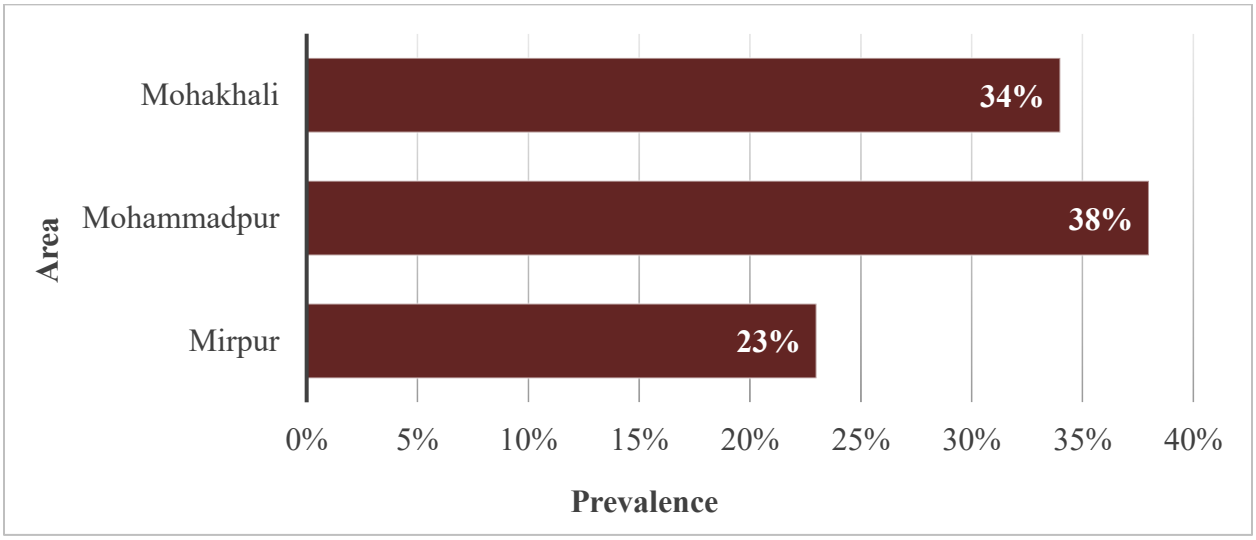


Figure 36: Prevalence of *Staphylococcus* spp. of sixty different restaurants according to the result of biochemical tests, (Mirpur, n=10; Mohammadpur, n=27; Mohakhali, n=25; n=isolate numbers).

4.1.10 Molecular analysis: Identification of Staphylococcus aureus isolates by PCR Assay and Gel Electrophoresis

After the biochemical test results, out of 178 isolates, only 61 were assumed to be *Staphylococcus* spp. (according to biochemical tests). Only these isolates were further prepared for molecular analysis. For this analysis, TStag (to detect *Staphylococcus* spp.) and nucA (to detect *Staphylococcus aureus*) primers were used.

The TStag and nucA primers were utilized to detect *Staphylococcus* spp. (370 band size) (**Figure 40**) and *Staphylococcus aureus* (270 band size) (**Figure 41**). According to the molecular analysis, 26.4% of the 60 samples were positive for *Staphylococcus* spp., and 2.24% of them were identified as *Staphylococcus aureus* (**Figure 37**). Interestingly, the prevalence of *Staphylococcus* spp. in the Mirpur area had the lowest incidence at 22.5%, while Mohammadpur had the highest at 30%. Mohakhali had an incidence of 25% (**Figure 38**). Notably, only four of the isolates were confirmed to be *Staphylococcus aureus.* Mohakhali was the only area where *Staphylococcus aureus* strains were found, with four restaurants testing positive (**Figure 39**).

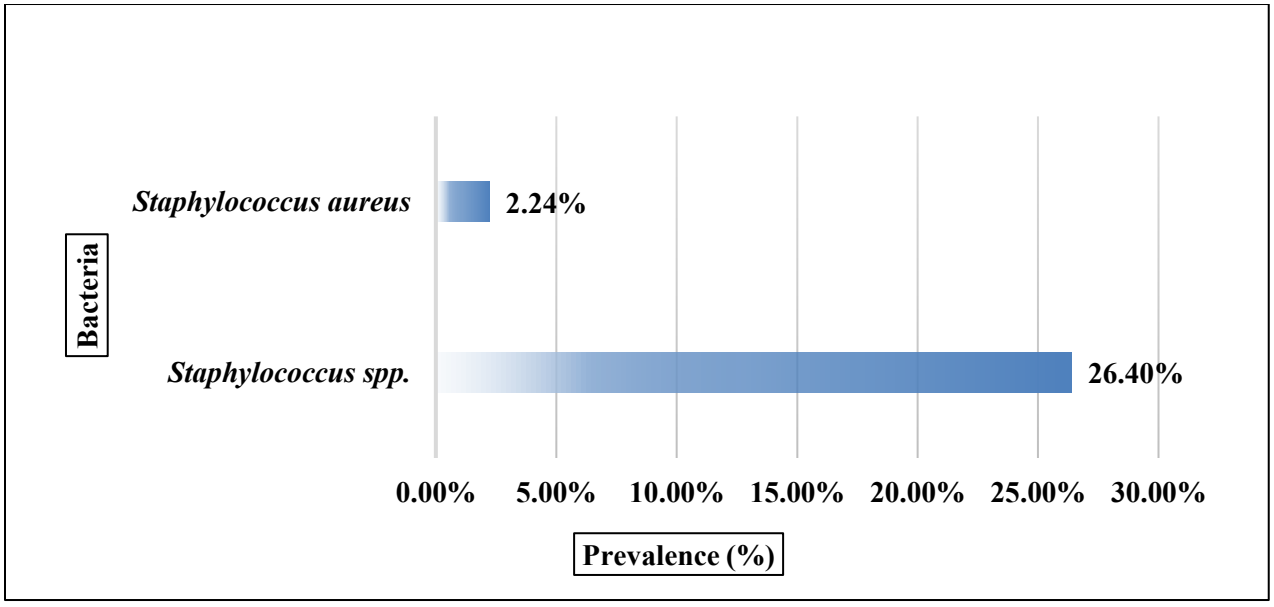


Figure 37 : Contamination rate of *Staphylococcus* spp. and *Staphylococcus aureus* after molecular analysis.

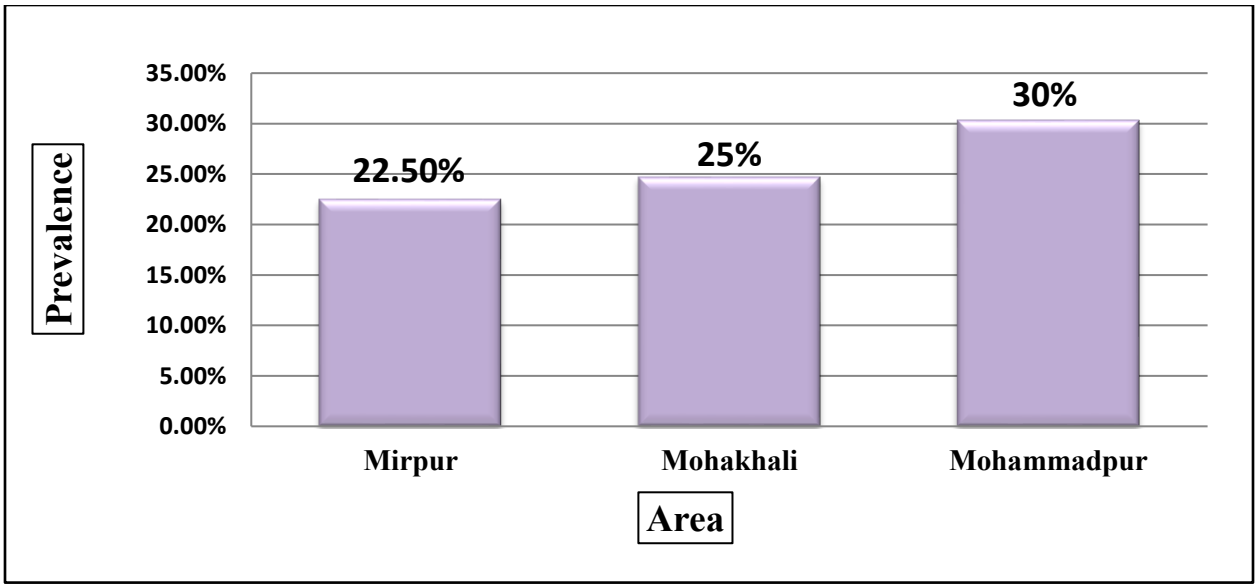


Figure 38: Prevalence of *Staphylococcus* spp. in three different areas according molecular analysis (among isolates).

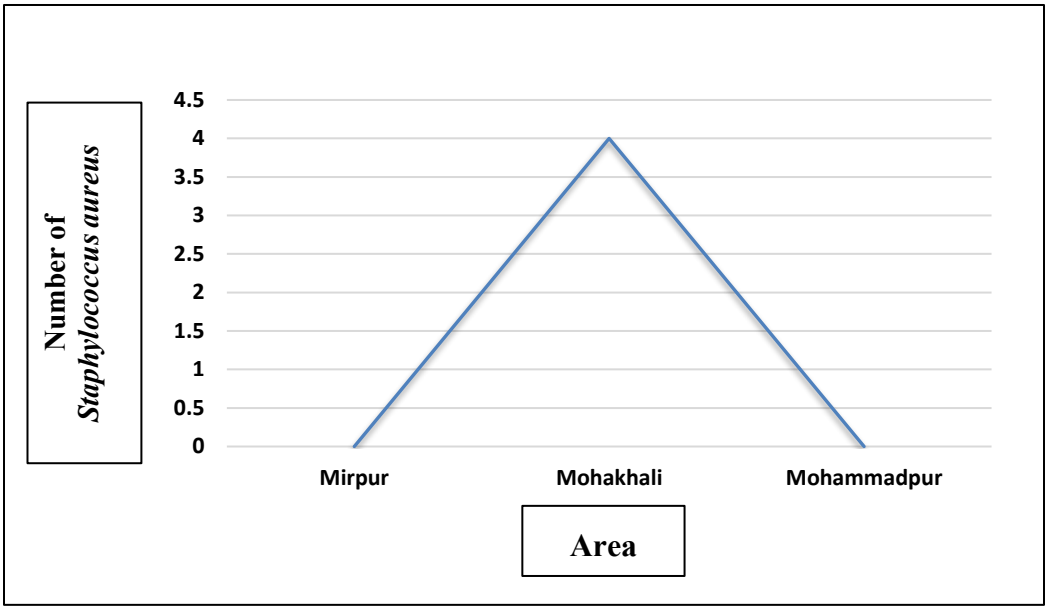


Figure 39: Prevalence of *Staphylococcus aureus* in four restaurants in Mohakhali.

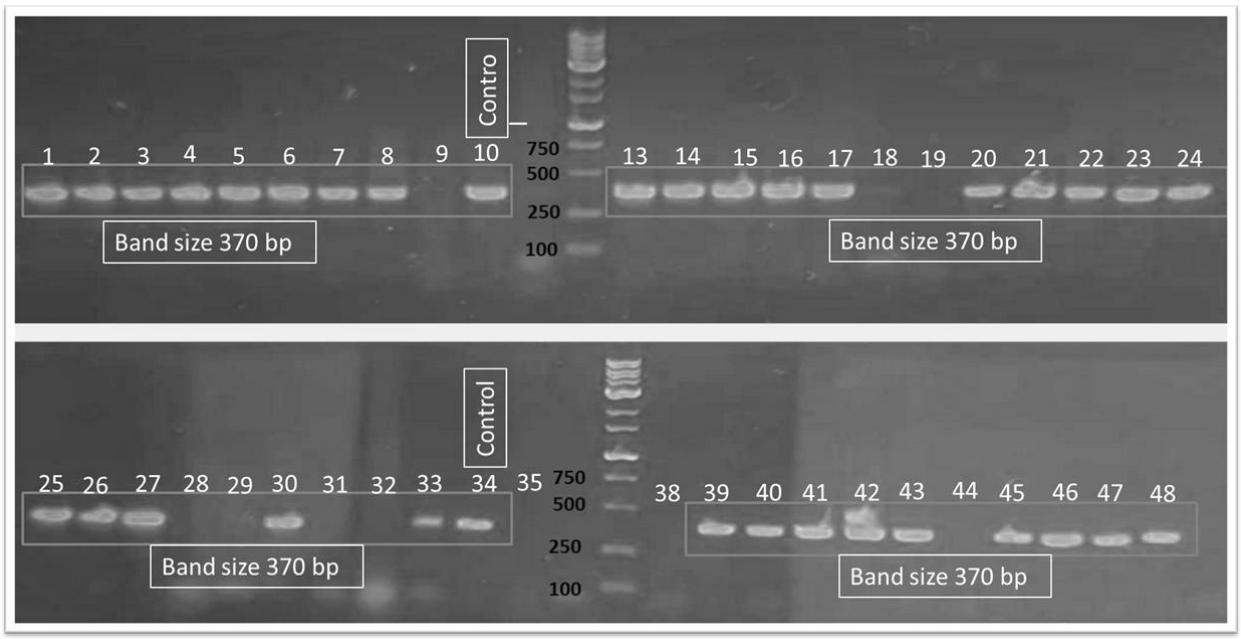


Figure 40: identification of *Staphylococcus* spp. by the TStag primer targeting the *tuf* gene (370 bp). Lane 12 indicates the ladder DNA. Lane 1 to 11 and 13 to 24 in the first agarose gel, in the next agarose gel- 25 to 35 and 38 to 48: *Staphylococcus* spp. isolates from food samples.

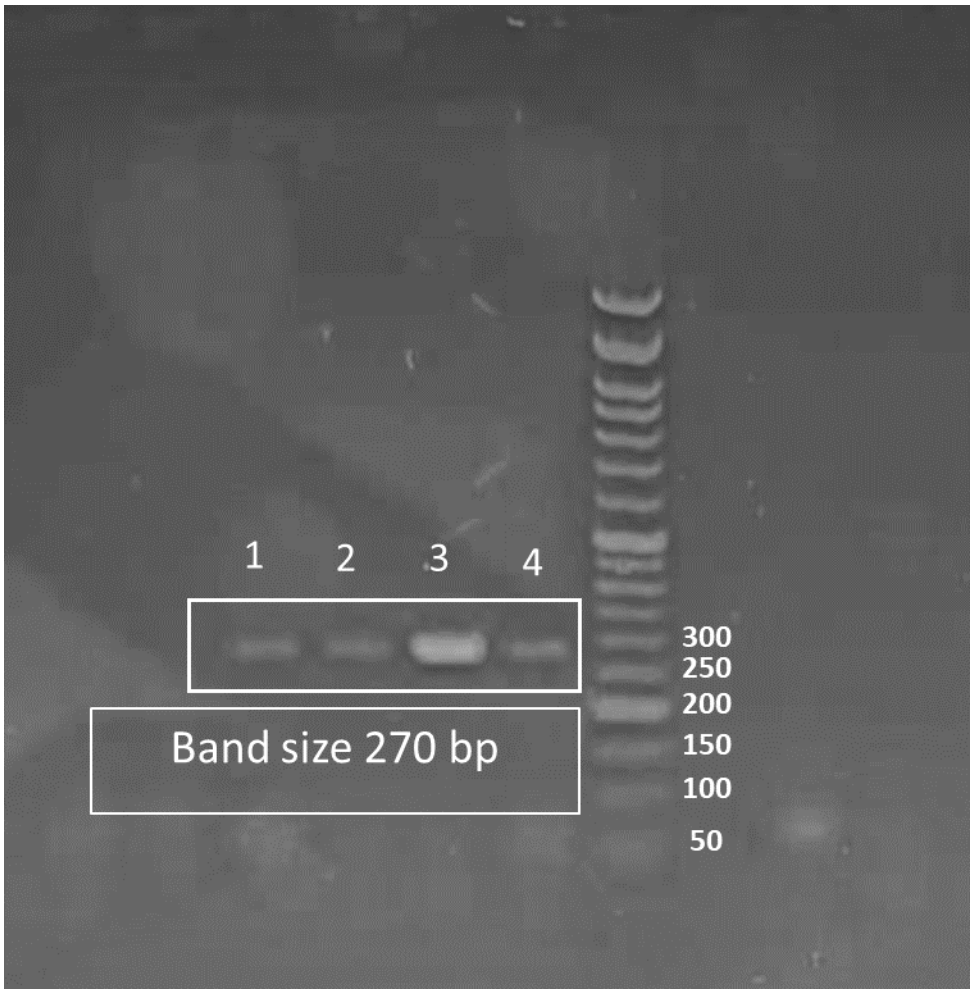


Figure 41: Identification of *Staphylococcus aureus* by nucA primer targeting *nucA* gene (270 bp). Here lane 12 indicated the ladder and lanes 1 to 11 contain the isolates isolated from the sample.

4.1.11 Antibiotic susceptibility test:

The antibiotic susceptibility test was conducted on *Staphylococcus aureus* to determine its resistance to antibiotics. For the test, the antibiotics used are the following- methicillin (MET, 5mcg), cefepime (CPM, 30mcg), doxycycline (DO, 30mcg), gentamicin (GEN, 120mcg), amoxicillin (AMC, 30mcg), erythromycin (E, 15mcg), vancomycin (VA, 30mcg), penicillin (P, 10 units), and tetracycline (TE, 30mcg). Surprisingly, *S. aureus* only showed resistance to **Amoxicillin (AMC) and Penicillin (PCN)** and exhibited equal or greater sensitivity to the rest of the antibiotics **(Table 8)**. Due to resistance to two antibiotics, all *S. aureus* isolates were identified as Multi-Drug-Resistant bacteria.

4.1.12 Blood hemolysis and salt tolerant tests:

Lastly, the *S. aureus* isolates were tested on blood agar, and the results indicated that they were Gamma-hemolytic bacteria (**Figure 42**). Furthermore, to assess the salt tolerance levels of the isolates, 10% salt was added to the Mannitol Salt Agar (MSA) media. The data revealed that among the confirmed *Staphylococcus* spp., 28% were highly salt tolerant, and 72% were moderately salt tolerant bacteria (**Figure 43**).

Antibiotics	Results
Methicillin (MET)	Susceptible
Cefepime (CPT)	Susceptible
Doxycycline (DO)	Susceptible
Gentamicin (GEN)	Susceptible
Amoxicillin (AMX)	**Resistant**
Tetracycline (TE)	Susceptible
Erythromycin (E)	Susceptible
Vancomycin (VAN)	Susceptible
Penicillin (PCN)	**Resistant**

Table 8: Antibiotic susceptibility result of *Staphylococcus aureus* (4 isolates).

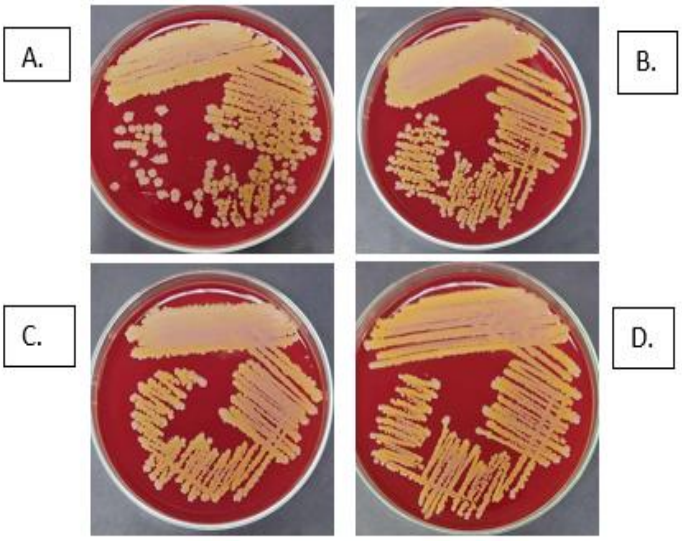


Figure 42: Gamma hemolysis result of *Staphylococcus aureus*

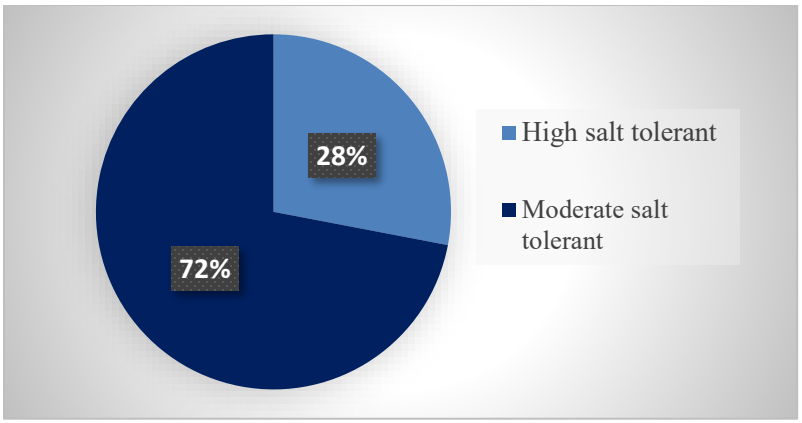


Figure 43: An additional 10% salt was added in MSA media to check for salt tolerance of *Staphylococcus* spp. and it showed that 28% and 72% are high salt and moderate salt tolerant isolates respectively.

4.2 Observation and result (Assessment of *Staphylococcus spp.* Contamination in home-prepared foods: microbiological and molecular investigations):

Along with the 60 samples collected from the restaurants, 10 more were collected as controls from 10 different homes. These samples were collected aseptically using sterile instruments. 25 grams of each sample was homogenized using mortal pastel (Comi et al., 2005) and was transferred to 100 ml peptone broth (0.85%) (Ranucci et al., 2004; Heo et al., 2020; Chung et al., 2021) and was in the incubator for 24 hours at 37° C.

From the broth, 100 µl was transferred into MSA media plates. the plates were in incubation for 24 hours at 37°C (Rhee and Woo, 2010; San Moon et al., 2007; Oh et al., 2007). All the plates were examined and morphologically distinct colonies (**Figure 44**) (Comi et al., 2005). Strains that gave yellow and pink color in MSA media were streaked on NA media and then were chosen for molecular analysis. **Table 9** contains the observational on MSA and NA media. Lastly, **Figure 45** presents the result of molecular analysis. Here, TStag primer was used. As the *tuf* gene was not detected, further test for the detection of the *nucA* gene was not performed.

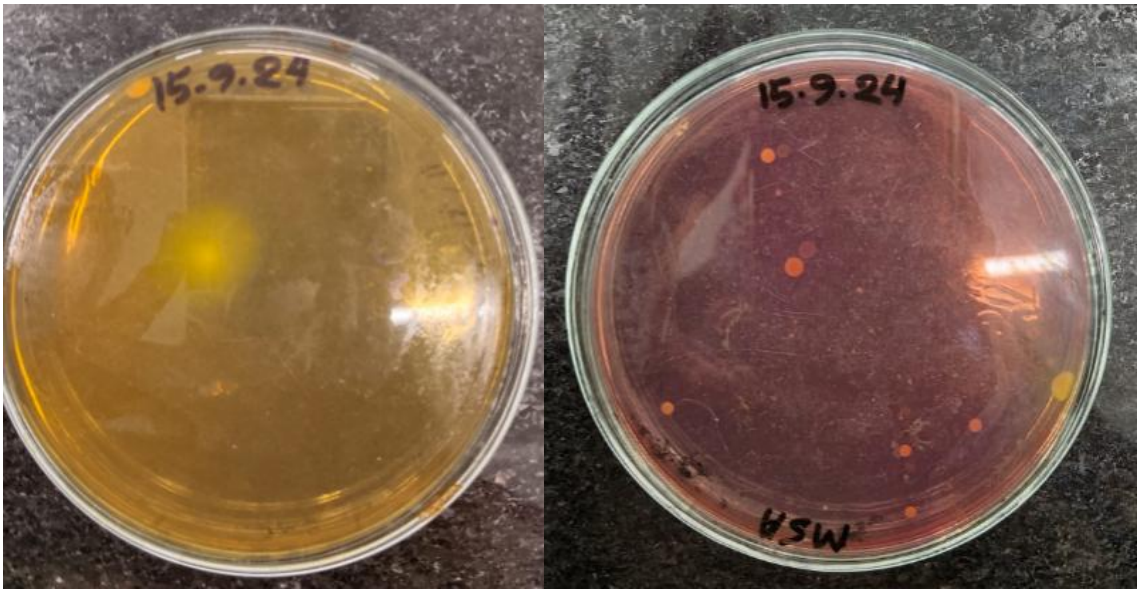


Figure 44: Dilution results on MSA media.

Table 9: observational result for NA and MSA media

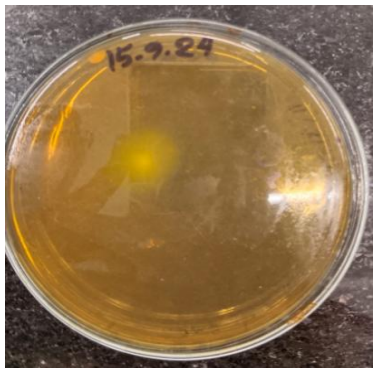
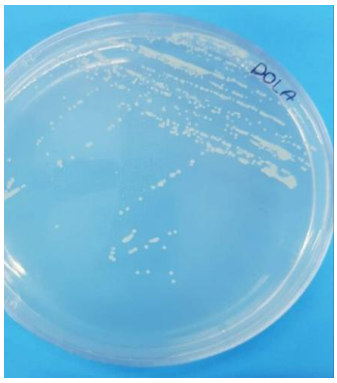
Media	Total number of isolates	Observation	Picture	Hypothesis	Prevalence of positive presumptive
NA	4	Yellow colonies		*Staphylococcus* spp.	After testing on MSA media, only 4 isolates were tested for molecular analysis.
MSA		White colonies			

Figure 45: Four PCR amplicons with TStag primer (from the control group) were in the first 4 wells (no bands were observed). L is the ladder.

Table 10: Sample collection sites and the number of isolates collected from the Mohakhali area

SL no	Area	Restaurant name	Total isolates	Bacteria identified by *tuf* gene (*staphylococcus* spp.)	Percentage	Bacteria identified by *nuca* gene (*staphylococcus aureus*.)	Percentage
1	**Mirpur**	Saudia	2	0	0%	0	0%
2		Purnima	2	0	0%	0	0%
3		Maer doa restaurant	1	1	100%	0	0%
4		Chadpur chanmia restaurant	2	2	100%	0	0%
5		Nameless	3	0	0%	0	0%
6		Dastarkhana	1	1	100%	0	0%
7		Saudia	3	0	0%	0	0%
8		Saudia	2	1	50%	0	0%
9		Nameless	3	0	0%	0	0%
10		Nameless	2	0	0%	0	0%
11		Ananna vojon bilas	3	0	0%	0	0%
12		Shariat pur restaurant	2	0	0%	0	0%
13		Pakghar restaurant	3	1	33%	0	0%
14		Saiad miran shah	2	0	0%	0	0%
15		The sun moon hotel and restaurant	1	0	0%	0	0%
16		Manik hotel and restaurant	2	0	0%	0	0%
17		Kolapata restaurant	1	1	100%	0	0%
18		Nameless	2	0	0%	0	0%
19		"	1	0	0%	0	0%
20		"	2	2	100%	0	0%
		Total	40	9			

Table 11: Sample collection sites and the number of isolates collected from the Mohakhali area

Sl no	Area	Restaurant name	Total isolates	Bacteria identified by *tuf* gene (*staphylococcus* spp.)	Percentage	Bacteria identified by *nuca* gene (*staphylococcus aureus*.)	Percentage
21	**Mohakhali**	Nameless	2	2	100%	0	0%
22		Chadni restaurant	4	0	0%	1	25%
23		Nameless	3	0	0%	0	0%
24		Niribili restaurant	5	2	40%	0	0%
25		Long restaurant	2	1	50%	0	0%
26		Nameless	5	1	20%	1	20%
27		Khabar ghor	3	0	0%	0	0%
28		Vai vai biriani ghor and restaurant	4	2	50%	0	0%
29		Mamoni restaurant	4	0	0%	0	0%
30		Vater hotel	4	0	0%	1	25%
31		Rumali ruti	4	0	0%	0	0%
32		Nameless	3	2	67%	0	0%
33		Noakhali hotel and restaurant	3	0	0%	0	0%
34		Nameless	4	0	0%	0	0%
35		Astagfirullah restaurant	3	1	33%	0	0%
36		Bismillah restaurant	4	2	50%	0	0%
37		Nameless	2	0	0%	0	0%
38		Khabardabar restaurant	2	1	50%	0	0%
39		Oriental	3	0	0%	1	33%
40		Ghoroa	4	3	75%	0	0%
		Total	68	17		4	

Table 12: Sample collection sites and the number of isolates collected from the Mohammadpur area

SL no	Area	Restaurant name	Total isolates	Bacteria identified by *tuf* gene (*staphylococcus* spp.)	Percentage	Bacteria identified by *nuca* gene (*staphylococcus aureus*.)	Percentage
41	**Mohammadpur**	Mon khushi restaurant	3	2	67%	0	0%
42		Chayanir restaurant	4	0	0%	0	0%
43		Bagdad restaurant	4	0	0%	1	25%
44		Nameless	3	0	0%	0	0%
45		Mona mia	5	3	60%	1	20%
46		Selim kabab	2	0	0%	0	0%
47		Nahar restaurant	3	2	67%	0	0%
48		Nadim restaurant	2	0	0%	0	0%
49		Nameless	5	3	60%	0	0%
50		Vhojon	3	1	33%	0	0%
51		Mamun soup house	2	0	0%	0	0%
52		Maza food corner	4	2	50%	0	0%
53		Town hall	3	2	67%	0	0%
54		Lal bagh restaurant	4	0	0%	0	0%
55		Smile food corner	5	0	0%	1	20%
56		Nameless	2	1	50%	0	0%
57		Nameless	3	2	67%	1	33%
58		Mohammadia hotel	4	0	0%	0	0%
59		Nameless	4	0	0%	0	0%
60		Manik mia restaurant	5	3	60%	0	0%
		Total	70	21		0	

Table 13: Sample collection sites and the number of isolates collected from the Mirpur area

Control Group							
SL No	**Area/Restaurant**		**Total isolates**	**Bacteria identified by *tuf* gene (*Staphylococcus* spp.)**	**Percentage**	**Bacteria identified by *nucA* gene (*Staphylococcus aureus*.)**	**Percentage**
1	**Home Cooked**	D-1	1	0	0%	0	0%
2		M-1	0	0	0%	0	0%
3		DD-2	2	0	0%	0	0%
4		DD-3	0	0	0%	0	0%
5		S-1	0	0	0%	0	0%
6		S-2	0	0	0%	0	0%
7		R-1	0	0	0%	0	0%
8		R-2	0	0	0%	0	0%
9		R-3	0	0	0%	0	0%
10		R-4	1	0	0%	0	0%
		Total	4				

Table 10, Table 11, Table 12, and Table 13 contain the names of the restaurants and homes, the number of isolated samples for the analysis, and the result of the molecular analysis.

Chapter 5

5. Discussion

In this experiment, we tried to investigate the presence of both *Staphylococcus* spp. and *Staphylococcus aureus* in cooked meat (chicken curry). For this purpose, we collected 60 samples from 60 different restaurants situated in three different locations, along with 10 samples cooked at home. A total of 178 isolates were selected (from restaurant samples) for biochemical tests and molecular analysis. The results were quite substantial, revealing that almost 26.4% of the isolates were found to be *Staphylococcus* spp. and 2.24% were *Staphylococcus aureus*. Interestingly, after all the analysis, we found that the occurrence of *Staphylococcus* spp. was notably higher in Mohammadpur, while *Staphylococcus aureus* was exclusively found only in the Mohakhali area. However, the samples collected from home did not show any presence of *Staphylococcus* spp. Additionally, the antibiotic susceptibility tests revealed that every *Staphylococcus aureus* isolate exhibited resistance to Amoxicillin (AMC) and Penicillin (PCN), pointing to a concerning multidrug resistance pattern. These isolates were also found to be gamma-hemolytic, which denotes valuable information for the understanding of potential health risks associated with similar strains. All *Staphylococcus* spp. were also tested for salt tolerance, and it was found that 28% of the isolates were highly salt tolerant (17.5% salt concentration).

5.1 Prevalence of *Staphylococcus* spp. and *Staphylococcus aureus*

Each year, around 30 million people in Bangladesh suffer from foodborne illnesses. Among them, diarrheal diseases are the most common cases of food poisoning, and in severe cases, these can cause death. The diseases are caused either by toxins produced by the microbe or by the human body's reactions to the microbe (Banik et al., 2018). A large number of cases have revealed that Staphylococci are the third most important cause of food-borne illnesses (Morandi et al., 200&) and are involved in food-related infections and food poisoning (Batista et al., 2013; Chajęcka-Wierzchowska et al., 2015; Fowoyo and Ogunbanwo, 2016; Podkowik et al., 2016; Vasconcelos et al., 2011; Zell et al., 2008). Minimal amounts of SEs, a concentration as low as 0.5 ng/ml, can

cause food poisoning and even cause a large outbreak (Kadariya et al., 2014). Hence, several investigations were done on animal-originated foods.

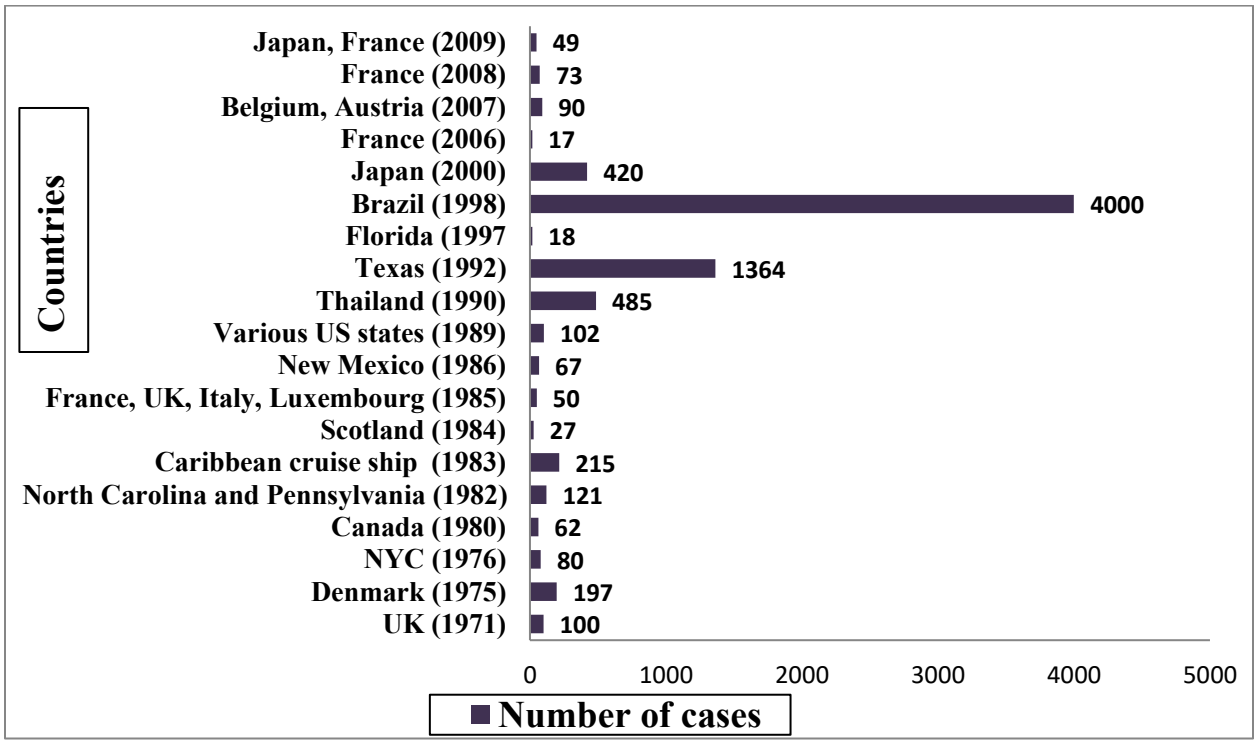


Figure 46: Number of Staphylococcal food poisoning (SFP) cases from ready-to-eat food in 19 countries and years (1971-2009).

Figure 46 presents the report of Staphylococcal food poisoning during different periods in different countries (from 1971 to 2008) (Hennekinne et al., 2012). Throughout this investigation, many types of samples were tested, such as chicken salad, sausage rolls, ham and cheese sandwiches, stuffed chicken, turkey, poultry, gravy, hamburger, etc. This data clearly shows that ready-to-eat meat can be a life-threatening source of illness for the general population. Besides, a report from the West of Iran revealed that a total of 98 (9.3%) *S. aureus* strains were identified from 1050 animal-originated food samples like cream, yogurt, raw red meat, raw poultry, cheese, etc. (Mashouf et al., 2015). Another report from Turkey stated that a total of one hundred thirty-eight samples (33.4%) were contaminated with *S. aureus* (Guven et al., 2010). In Italy, a survey was conducted with 176 raw meat samples; among them, *S. aureus* was present in 42 of the 176 samples (23.86%). (Pesavento et al., 200&). All these reports show that studies were done on raw

animal-originated food items, and there was the presence of *Staphylococcus* spp. and *S. aureus.* But at the same time, it can be said that very little research was done on cooked meat items (Lakhanpal et al., 2019; Hoveida et al., 2019; Dehkordi et al., 2019; Chajęcka-Wierzchowska et al., 2014; Argudin et al., 2010; Oh et al., 2007; Yildirim et al., 2017). Moreover, experiments were conducted by a group of students at BRAC University, where they collected cooked meat (chicken curry) from different government medical cafeterias (inside the hospital area), and they found the presence of MDR *Staphylococcus aureus* in it. In our study, we also found the presence of *Staphylococcus* spp. and *Staphylococcus aureus.* A total of 10 (50%) samples from Mohammadpur and 10 (50%) samples from Mohakhali tested positive for *Staphylococcus* spp., while Mirpur yielded only 7 (35%) positive samples. The samples analyzed in our study were also animal-originated food (cooked chicken), which contextualizes our findings. The rate of *Staphylococcus aureus* strains across the entire study was notably low, which aligns with the findings of our study. It is important to note that our outcome reflects a significantly lower occurrence compared to previous studies, which is likely because of the limited sample size.

Several reasons could be responsible for the contamination. 5 possible conditions are required to induce SFP. They are- any source that contains enterotoxin-producing staphylococci, such as raw materials, any carrier, transfer of staphylococci from source to food, or consuming food that has favorable physicochemical characteristics for staphylococci, favorable temperature and time that is suitable for bacterial growth and toxin production, and lastly, ingestion of those foods. Several other reasons can be speculated for the prevalence of cooked chicken as well. For instance, inadequate cooking temperature or heating cooked food at a low temperature, cross-contamination after cooking, etc. On the other hand, our collected samples can also be contaminated from the equipment or utensils that are used.

As observed from earlier data, the prevalence rates of *Staphylococcus* spp. are quite similar in all three places - Mohakhali (25%), Mohammadpur (30%), and Mirpur (22.5%). Several factors may contribute to this phenomenon, including the population in the restaurants, the working environment, and the degree of hygienic practices adhered to. Furthermore, the handling and serving methods of food play an important role in food safety. As cooked food is not thermally treated anymore, or even if it's treated, the temperature is quite low, which allows bacterial strains to survive and potentially produce enterotoxins. This increases the risk of food poisoning. Given

the geographical proximity of these areas, there could be common underlying causes that are responsible for the foodborne illnesses.

One of the common foodborne illnesses that affects people worldwide is caused by *Staphylococcus* spp. However, most of the time, investigations are often incomplete, and data are frequently underreported. Thus, it has become challenging to identify the causative agents of outbreaks and take appropriate measures to prevent future occurrences. Ignoring medical attention can lead to severe complications, so it's crucial to seek medical attention if there are any symptoms of food poisoning (Freitas et al., 2023). Bangladesh is no exception to that. As a result, the origin of the poisoning remains hidden. Reviewing previous literature and the results of this experiment, it is evident that having microorganisms that can cause foodborne illnesses in the food has become quite common (Safarpoor et al., 2017; Dehkordi et al., 2019; Fijałkowski et al., 2016; Chajęcka-Wierzchowska et al., 2014; Jofre et al., 2008; Ranucci et al., 2004; Kruse and Sorum, 1994). So, unless we find concrete evidence of pathogenic bacteria causing FBD, we cannot go for the treatment.

5.2 Blood hemolysin strains

Staphylococcus aureus remains a predominant pathogen globally, responsible for infection. The virulence potential of this bacterium lies in the secreted proteins, which have a toxic effect on humans. Notable among these are super antigenic toxins and leukocyte-specific cytotoxins that interfere with the body's defense system (Hennekinne et al., 2012). Moreover, some of the strains produce a series of hemolysins classified by their blood hemolysis ability. Among these, some can completely lyse the red blood cells (RBC), some do that partially, and some do not lyse the RBC at all (G.M. Wiseman, 1975). In the findings of Wang et al., 2020; a positive prevalence of beta hemolytic activity was observed in the *Staphylococcus aureus* samples analyzed. Conversely, our study yielded no significant results as all the strains of *Staphylococcus aureus* showed gamma hemolysis, indicating a non-hemolytic profile. But this does not reduce the virulence risk of the bacterium.

5.3 Antibiotic susceptibility test result of *Staphylococcus aureus*

The global increase in bacterial antibiotic resistance has emerged, and it has become a serious health issue (Hassan et al., 2021). In our experiment, the isolated *S. aureus* strains have shown resistance to only two antibiotics (AMX and PCN), while remaining susceptible to others. This report emphasizes the need for immediate and necessary actions to prevent them from posing a more significant threat. Due to the adaptive response to environmental stress and antibiotic selective pressure, the epidemiology of pathogens can change (Dehkordi et al., 2019). Numerous reports on multidrug-resistant bacteria have been documented, including studies highlighting *Staphylococcus aureus* strains sourced from animal-originated food. For instance, a report from India identified that their isolates were resistant to methicillin (93.7%), clindamycin (68.8%), erythromycin (56.3%), and vancomycin (43.8%) (Lakhanpal et al., 2019). Additionally, research from Iran revealed that out of 98 isolates examined, the most frequent resistance was observed against erythromycin, tetracycline, clindamycin, and gentamicin (Mashouf et al., 2015). Compared to these studies, our strains showed resistance to only 2 of the antibiotics. In contrast, our isolated strains of *S. aureus*, were still resistant to only two antibiotics. This situation remains concerning as it highlights the potential for future resistance development, warranting close monitoring and further investigation.

Staphylococcus aureus is a major cause of infections that have been acquired in hospitals and food poisoning. It has been found that they can survive and produce toxins in cooked food, leading to instances of food poisoning. Another concern is that virulence and antibiotic-resistance genes of *S. aureus* or *Staphylococcus* spp. can be transmitted through mobile genetic elements such as plasmids (McCarthy and Lindsay, 2012), prophages (McCarthy, Witney, & Lindsay, 2012), and Staphylococcal pathogenic islands (SaPIs) (Lindsay, 2019). This transmission can be done by a horizontal gene transfer process between strains. This is a cause for concern because horizontal gene transfer (HGT) could facilitate the spread of virulence genes between species, potentially leading to an epidemic.

Chapter 6

6. Conclusion

As the demand for processed meat products is rising, and around one-third of SFP outbreaks are triggered by meat and meat products, this experiment was carried out to observe the presence of *S. aureus* and *Staphylococcus* spp. in cooked meat (chicken curry). The result revealed that the presence of *Staphylococcus* spp. is higher than *S. aureus* in all the samples, and *S. aureus* was susceptible to most antibiotics.

Pathogenic microorganisms can grow on, adhere to, and form biofilms on food products, utensils, and processing environments; consequently, the presence of pathogenic microorganisms on food and equipment for the foodstuff industries elevates the food safety risk and it has become an important challenge in the public health care in the past several years. In recent surveys, it has been proven that the resistance of bacteria is a major threat to public health. Several reasons are responsible for the resistance pattern in bacteria. For instance, the wide application of antimicrobials in medical and veterinary shops, excessive use of antibiotics in agriculture, and common use of antiseptics and disinfectants have caused selective pressure on the bacteria. Moreover, HGT of the mobile genetic elements carrying antibiotic resistance genes is also the cause of MDR cases. So, the screening process for antibiotic resistance in bacteria is crucial. Though the current experiment showed that *S. aureus* is susceptible to most antibiotics, two antibiotics were resistant to the bacteria.

Foodborne diseases are a serious threat to public health and one of the main causes of morbidity and mortality. If cooked food is kept at room temperature for an ample amount of time, it can allow exponential growth of *S. aureus* and *Staphylococcus* spp., resulting in the production of enterotoxin. Thus, it can lead to SFP. The number of SFP cases is most likely underestimated as people do not seek medical services because of the short duration of the disease or mild symptoms. Also, the improper way of sample collection and laboratory examination may have caused the negligence. To avoid contamination, cooked food should be kept away from contact with extrinsic factors like insects, dust, cross-contamination, respiratory secretion, etc. and proper decontamination and disinfection measures should be taken into account. Additionally, new

technologies should be developed that can effectively inactivate the microorganisms. Lastly, regular inspection must be made compulsory by food specialists, microbiologists, and nutritionists.

Chapter 7

7. Limitations:

There have been many limitations in this experiment. One of them is the small number of sample size. This results in a higher risk of error and it also may overestimate the validity and reliability of the result. Moreover, the prevalence found in the sample may vary from time to time as the contamination rate and the probability of being contaminated depends on the restaurant environment and hygiene. Lastly, these bacteria were found in cooked meals. It generates the hypothesis that the food was contaminated after cooking in an unhygienic environment or that the strains were thermophilic and resistant to the spices that hold antimicrobial properties. Investigations were not done on this matter.

Chapter 8

8. Reference

- Sarker, N., Islam, S., Hasan, M., Kabir, F., Uddin, M. A., & Noor, R. (2013). Use of multiplex PCR assay for detection of diarrheagenic Escherichia coli in street vended food items. *Am J Life Sci*, *1*(6), 267-272.
- Akhtar, S., Sarker, M. R., & Hossain, A. (2014). Microbiological food safety: a dilemma of developing societies. *Critical reviews in microbiology*, *40*(4), 348-359.
- Happy, A. H., Bishwabidyalay, G., Bishwabidyalay, G., Bishwabidyalay, G., Bishwabidalay, G., Bishwabidyalay, G., ... & Bishwabidyalay, G. (2018). Isolation, identification and characterization of Gram negative bacteria from popular street food (chotpoti) at savar area, Dhaka, Bangladesh. *Open Access Library Journal*, *5*(11), 1.
- Paudyal, N., Anihouvi, V., Hounhouigan, J., Matsheka, M. I., Sekwati-Monang, B., Amoa-Awua, W., ... & Fang, W. (2017). Prevalence of foodborne pathogens in food from selected African countries–A meta-analysis. *International journal of food microbiology*, *249*, 35-43.
- Hoque, A., Khatun, A., Mohammad, A., Muhammad, A., Masood, T., Khan, A., & Faruquee, H. (2015). Microbiological hazard analysis and exposure assessment of street vended ready-to-eat foods in Dhaka City, Bangladesh. *American-Eurasian Journal of Agricultural and Environmental Sciences*, *15*(9), 1725-1731.
- Safarpoor Dehkordi, F., Gandomi, H., Basti, A. A., Misaghi, A., & Rahimi, E. (2017). Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. *Antimicrobial resistance & infection control*, *6*, 1-11.
- Shahraz, F., Dadkhah, H., Khaksar, R., Mahmoudzadeh, M., Hosseini, H., Kamran, M., & Bourke, P. (2012). Analysis of antibiotic resistance patterns and detection of mecA gene in Staphylococcus aureus isolated from packaged hamburger. *Meat science*, *90*(3), 759-763.
- Lakhanpal, P., Panda, A. K., Chahota, R., Choudhary, S., & Thakur, S. D. (2019). Incidence and antimicrobial susceptibility of Staphylococcus aureus isolated from ready-to-eat foods of animal origin from tourist destinations of North-western Himalayas, Himachal Pradesh, India. *Journal of food science and technology*, *56*, 1078-1083.

- Young, C. P., O'Donoghue, M. M., Ho, J., & Boost, M. V. (2014). High levels of Staphylococcus aureus contamination in Chinese-style roast pork. *Foodborne pathogens and disease*, *11*(7), 552-554.
- Hoveida, L., Halaji, M., Rostami, S., & Mobasherizadeh, S. (2019). Biofilm-producing ability of Staphylococcus spp isolated from different foodstuff products. *Annali Di Igiene Medicina Preventiva E Di Comunita*, *31*(2), 140-147.
- Madahi, H., Rostami, F., Rahimi, E., & Dehkordi, F. S. (2014). Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. *Jundishapur journal of microbiology*, *7*(8), e10237.
- Haghi, F., Zeighami, H., Hajiloo, Z., Torabi, N., & Derakhshan, S. (2021). High frequency of enterotoxin encoding genes of Staphylococcus aureus isolated from food and clinical samples. *Journal of Health, Population and Nutrition*, *40*(1), 27.
- Soltan-Dallal, M. M., Salehipour, Z., & Mehrabadi, J. F. (2010). Molecular epidemiology of Staphylococcus aureus in food samples based on the protein A gene polymorphic region DNA sequence. *Canadian Journal of Microbiology*, *56*(1), 18-21.
- Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., & Cocolin, L. (2005). Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science*, *69*(3), 381-392.
- Ranucci, D., Miraglia, D., Branciari, R., D'Ovidio, V., & Severini, M. (2004). Microbiological characteristics of hamburgers and raw pork sausages, and antibiotic-resistance of isolated bacteria. *Veterinary research communications*, *28*, 269-272.
- Heo, S., Lee, J. H., & Jeong, D. W. (2020). Food-derived coagulase-negative Staphylococcus as starter cultures for fermented foods. *Food science and biotechnology*, *29*, 1023-1035.
- Chung, H. Y., Kim, Y. T., Kwon, J. G., Im, H. H., Ko, D., Lee, J. H., & Choi, S. H. (2021). Molecular interaction between methicillin-resistant Staphylococcus aureus (MRSA) and chicken breast reveals enhancement of pathogenesis and toxicity for food-borne outbreak. *Food Microbiology*, *93*, 103602.
- Rhee, C. H., & Woo, G. J. (2010). Emergence and Characterization of Foodborne Methicillin-Resistant Staphylococcus aureusin Korea. *Journal of food protection*, *73*(12), 2285-2290.
- San Moon, J., Lee, A. R., Jaw, S. H., Kang, H. M., Joo, Y. S., Park, Y. H., ... & Koo, H. C. (2007). Comparison of antibiogram, staphylococcal enterotoxin productivity, and coagulase

genotypes among Staphylococcus aureus isolated from animal and vegetable sources in Korea. *Journal of food protection*, *70*(11), 2541-2548.

- Oh, S. K., Lee, N., Cho, Y. S., Shin, D. B., Choi, S. Y., & Koo, M. (2007). Occurrence of toxigenic Staphylococcus aureus in ready-to-eat food in Korea. *Journal of food protection*, *70*(5), 1153-1158.
- Krupa, P., Bystroń, J., Bania, J., Podkowik, M., Empel, J., & Mroczkowska, A. (2014). Genotypes and oxacillin resistance of Staphylococcus aureus from chicken and chicken meat in Poland. *Poultry Science*, *93*(12), 3179-3186.
- Podkowik, M., Bystroń, J., & Bania, J. (2012). Prevalence of antibiotic resistance genes in staphylococci isolated from ready-to-eat meat products. *Polish Journal of Veterinary Sciences*, (2).
- Rodríguez-Lázaro, D., Oniciuc, E. A., García, P. G., Gallego, D., Fernández-Natal, I., Dominguez-Gil, M., ... & Hernández, M. (2017). Detection and characterization of Staphylococcus aureus and methicillin-resistant S. aureus in foods confiscated in EU borders. *Frontiers in microbiology*, *8*, 1344.
- Gousia, P., Economou, V., Sakkas, H., Leveidiotou, S., & Papadopoulou, C. (2011). Antimicrobial resistance of major foodborne pathogens from major meat products. *Foodborne pathogens and disease*, *8*(1), 27-38.
- Khater-Dalia, F., Heikal, G. E., Shehata, A. A., & El-Hofy, F. I. (2013). The microbiological assessment of ready-to-eat-food (liver and kofta sandwiches) in Tanta City, Egypt. *Benha Vet. Med. J*, *25*(2), 187-197.
- Lindsay, J. A. (2019). Staphylococci: evolving genomes. *Microbiology Spectrum*, *7*(6), 10-1128.
- Freitas, J. K. G. R., Assis, C. F. D., Oliveira, T. R. M. D., Maia, C. M. D. M., de Sousa, B. J., Medeiros, G. C. B. S. D., ... & Chaves Damasceno, K. S. F. D. S. (2023). Prevalence of staphylococcal toxin in food contaminated by Staphylococcus spp.: Protocol for a systematic review with meta-analysis. *PLoS One*, *18*(2), e0282111.
- Argudín, M. Á., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and Staphylococcus aureus enterotoxins. *Toxins*, *2*(7), 1751-1773.

- Shahraz, F., Dadkhah, H., Khaksar, R., Mahmoudzadeh, M., Hosseini, H., Kamran, M., & Bourke, P. (2012). Analysis of antibiotic resistance patterns and detection of mecA gene in Staphylococcus aureus isolated from packaged hamburger. *Meat science*, *90*(3), 759-763.
- Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M., & Łaniewska-Trokenheim, Ł. (2014). Retail ready-to-eat food as a potential vehicle for Staphylococcus spp. harboring antibiotic resistance genes. *Journal of food protection*, *77*(6), 993-998.
- Mashouf, R. Y., Hosseini, S. M., Mousavi, S. M., & Arabestani, M. R. (2015). Prevalence of enterotoxin genes and antibacterial susceptibility pattern of Staphylococcus aureus strains isolated from animal originated foods in West of Iran. *Oman medical journal*, *30*(4), 283.
- Ho, J., O'Donoghue, M. M., & Boost, M. V. (2014). Occupational exposure to raw meat: a newly-recognized risk factor for Staphylococcus aureus nasal colonization amongst food handlers. *International Journal of Hygiene and Environmental Health*, *217*(2-3), 347-353.
- Odetokun, I. A., Adetona, M. A., Ade-Yusuf, R. O., Adewoye, A. O., Ahmed, A. N., Ghali-Mohammed, I., ... & Fetsch, A. (2023). S taphylococcus aureus contamination of animal-derived foods in Nigeria: a systematic review, 2002—2022. *Food Safety and Risk*, *10*(1), 6.
- Pesavento, G., Ducci, B., Comodo, N., & Nostro, A. L. (2007). Antimicrobial resistance profile of Staphylococcus aureus isolated from raw meat: A research for methicillin resistant Staphylococcus aureus (MRSA). *Food control*, *18*(3), 196-200.
- Archer, G. L. (1998). Staphylococcus aureus: a well-armed pathogen. *Reviews of Infectious Diseases*, *26*(5), 1179-1181.
- García-Lara, J., Masalha, M., & Foster, S. J. (2005). Staphylococcus aureus: the search for novel targets. *Drug Discovery Today*, *10*(9), 643-651.
- Sokari, T. G., & Anozie, S. O. (1990). Occurrence of enterotoxin producing strains of Staphylococcus aureus in meat and related samples from traditional markets in Nigeria. *Journal of food protection*, *53*(12), 1069-1071.
- Guven, K., Mutlu, M. B., Gulbandilar, A., & Cakir, P. (2010). Occurrence and characterization of Staphylococcus aureus isolated from meat and dairy products consumed in Turkey. *Journal of Food safety*, *30*(1), 196-212.
- Thomas, R., Anjaneyulu, A. S. R., & Kondaiah, N. (2008). Development of shelf stable pork sausages using hurdle technology and their quality at ambient temperature (37±1 C) storage. *Meat science*, *79*(1), 1-12.

- Dehkordi, M. K., Shamsabadi, M. G., & Banimehdi, P. (2019). The occurrence of Staphylococcus aureus, enterotoxigenic and methicillin-resistant strains in Iranian food resources: a systematic review and meta-analysis. *Annali di Igiene, Medicina Preventiva e di Comunita*, *31*(4).
- Fijałkowski, K., Peitler, D., & Karakulska, J. (2016). Staphylococci isolated from ready-to-eat meat–identification, antibiotic resistance and toxin gene profile. *International journal of food microbiology*, *238*, 113-120.
- Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M., & Łaniewska-Trokenheim, Ł. (2014). Retail ready-to-eat food as a potential vehicle for Staphylococcus spp. harboring antibiotic resistance genes. *Journal of food protection*, *77*(6), 993-998.
- Jofré, A., Garriga, M., & Aymerich, T. (2008). Inhibition of Salmonella sp. Listeria monocytogenes and Staphylococcus aureus in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat science*, *78*(1-2), 53-59.
- Argudín, M. Á., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and Staphylococcus aureus enterotoxins. *Toxins*, *2*(7), 1751-1773.
- Pepe, O., Blaiotta, G., Bucci, F., Anastasio, M., Aponte, M., & Villani, F. (2006). Staphylococcus aureus and staphylococcal enterotoxin A in breaded chicken products: detection and behavior during the cooking process. *Applied and environmental microbiology*, *72*(11), 7057-7062.
- Gallina, S., Bianchi, D. M., Bellio, A., Nogarol, C., Macori, G., Zaccaria, T., ... & Decastelli, L. (2013). Staphylococcal poisoning foodborne outbreak: Epidemiological investigation and strain genotyping. *Journal of food protection*, *76*(12), 2093-2098.
- Oh, S. K., Lee, N., Cho, Y. S., Shin, D. B., Choi, S. Y., & Koo, M. (2007). Occurrence of toxigenic Staphylococcus aureus in ready-to-eat food in Korea. *Journal of food protection*, *70*(5), 1153-1158.
- Rodríguez-Lázaro, D., Oniciuc, E. A., García, P. G., Gallego, D., Fernández-Natal, I., Dominguez-Gil, M., ... & Hernández, M. (2017). Detection and characterization of Staphylococcus aureus and methicillin-resistant S. aureus in foods confiscated in EU borders. *Frontiers in microbiology*, *8*, 1344.

- Yildirim, Y. E. L. İ. Z., Onmaz, N. E., Gonulalan, Z., Al, S. E. R. H. A. T., Yildirim, A., Karadal, F., ... & Pamuk, Ş. (2017). Microbiological quality of pastrami and associated surfaces at the point of sale in Kayseri, Turkey. *Public health*, *146*, 152-158.
- Ma, Y., Lan, G., Li, C., Cambaza, E. M., Liu, D., Ye, X., ... & Ding, T. (2019). Stress tolerance of Staphylococcus aureus with different antibiotic resistance profiles. *Microbial Pathogenesis*, *133*, 103549.
- Pexara, A., Bourriel, A., & Govaris, A. (2010). Staphylococcus aureus and Staphylococcal enterotoxins in foodborne diseases. *Journal of the Hellenic Veterinary Medical Society*, *61*(4), 316-322.
- Rolain, J. M., Canton, R., & Cornaglia, G. (2012). Emergence of antibiotic resistance: need for a new paradigm. *Clinical Microbiology and Infection*, *18*(7), 615-616.
- Hassan, M. M., El Zowalaty, M. E., Lundkvist, Å., Järhult, J. D., Nayem, M. R. K., Tanzin, A. Z., ... & Ashour, H. M. (2021). Residual antimicrobial agents in food originating from animals. *Trends in food science & technology*, *111*, 141-150.
- Lakhanpal, P., Panda, A. K., Chahota, R., Choudhary, S., & Thakur, S. D. (2019). Incidence and antimicrobial susceptibility of Staphylococcus aureus isolated from ready-to-eat foods of animal origin from tourist destinations of North-western Himalayas, Himachal Pradesh, India. *Journal of food science and technology*, *56*, 1078-1083.
- Klein, G. (1999). Food as a potential vector for antibiotic resistance. 1. Relevance of residues and selected foodborne infections and intoxicants. *Berliner und Munchener Tierarztliche Wochenschrift*, *112*(10-11), 365-369.
- Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N. C., Corrente, M., Parisi, A., ... & Celano, G. V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. *International journal of food microbiology*, *115*(3), 290-296.
- Selvanathan, E. A., Jayasinghe, M., Hossain, M. M., & Selvanathan, S. (2020). Modelling the demand for meat in Bangladesh. *Science and Technology Innovation for a Sustainable Economy*, 135-151.
- Goja, A. M., Ahmed, T. A. A., Saeed, S. A. M., & Dirar, H. A. (2013). Isolation and identification of Staphylococcus spp. in fresh beef. *Pakistan journal of Nutrition*, *12*(2), 114.

- Organji, S. R., Abulreesh, H. H., Elbanna, K., Osman, G. E., & Almalki, M. H. (2018). Diversity and characterization of Staphylococcus spp. in food and dairy products: a foodstuff safety assessment. *The Journal of Microbiology, Biotechnology and Food Sciences*, *7*(6), 586.
- Igbinosa, E. O., Beshiru, A., Akporehe, L. U., Oviasogie, F. E., & Igbinosa, O. O. (2016). Prevalence of methicillin-resistant Staphylococcus aureus and other Staphylococcus species in raw meat samples intended for human consumption in Benin City, Nigeria: implications for public health. *International journal of environmental research and public health*, *13*(10), 949.
- Happy, A. H., Bishwabidyalay, G., Bishwabidyalay, G., Bishwabidyalay, G., Bishwabidalay, G., Bishwabidyalay, G., ... & Bishwabidyalay, G. (2018). Isolation, identification and characterization of Gram negative bacteria from popular street food (chotpoti) at savar area, Dhaka, Bangladesh. *Open Access Library Journal*, *5*(11), 1.
- Sarker, Y. A., Hasan, M. M., Paul, T. K., Rashid, S. Z., Alam, M. N., & Sikder, M. H. (2018). Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. *Journal of Advanced Veterinary and Animal Research*, *5*(2), 140-145.
- Popelka, P., Nagy, J., Germuška, R., Marcinčák, S., Jevinova, P., & De Rijk, A. (2005). Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. *Food additives and contaminants*, *22*(6), 557-562.
- Barman, A. K. A., Hossain, M. M., Rahim, M. M., Hassan, M. T., & Begum, M. (2018). Oxytetracycline residue in Tilapia. *Bangladesh Journal of Scientific and Industrial Research*, *53*(1), 41-46.
- Sarker, N., Islam, S., Hasan, M., Kabir, F., Uddin, M. A., & Noor, R. (2013). Use of multiplex PCR assay for detection of diarrheagenic Escherichia coli in street vended food items. *Am J Life Sci*, *1*(6), 267-272.
- Hoque, A., Khatun, A., Mohammad, A., Muhammad, A., Masood, T., Khan, A., & Faruquee, H. (2015). Microbiological hazard analysis and exposure assessment of street vended ready-to-eat foods in Dhaka City, Bangladesh. *American-Eurasian Journal of Agricultural and Environmental Sciences*, *15*(9), 1725-1731.
- Adjrah, Y., Soncy, K., Anani, K., Blewussi, K., Karou, D. S., Ameyapoh, Y., ... & Gbeassor, M. (2013). Socio-economic profile of street food vendors and microbiological quality of ready-to-eat salads in Lomé. *International Food Research Journal*, *20*(1).

- Aneja, K. R. (2007). *Experiments in microbiology, plant pathology and biotechnology*. New Age International.
- Haghi, F., Zeighami, H., Hajiloo, Z., Torabi, N., & Derakhshan, S. (2021). High frequency of enterotoxin encoding genes of Staphylococcus aureus isolated from food and clinical samples. *Journal of Health, Population and Nutrition*, *40*(1), 27.
- Canizalez-Roman, A., Gonzalez-Nuñez, E., Vidal, J. E., Flores-Villaseñor, H., & León-Sicairos, N. (2013). Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. *International journal of food microbiology*, *164*(1), 36-45.
- Morot-Bizot, S. C., Talon, R., & Leroy, S. (2004). Development of a multiplex PCR for the identification of Staphylococcus genus and four staphylococcal species isolated from food. *Journal of Applied Microbiology*, *97*(5), 1087-1094.
- Wayne, P. A. (2015). Clinical and laboratory standards institute (CLSI). *Performance standards for antimicrobial susceptibility testing*.
- Ranucci, D., Miraglia, D., Branciari, R., D'Ovidio, V., & Severini, M. (2004). Microbiological characteristics of hamburgers and raw pork sausages, and antibiotic-resistance of isolated bacteria. *Veterinary research communications*, *28*, 269-272.
- Gousia, P., Economou, V., Sakkas, H., Leveidiotou, S., & Papadopoulou, C. (2011). Antimicrobial resistance of major foodborne pathogens from major meat products. *Foodborne pathogens and disease*, *8*(1), 27-38.
- Banik, A., Abony, M., Datta, S., & Towhid, S. T. (2018). Microbial status and multidrug resistance pattern of pathogenic bacteria isolated from street food in Dhaka city, Bangladesh. *Journal of Advances in Microbiology*, *13*(1), 1-13.
- Morandi, S., Brasca, M., Lodi, R., Cremonesi, P., & Castiglioni, B. (2007). Detection of classical enterotoxins and identification of enterotoxin genes in Staphylococcus aureus from milk and dairy products. *Veterinary microbiology*, *124*(1-2), 66-72.
- Batista, J. E. C., Ferreira, E. L., Nascimento, D. C. D. O., Ventura, R. F., de Oliveira, W. L. M., Leal, N. C., & Lima-Filho, J. V. (2013). Antimicrobial resistance and detection of the mec A gene besides enterotoxin-encoding genes among coagulase-negative Staphylococci isolated from clam meat of Anomalocardia brasiliana. *Foodborne pathogens and disease*, *10*(12), 1044-1049.

- Chajęcka-Wierzchowska, W., Zadernowska, A., Nalepa, B., Sierpińska, M., & Łaniewska-Trokenheim, Ł. (2015). Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin–phenotypic and genotypic antibiotic resistance. *Food microbiology*, *46*, 222-226.
- Fowoyo, P. T., & Ogunbanwo, S. T. (2016). Virulence and toxigenicity of coagulase-negative staphylococci in Nigerian traditional fermented foods. *Canadian journal of microbiology*, *62*(7), 572-578.
- Podkowik, M., Seo, K. S., Schubert, J., Tolo, I., Robinson, D. A., Bania, J., & Bystroń, J. (2016). Genotype and enterotoxigenicity of Staphylococcus epidermidis isolate from ready to eat meat products. *International journal of food microbiology*, *229*, 52-59.
- Vasconcelos, N. G., Pereira, V. C., Araújo Júnior, J. P., & da Cunha, M. D. L. (2011). Molecular detection of enterotoxins E, G, H and I in Staphylococcus aureus and coagulase-negative staphylococci isolated from clinical samples of newborns in Brazil. *Journal of Applied Microbiology*, *111*(3), 749-762.
- Zell, C., Resch, M., Rosenstein, R., Albrecht, T., Hertel, C., & Götz, F. (2008). Characterization of toxin production of coagulase-negative staphylococci isolated from food and starter cultures. *International journal of food microbiology*, *127*(3), 246-251.
- Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed research international*, *2014*(1), 827965.
- Kruse, H., & Sørum, H. (1994). Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied and environmental microbiology*, *60*(11), 4015-4021.
- Hennekinne, J. A., De Buyser, M. L., & Dragacci, S. (2012). Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. *FEMS microbiology reviews*, *36*(4), 815-836.
- McCarthy, A. J., & Lindsay, J. A. (2012). The distribution of plasmids that carry virulence and resistance genes in Staphylococcus aureus is lineage associated. *BMC microbiology*, *12*, 1-8.
- McCarthy, A. J., Witney, A. A., & Lindsay, J. A. (2012). Staphylococcus aureus temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. *Frontiers in cellular and infection microbiology*, *2*, 6.

- Lindsay, J. A. (2019). Staphylococci: evolving genomes. *Microbiology Spectrum*, *7*(6), 10-1128.
- Cieszyński, P. (2012). Occurrence and antibiotic resistance of enterococci in ready-to-eat food of animal origin. *African Journal of Microbiology Research*, *6*(39), 6773-6780.
- Guran, H. S., & Kahya, S. (2015). Species Diversity and Pheno-and Genotypic Antibiotic Resistance Patterns of Staphylococci Isolated from Retail Ground Meats. *Journal of food science*, *80*(6), M1291-M1298.
- Abdelfatah, M. M., Salem, A., & Shawky, N. (2019). Assessment of some food poisoning bacteria in ready-to-eat meals. *Benha Veterinary Medical Journal*, *37*(1), 46-52.
- Ferdous, J., Bradshaw, A., Islam, S. A., Zamil, S., Islam, A., Ahad, A., ... & Hoque, M. A. (2019). Antimicrobial residues in chicken and fish, Chittagong, Bangladesh. *EcoHealth*, *16*, 429-440.
- G.M. Wiseman, The hemolysins of Staphylococcus aureus, Bacteriol. Rev. 39 (1975) 317e344.
- Wang, L. J., Yang, X., Qian, S. Y., Liu, Y. C., Yao, K. H., Dong, F., & Song, W. Q. (2020). Identification of hemolytic activity and hemolytic genes of Methicillin-resistant Staphylococcus aureus isolated from Chinese children. *Chinese medical journal*, *133*(01), 88-90.