Bacteriological Assessment of Common Fly (*Musca domestica)* As a Potential Carrier for Public Health Epidemics

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of B.Sc. in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing my 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 complete and accurate referencing.
3. The thesis does not contain material accepted or submitted for any other degree or diploma at a university or other institution.
4. I have acknowledged all primary sources of help.
5. I request the embargo of our thesis for 24M from the submission date, as I intend to submit our findings to a peer-reviewed journal for publication.

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Approval

The thesis titled “Can Bacteria Fly? -Bacteriological Assessment of Common Fly (*Musca domestica*) As a Potential Carrier for Public Health Epidemics”

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Ethics Statement

This research is done under proper supervision and is the author's original work. No animal was harmed during experiments. The article is written in such a manner that the write-up does not contain material previously published or written by a third party, except where this is appropriately cited through complete and accurate referencing. The study was conducted following the Declaration of Helsinki, and ethical permission was obtained from the Ethical Review Board of BRAC University, and Marks Dental College and Hospital. After explaining the study to the participants, their verbal consent was obtained before the sample collection and Data Collection. The experiment was done by maintaining all the rules and regulations of the Biotechnology and Microbiology Laboratory, Department of Mathematics and Natural Sciences at BRAC University.

Abstract:

House fly (*Musca domestica*) plays a pivotal role in the transmission of various antimicrobial-resistant pathogens, posing a huge risk to public health. Both global and national research has shown that *Musca domestica* is an important vector of pathogens that is directly and indirectly responsible for the rapid increase of multidrug-resistant pathogens. The purpose of this study was to identify and analyze the types of bacteria commonly associated with houseflies, with a specific focus on enteric pathogens such as *Escherichia coli, Salmonella spp., Shigella spp*., and others. Along with that, this study has also seen the resistance pattern of these bacteria. The study was conducted primarily divided into two seasons (October-February) and (March-July) in 3 different settings (Residential area, Fish Markets, and Street food) among 7 major areas (Ramna, Shahbag, Khilgaon, Paltan, Motijheel, Jatrabari, and Dhanmondi) of Dhaka city. From these 7 different areas, 53 samples of house flies were obtained, and from those 53 specimens, around 159 isolates of different bacteria were found. From this study, the highest number of bacterial isolates was found to be of *Vibrio spp*. (44.65%) and *Klebsiella pneumoniae* (23.27%). Again lowest isolates found were Salmonella *spp*. (1.26%) and *Pseudomonas aeruginosa* (5.66%). Among the isolates, *Escherichia coli* showed resistance to cefixime, amixoclav and azithromycin at an alarming rate 83%, 67%, and 83% respectively. *Pseudomonas aeruginosa* showed 100% resistance to amixoclav and cefixime, but *Klebsiella pneumoniae* raised an alarm as it is 83% resistant to cefixime. *Vibrio spp* and *Shigella spp*. Showed moderate mixed results.

Keywords:

House fly, *Musca domestica*, Antibiotic resistance, Multidrug resistance, residential area, fish market, Street food, Dhaka city,

Dedication

To our supervisor, Akash Ahmed, who lit up the path beyond shadows, urging us to find meaning and pursue it with heart.

As জীবনানন্দ দাশ said,

“যে জীবন ফড়িংয়ের দোয়েলের –

মানুষের সাথে তার হয় নাকো দেখা”—

you led us closer to that unseen life.

Thank you!

Acknowledgment

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Finally, we are thankful to all the researchers, academics, and writers whose work we have cited in this thesis. Their contributions have shaped our thinking and provided the foundation for this study.

We remain deeply grateful for all the support, encouragement, and guidance we have received and feel truly privileged to have had the opportunity to carry out this research project.

— Rokaiya Raisa (21226007)

— Mizanur Rahman (20326018)

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Abbreviation

PBS - Phosphate buffered saline

NA - Nutrient Agar

MHA - Muller Hinton Agar

MSA - Mannitol Salt Agar

TCBS - Thiosulfate Citrate Bile Salts Sucrose

XLD - Xylose Lysine Deoxycholate

PCR - Polymerase chain reaction

NFW - Nuclease-free water

EtBr - Ethidium Bromide

TAE - Tris-acetic acid

AST - Antibiotic Susceptibility Test

DNA - Deoxyribonucleic acid

bps - base pairs

K. pneumoniae - *Klebsiella pneumoniae*

E. coli - *Escherichia coli*

S. aureus - *Staphylococcus aureus*

P. aeruginosa - *Pseudomonas aeruginosa*

AMR - Anti Microbial Resistance

MDR - Multi Drug Resistance

CFU - Colony forming unit

Fig - Figure

µL - Microliter

ml - Milliliter

Chapter 1:

Introduction

1.1 Background:

Musca domestica commonly known as housefly is one of the most common and important arthropod vectors for causing disease in the world. The Editors of Encyclopaedia Britannica, (2025) mentioned that it is a tiny insect about 6-7 mm long with a gray body, four dark stripes on the thorax, and huge red compound eyes. It thrives in warm, moist, and unsanitary environments, making it common in homes, markets, street food areas, garbage dumps, fish markets etc. (Encyclopaedia Britannica, 2025) These synanthropic insects have become mechanical carriers of many infections and pose serious health risks to the common people especially in developing countries with underdeveloped sanitation systems (Khalil et al., 1994).

Because of their close association with humans, *Musca domestica* flies play an important role in pathogen transmission cycles. *Musca domestica's* behavioral and ecological characteristics make it an effective vector for bacterial pathogens. Research by Khamesipour et al. (2018) stated that flies spend a large portion of their life cycle in organic matter rich settings like human and animal waste, where they acquire pathogenic microbes by direct contact and eating. This is where they accumulate bacteria such as *E. coli, Salmonella,* and *Shigella* on their bodies and legs. These microbes can be transferred by flies to surfaces and food, posing significant health risks when ingested by humans. To tackle this public health challenge effectively, it is essential to understand the complex interactions between houseflies and pathogens. In recent years, more people are getting sick with diarrhea, and part of that can be linked to this kind of fly-borne transmission (Issa, 2019). These flies carry pathogens both on their bodies and inside their guts, making them potential carriers of harmful and even antibiotic-resistant bacteria. In a study it showed that approximately 700,000 people worldwide lose their lives each year to drug-resistant infections (Fongang et al., 2023). A recent study conducted by Singh et al. (2025) in north India revealed that

bacteria carried by houseflies are already significantly resistant to key antibiotics. Their study showed > 70% of isolates of *E. coli* resistant to ampicillin and > 50% resistant to tetracycline. Another study in Bangladesh discovered that in the antibiotic resistance patterns of *S. aureus, Salmonella spp.,* and *E. coli*, *S. aureus* showed 100% resistance to penicillin, with high resistance to amoxicillin (94%), streptomycin (94%), erythromycin (93%), and tetracycline (84%). However, most isolates remained sensitive to chloramphenicol (83%), ceftriaxone (80%), ciprofloxacin (78%), and gentamycin (70%) (Akter et al., 2020). *Musca domestica* is also a potential vector for *Vibrio cholerae* and *V. cholerae* non-O1 (Fotedar, 2001). It imposes a serious concern as Paul et al. (2016) reported 589,854 cholera cases and 7,816 deaths across 58 countries in 2011. Moreover, *Klebsiella pneumoniae* can also be transmitted by houseflies, and alarmingly, this pathogen carries more than 100 different AMR-required genes (Moya & Maicas, 2020).

1.2 State of Bacterial Study of Housefly

Recent scientific studies have shown that houseflies serve as reservoirs for a wide range of clinically significant bacterial infections. Housefly (*Musca domestica)* is an ideal vector for the spread of susceptible and resistant bacterial strains that may risk public health. Housefly-associated bacterial communities are varied, comprising both commensal and pathogenic species.

Contemporary studies have proven that houseflies can carry bacteria throughout their lifetime. Housefly is an essential host for a wide range of bacteria, including pathogenic and antibiotic-resistant strains. They carry various microorganisms, including *E. coli, Salmonella,* and *Shigella*, on their body surfaces and in their gut, spreading them across farms, markets, hospitals, and households. Because of this, serious diseases like diarrhea, food poisoning, and dysentery are becoming common and hard to treat. This data implies that the potential for bacterial acquisition

and transmission varies with the fly's life stage and environmental exposure (Bahrndorff et al., 2017).

Moreover, Many of these bacteria have been identified to harbor antibiotic resistance genes. The problem extends when houseflies carry non-pathogenic bacteria into an environment where pathogenic bacteria are present, such as a fish market or street food, horizontal gene transfer can occur. A review by Liu et al. (2021) mentioned that through processes like conjugation, transformation, or transduction, these commensal bacteria can acquire AMR genes or virulence factors from the surrounding microbial community. Once these genes are transferred, the bacteria can become opportunistic pathogens themselves or act as mobilizing reservoirs of AMR, facilitating the transfer of resistance genes into more harmful, pathogenic species (Liu et al., 2021). These transfers of genes among non-pathogens and pathogens can accelerate the evolution and spread of multidrug-resistant organisms. Thus, houseflies are not only passive carriers of bacteria, but they can actively promote the emergence and dissemination of resistant pathogens, posing a complex and growing public health risk.

1.3 Literature Review

Global Perspective

International study has repeatedly shown that *Musca domestica* plays an important function as mechanical vectors for bacterial infections in a variety of geographical settings. The global distribution and medical significance of housefly associated pathogens have been well documented in systematic studies covering multiple continents and environmental settings.

Khamesipour et al. (2018) made the case that "the synanthropic house fly, *Musca domestica* is a mechanical vector of pathogens (bacteria, fungi, viruses, and parasites), some of which cause

serious diseases in humans and domestic animals". This diverse group of harmful organisms demonstrates the broad spectrum significance of houseflies in disease transmission.

Recent extensive analyses have shown the global increase in housefly associated diseases. With the exception of a single specimen isolation, *Salmonella* prevalence in flies in various agricultural contexts ranged from 2.2 to 15.2%. Such variance in pathogen prevalence shows that environmental conditions, seasonal patterns, and local sanitation methods all have major effects on housefly vector potential. In 2017, a study at Hamadan University of Medical Sciences discovered that house flies contain a large amount of harmful microorganisms. According to the study, 394 bacterial isolates contained 22.9% *Staphylococcus aureus* and 11.6% *Escherichia coli* (Nazari, 2017).

Bangladesh Perspective

In Bangladesh, research into fly associated bacterial pathogens is still limited. Research conducted here has provided much evidence for the role of *Musca domestica* in bacterial pathogen transmission, particularly in rural and urban settings where sanitation challenges are higher. A fish-market investigation found *Salmonella* spp. in 56.7% of houseflies, with 91.2% of isolates being multidrug resistant and containing *tetA*, *tetB*, and *qnrA* genes (Sobur et al., 2019).

Another study in Dinajpur found *E. coli, Salmonella typhimurium*, and *Pseudomonas spp*. in local flies. The majority of the isolates were resistant to erythromycin, gentamicin, and bacitracin, but mostly susceptible to ciprofloxacin and azithromycin (Zuhora et al., 2023). Akter et al. published an article in 2020 that discovered the presence of *Escherichia coli, Staphylococcus aureus*, and *Salmonella* spp. in houseflies in Mymensingh, Bangladesh. They also found antibiotic-resistant microorganisms in that area. One study conducted in Dhaka revealed resistance patterns among bacterial isolates identified from houseflies. While ciprofloxacin showed the highest sensitivity

(81.08%), and tetracycline showed moderate sensitivity (73.65%), several antibiotics displayed high resistance rates against their isolated organisms from houseflies. Erythromycin (26.35%) and amoxiclav (64.19%) showed reduced effectiveness, and amoxicillin and cefixime demonstrated a high level of resistance against all isolates. (Tabassum & Chowdhury, 2023).

1.4 Knowledge Gap

Despite global evidence that houseflies can transmit various harmful and antibiotic-resistant bacteria, data from urban areas in Bangladesh like Dhaka, Chittagong, or other big cities still remain scarce. Existing studies have mostly focused on rural settings or examined only a limited range of bacterial species, leaving the broader pathogen diversity underexplored.

The influence of environmental factors and sanitation practices on the types and spread of flyborne pathogens in urban Bangladesh has not been fully characterized. Additionally, while clinical reports highlight rising antibiotic resistance, little is known about the role of houseflies in carrying and spreading these resistant strains in the environment.

There is also a lack of integrated studies that combine molecular identification with antibiotic resistance profiling in housefly-associated bacteria. Pathogens beyond commonly studied ones like *Salmonella*, *Shigella, Klebsiella*, and *Pseudomonas* have received limited attention. Seasonal trends, environmental effects, and the effectiveness of antibiotics remain largely uninvestigated. These gaps highlight the need for more focused and comprehensive research.

1.5 Objective

This research aims to investigate the role of houseflies (*Musca domestica)* as carriers of bacterial pathogens in urban areas of Dhaka, Bangladesh. The primary objective is to identify and analyze the types of bacteria commonly associated with houseflies, with a specific focus on enteric pathogens such as *Escherichia coli, Salmonella spp., Shigella spp.*, and others. The study will assess the prevalence and seasonal trends of these bacteria across different environments, including street food areas, residential zones, and fish markets.

Another key focus is to evaluate the potential for these flies to act as vectors in the transmission of antibiotic-resistant bacteria. Understanding the patterns of antibiotic resistance among housefly-associated pathogens is essential for assessing public health risks, especially in densely populated urban settings where sanitation and waste management are often inadequate.

Additionally, this research will help fill critical knowledge gaps by combining bacterial identification with antibiotic susceptibility profiling. The findings aim to inform effective public health interventions and support the development of targeted strategies to minimize the risk of housefly-mediated bacterial disease transmission in Bangladesh and similar environments

Chapter 2:

Methods and Materials

2.1 Study design flowchart and setting

This study was conducted at the Microbiology Laboratory of BRAC University. All necessary materials required for the sample collection were sterilised and obtained from the laboratory, including icebox, zip lock bags, polythene bags, fly catcher, gloves, and scissors.

Sample was collected from 7 major areas of Dhaka city: Ramna, Shahbag, Khilgaon, Paltan, Motijheel, Jatrabari, and Dhanmondi. From each area, houseflies (*Musca domestica*) were collected from three distinct environments: Residential areas, street food stalls, and Fish markets The sample collection process was divided into two parts covering two major seasons in Bangladesh. Summer and Winter. The first part was performed between October and February marked as the winter season where temperature in Dhaka was low, ranging between 10°C-21°C. The second part of sample collection was done when temperature was as high as ≥ 31°C, from March to July officially known as summer season.

Captured houseflies were transported to the laboratory using iceboxes to maintain sample integrity. Upon arrival, flies were aseptically transferred into falcon tubes containing Phosphate Buffer Saline (PBS) solution for initial processing. Samples were inoculated onto various selective agar and differential media using the spread plate technique.

After 24 hours of incubation, distinct colonies were selected based on colony morphology and phenotypic characteristics and were subcultured on nutrient agar for further identification. These subcultures were then used for DNA extraction and simultaneously stored in soft agar for preservation. Following DNA extraction, biochemical tests and polymerase chain reaction (PCR) were performed for confirmation of bacterial species. Confirmed isolates were subjected to antibiotic susceptibility testing (AST) to give clear results on resistance patterns.

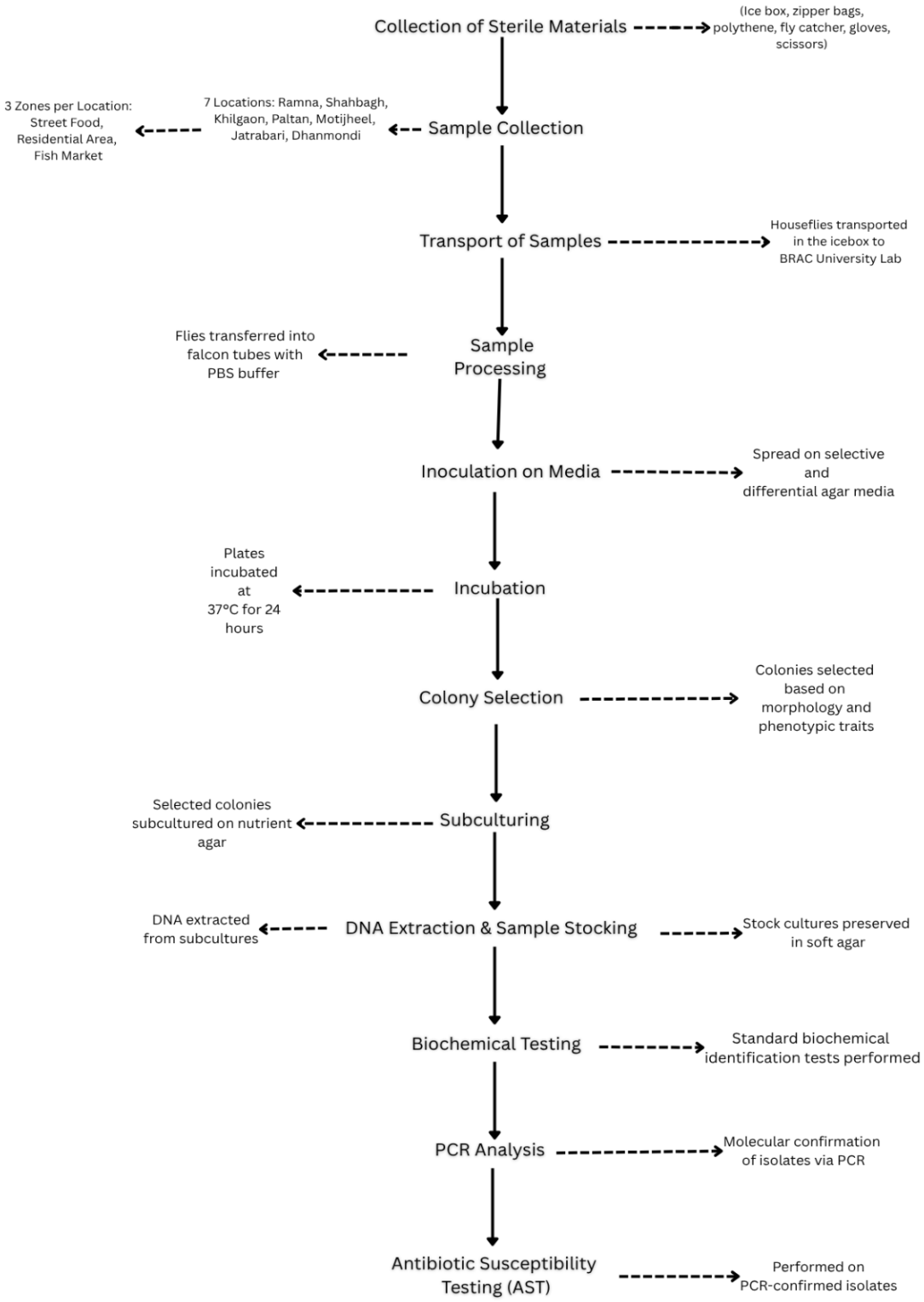

Figure 1: Flowchart Overview of Study Setting and Methodology

2.2 Sample Collection

Using a homemade fly catcher, the flies were captured using a polyethylene bag mounted like a net. Then, flies were caught by entrapping them in the polyethylene bag and transferred into a zipper bag aseptically by cutting the polyethylene bag into small chunks using scissors. The chunk of polyethylene containing the files was then moved into a zipper bag, while the polyethylene chunk was properly discarded. After that, the zipper bag containing entrapped flies was transferred into an icebox, where 4°C was maintained using ice packs. Then, the icebox was transferred to BRAC University within 1 hour, where further processing of the flies was done.

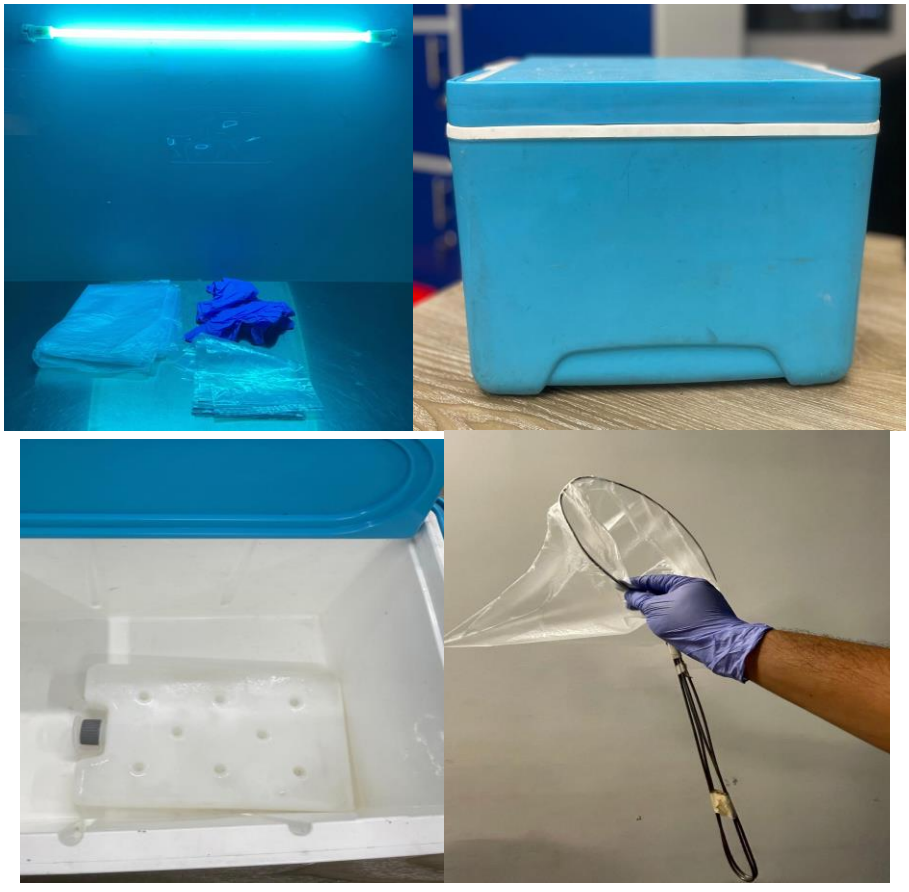

Figure 2: Sterilized Materials for sample collection

2.3 Sample Processing

Upon arriving at BRAC University Microbiology Laboratory, the alive, uncontaminated, and unimpaired housefly was transferred into a 15 ml Falcon, containing 10 ml phosphate-buffered saline (PBS). The PBS buffer was prepared using room temperature and pH 7.4 was maintained. The flies were transferred into the falcon in aseptic conditions under the laminar flow by using heat-sterilized forceps. Then the flacon containing fly submerged in the PBS buffer was vortexed for more than 5 minutes for a homogenized mixture. Subsequently, 80µl of the buffer solution was transferred using a micropipette into selective media for isolation of the desired bacteria.

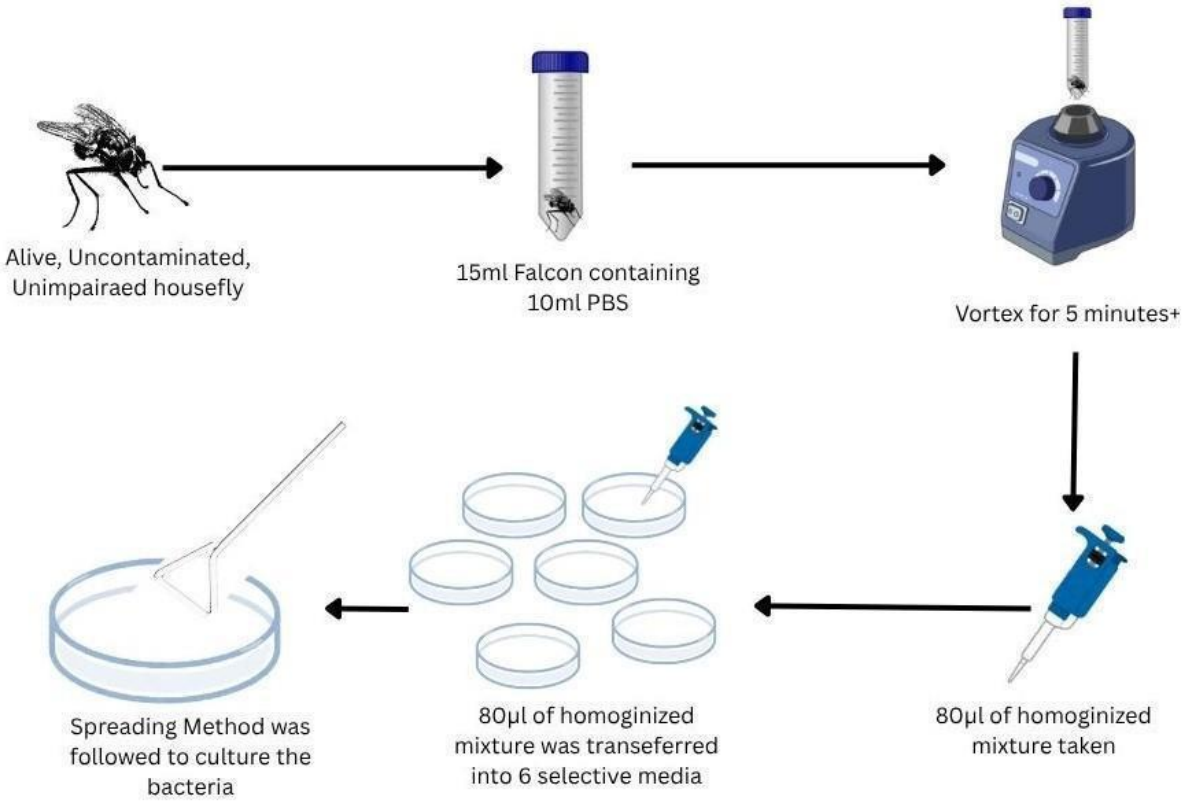

Figure 3: Flow of Sample Processing

2.4 Culture Media Used for Bacterial Isolation

To isolate a diverse range of bacterial species from the collected housefly stored in PBS solution, it was cultured in a variety of selective and differential culture media. These media were carefully chosen based on their ability to selectively promote the growth of specific bacterial groups while inhibiting unwanted microorganisms (Raymond, 2022). This approach helped to target both gram-positive and gram-negative bacteria commonly associated with environmental contamination.

MacConkey Agar

MacConkey agar was used to isolate gram-negative, lactose-fermenting, and non-fermenting enteric bacteria. This media contains bile salts and crystal violet which inhibit the growth of most gram-positive bacteria (Raymond, 2022). It also contains lactose as a fermentable carbohydrate and neutral red which works as a pH indicator. Lactose fermenters such as *Escherichia coli* or *Klebsiella pneumoniae* produce pink, dark pink, or red colonies due to acid production. For detecting enterobacteria that can potentially be transmitted by houseflies, MacConkey agar was essential

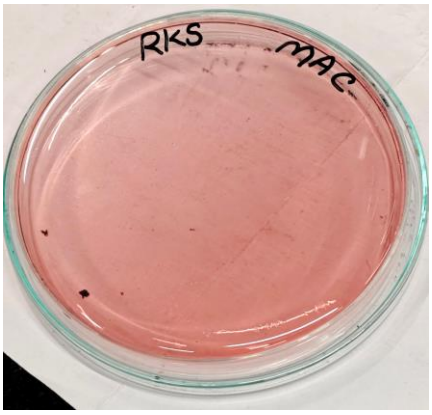

Figure 4: Fresh MacConkey agar plate

Cetrimide Agar

To specifically detect *Pseudomonas aeruginosa,* Cetrimide agar was used. This agar acts as a selective agent that inhibits the growth of most other bacteria but allows the growth of *P. aeruginosa* (Aryal, 2023). Isolating *P. aeruginosa* is important as it is a common opportunistic pathogen. Its colony appears blue-green and may fluoresce under UV light as the cetrimide media enhances the production of pyocyanin and pyoverdine, pigments characteristic of this strain, making the isolation easier (Aryal, 2023).

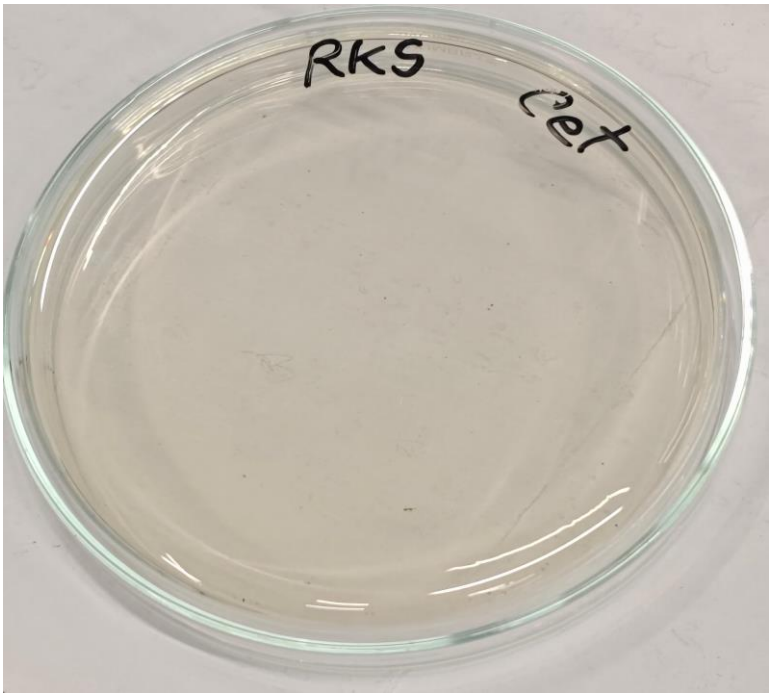

Figure 5: Fresh Cetrimide agar Plate

Mannitol Salt Agar (MSA)

To target gram-positive cocci, specifically *Staphylococcus aureus,* Mannitol Salt Agar (MSA) was utilized. This medium contains 7.5% sodium chloride, which inhibits the growth of most bacteria except salt-tolerant staphylococci species (Raymond, 2022). It also includes mannitol and phenol red as the fermentable sugar and pH indicator. *Staphylococcus aureus* ferments mannitol, leading to a yellow color change in the medium due to acid production. The inclusion of MSA enabled differentiation between pathogenic and non-pathogenic *staphylococci*.

Figure 6: Fresh Mannitol Salt Agar Plate

Thiosulfate Citrate Bile Salts Sucrose (TCBS)

Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar was used for the isolation of *Vibrio* species. This highly selective medium has a high pH of around 8.6 that suppresses the growth of most intestinal flora (Aryal, 2022). The presence of bile salts and citrate further inhibits non-*Vibrio* organisms. TCBS contains sucrose as a fermentable sugar, along with bromothymol blue and thymol blue as pH indicators. *Vibrio cholerae,* which ferments sucrose, produces large yellow colonies, whereas *Vibrio parahaemolyticus*, a non-sucrose fermenter, forms green to blue-green colonies. The use of TCBS was essential in detecting any *Vibrio* spp. possibly carried by houseflies from contaminated environments.

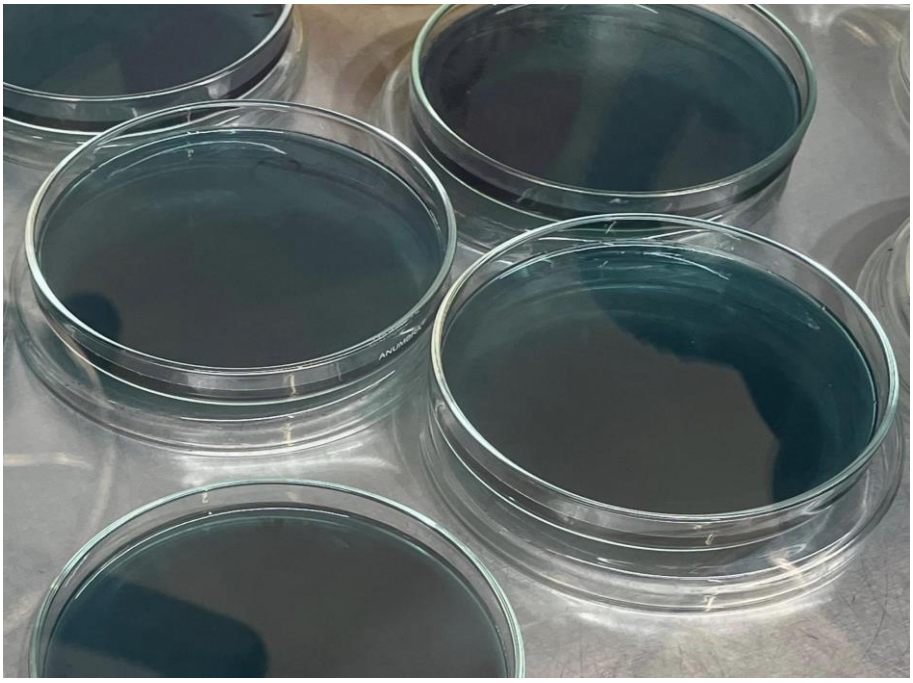

Figure 7: Fresh TCBS Agar Plate

Xylose Lysine Deoxycholate (XLD)

Finally, Xylose Lysine Deoxycholate (XLD) agar was used for isolating *Salmonella* and *Shigella* species. This medium selectively inhibits gram-positive organisms using sodium deoxycholate (Aryal, 2022b). It differentiates bacteria based on their ability to ferment sugars such as xylose, lactose, and sucrose, decarboxylate lysine, and produce hydrogen sulfide (H_2S). *Salmonella* typically appears as red colonies with black centers due to H_2S production, while *Shigella* appears as red or pink colonies without blackening. Other fermenting bacteria produce yellow colonies due to acid formation. XLD was especially important for detecting intestinal pathogens associated with food and water contamination.

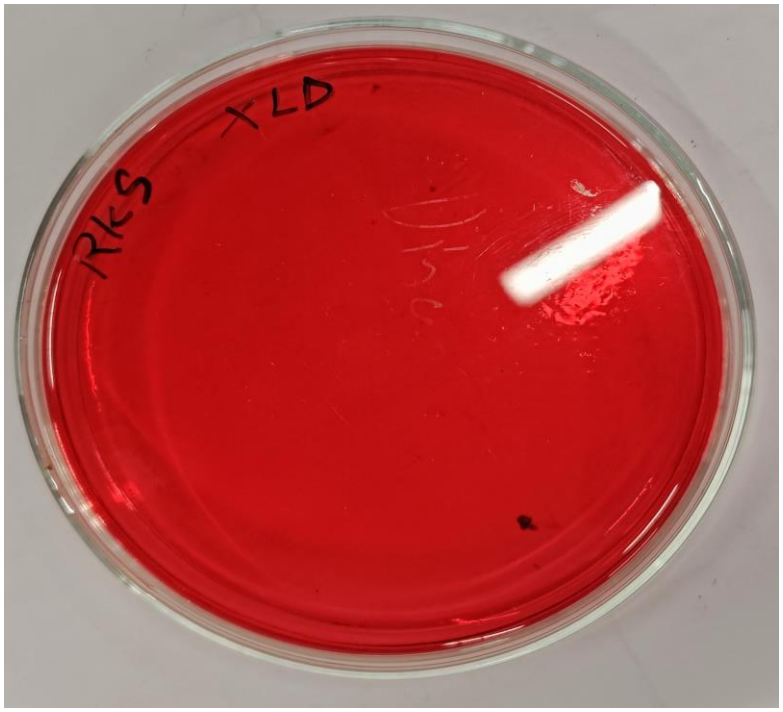

Figure 8: Fresh XLD Agar Plate

By plating on these five different selective and differential media, random, inclusive bacterial colonies were selected based on colony morphology, which were then subjected to further detection. These media were chosen strategically to cover major pathogenic groups likely to be transmitted by flies in urban environments, laying the foundation for further confirmation through biochemical and molecular analyses.

On these various selective and differential agar media, 80µL of sample was spread using a sterile glass spreader. To avoid any type of contamination, all inoculations were carried out aseptically inside a disinfected laminar flow cabinet. The spread plates were then kept in incubation for 24 hours at 37 °C. After incubation, colony morphology, such as color, shape, and size, was used for presumptive identification of bacterial types. Colonies with distinct appearances were selected for further analysis.

Each selected colony was streaked onto Nutrient Agar (NA) plates to isolate pure cultures. These purified colonies were then preserved and later used for biochemical and molecular tests to confirm the identity of the isolates.

2.5 Additional Media use

Nutrient Agar (NA)

Nutrient agar is a nutrient medium most effectively used for the cultivation of microorganisms, supporting the growth of a wide range of nonfastidious organisms (Sandle, 2019). This medium is one of the most accepted media for the growth of diverse non-fastidious bacteria. The preparation of these media was done by measuring 28 grams of nutrient agar powder, which was then suspended in 1 liter of distilled water in a clinical flask. Furthermore, the agar was properly dissolved in the distilled water by using a series of boiling and stirring using a glass rod. Subsequently, the clinical flask was covered with an ammonium foil and placed in an autoclave for sterilization. Afterwards, the lukewarm liquid media was carefully transferred onto a petri dish and stored in a fresh media refrigerator, and used after the agar base was solidified for streaking.

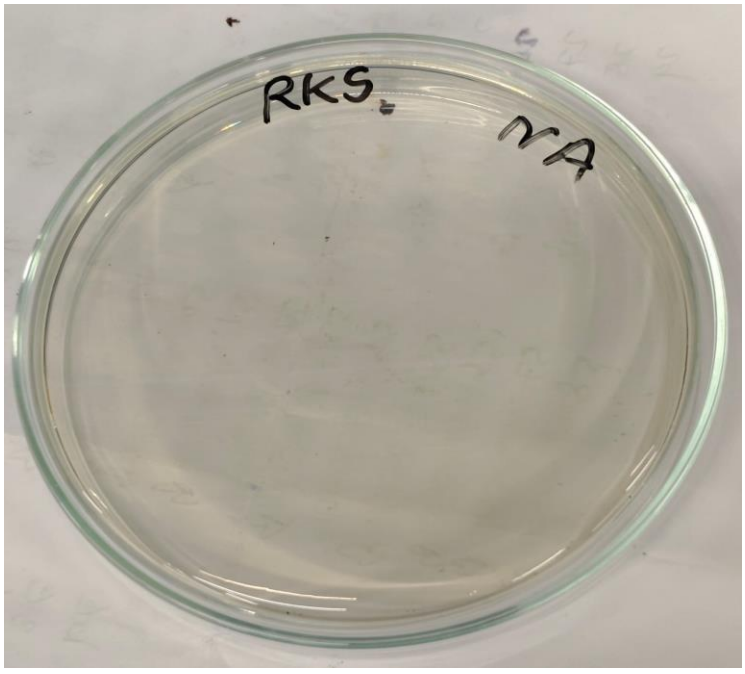

Figure 9: Fresh Nutrient Agar Media

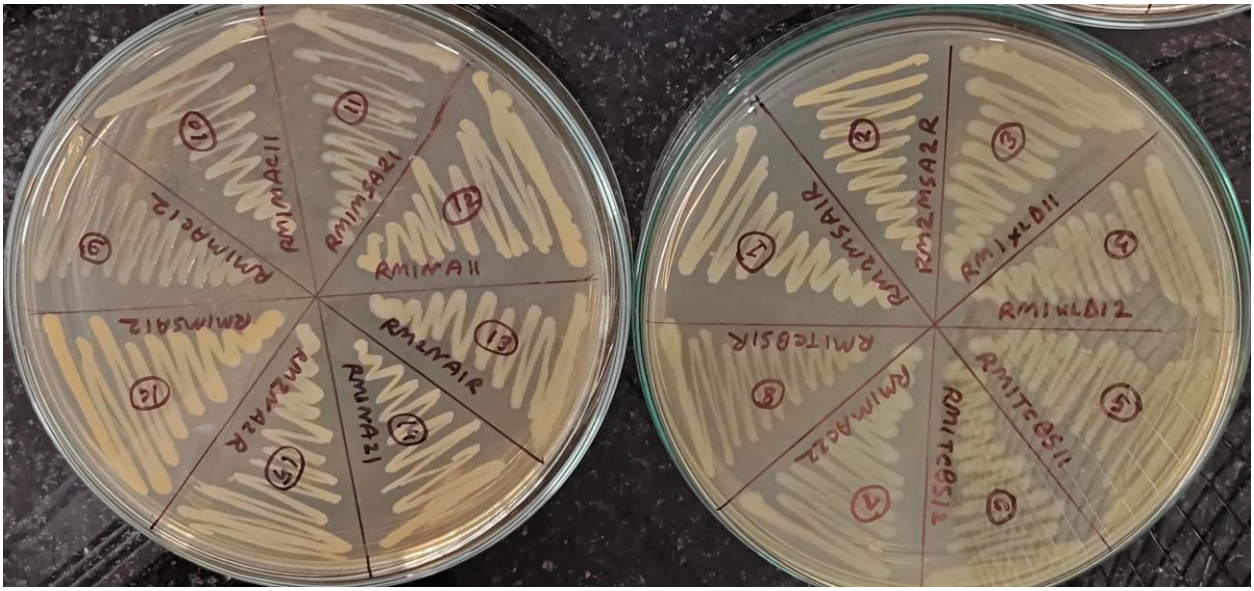

Figure 10: Sub-cultured Nurtient Agar Plate

Phosphate-buffered saline (PBS)

For preserving a solution's pH, phosphate-buffered saline, or PBS, is one of the most widely used buffers in various biological research applications. In addition to sodium chloride and sodium phosphate, this solution may also contain potassium chloride and potassium phosphate, depending on its intended use. This solution is isotonic because PBS's ion concentrations and osmolarity typically correspond to those of the human body.

Preparation:

Prepare 800 ml of Distilled Water in a Clean Container

↓

Add 8g Sodium Chloride (NaCl)

↓

Add 1.44g Sodium Phosphate Dibasic (Na_2HPO_4)

↓

Add 0.2g Potassium Chloride (KCl)

↓

Add 0.245 g Potassium Phosphate Monobasic (KH_2PO_4)

↓

Adjust pH to ~7.4 (using pH meter)

↓

Add Distilled Water to Make Final Volume 1 Liter

↓

Sterilize Using Autoclave (Liquid Cycle)

↓

Store at Room Temperature

Figure 11: PBS buffer solution

Muller Hinton Agar (MHA)

Mueller-Hinton agar (MHA) was initially developed by Mueller-Hinton in 1941 for the isolation of pathogenic *Neisseria* species. Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganisms by the Kirby-Bauer disk diffusion technique (Aryal, 2015). It is non-selective media; thus, any bacteria inoculated in this medium will have growth. MHA media was prepared by dissolving 38 grams of MHA powder in 1 liter of distilled water through boiling and stirring in a clinical flask. The conical flask's mouth was covered using aluminum foil and subjected to autoclaving to ensure sterility. After sterilization, the liquid media were transferred into sterilized Petri dishes.

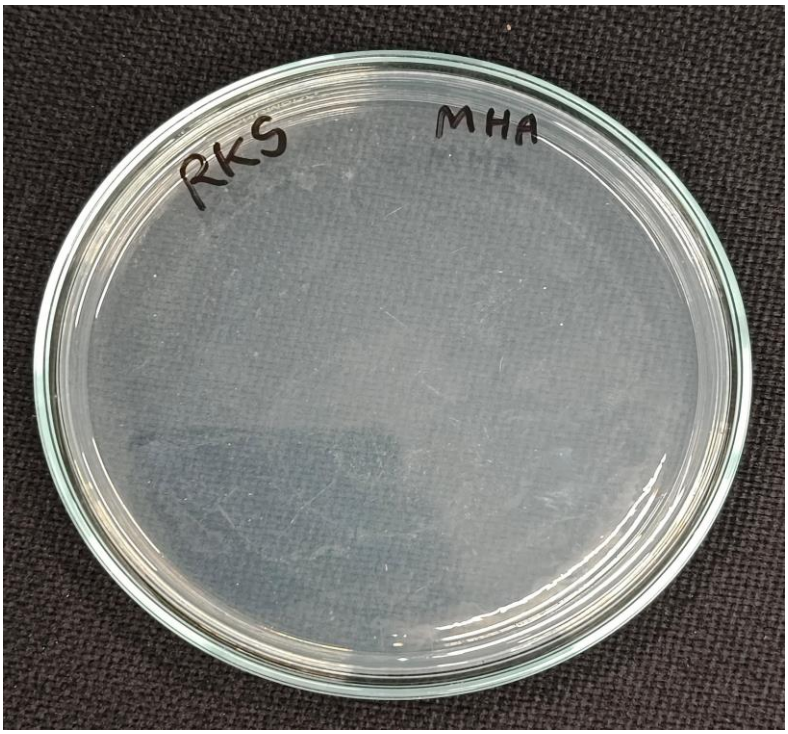

Figure 12: Fresh MHA plate

Serial Dilution

Serial dilution is one of the common methods used to reduce the number of bacterial concentrations to the required concentration for a particular test method or to a concentration where it is easier to count (Acharya Tankeshwar, 2022). Six autoclaved glass test tubes were taken for serial dilution, and 9ml of 1% autoclaved saline was incorporated into each tube. Then, 1 ml of the sample from the PBS buffer solution was added to 1 ml of saline solution. Then, this 10 ml solution was diluted 5 times using the same 10-fold dilution method, and each of the diluted samples was then plated onto the designated plates for further analysis.

Figure 13: 10-fold serial dilution

2.6 Phenotypic Characterization

Biochemical test

Biochemical tests are commonly used to identify bacteria based on their reactions with specific biochemical substances. These tests are part of traditional microbiological methods and are mainly focused on analyzing the phenotypic characteristics of microorganisms. After initial growth on selective media, the isolated bacterial colonies were transferred to nutrient agar (NA) plates for further cultivation. Before proceeding to molecular level identification, a series of confirmatory biochemical tests were carried out.

Table 1: List of Biochemical Tests

Biochemical Tests
Gram stain
Oxidase test
Catalase Test
Triple Sugar Iron (TSI) Test
Motility Indole Urease (MIU) Test

Gram Staining

Gram staining is a widely used method for distinguishing between two major types of bacteria based on differences in their cell wall structure. This technique classifies bacteria as either Gram-positive or Gram-negative by staining them violet or red, respectively. Gram-positive bacteria appear violet because their thick peptidoglycan layer retains the crystal violet dye. In contrast, Gram-negative bacteria have a thinner peptidoglycan layer that cannot hold the crystal violet during the decolorization step, resulting in them taking up the red counterstain instead. (Gram Staining, n.d.-b)

Table 2: Reagents Used in the Gram Staining Procedure:

Reagent	Function
Crystal Violet	Primary stain
Gram's Iodine	Mordant
95% Ethanol	De-colorizing agent
Safranin	Secondary dye

Procedure of Gram Staining

Begin with a clean, grease-free glass slide. Using a sterile loop, place a drop of the bacterial suspension onto the slide to create a thin smear. Allow the smear to air dry completely, then heat-fix it by briefly passing it through a flame.

Apply crystal violet stain to the smear and let it sit for 30 seconds to 1 minute. Rinse the slide gently with water. Next, cover the smear with Gram's iodine for 1 minute, then wash it off with water.

Decolorize the smear by applying 95% alcohol or acetone for 10–20 seconds, followed by an immediate water rinse. Counterstain with safranin for about 1 minute, then rinse again with water.

Finally, allow the slide to air dry or gently blot it with absorbent paper, and examine the stained smear under a microscope.

Result

Microscopic examination was performed to determine whether the bacteria were Gram-positive or Gram-negative and to observe their shape, such as rod-shaped (bacilli) or spherical (cocci). Gram-positive bacteria have a thick peptidoglycan layer in their cell walls, which retains the crystal violet stain, making them appear purple under the microscope. On the other hand, Gram-negative bacteria have a higher lipid content in their cell walls, which prevents them from retaining the crystal violet. Instead, they absorb the counterstain safranin and appear red or pink.

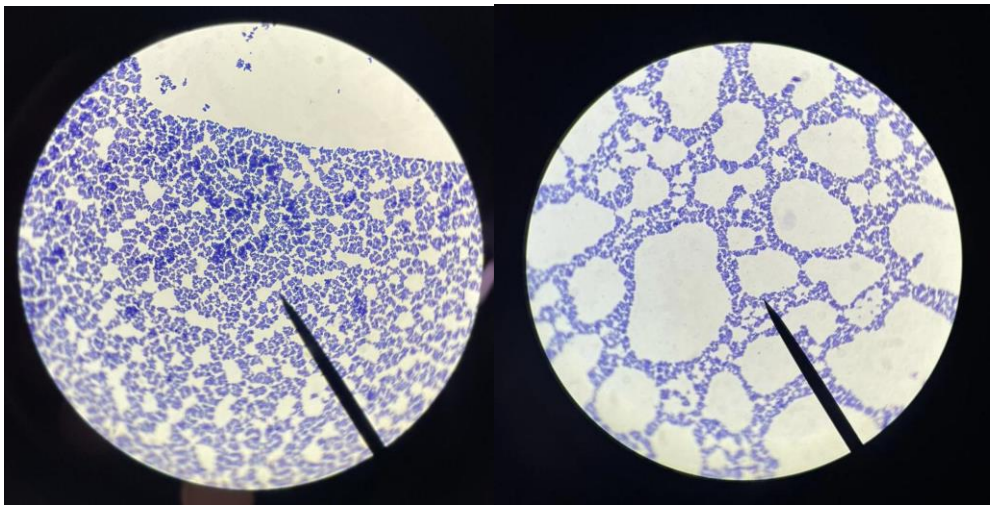

Figure 14: Gram Staining

Oxidase Test

The oxidase test is used to detect bacteria that produce the enzyme cytochrome c oxidase, which plays a role in the bacterial electron transport chain. This enzyme can oxidize a chemical reagent

called tetramethyl-p-phenylenediamine dihydrochloride, converting it into indophenols, which appear as a dark blue or purple color. If the enzyme is absent, the reagent remains in its reduced form and stays colorless. (Dahal, 2023)

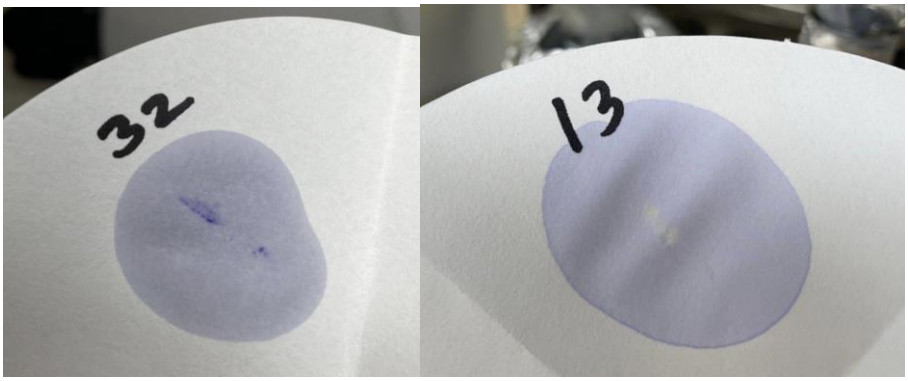

Figure 15: Oxidase Test

Filter Paper Method:

Prepare a Smear on Whatman No. 1 Filter Paper

↓

Add 1–2 Drops of Kovacs' Oxidase Reagent to the Smear

↓

Observe for Color Change (up to 60 seconds)

↓

Is there a Purple/Dark Blue Color?

↙ ↘

Yes No

↓ ↓

Positive for Oxidase Enzyme | Negative for Oxidase Enzyme

↓

Record Results

Catalase Test

The catalase test is a biochemical assay used to detect the presence of the catalase enzyme in aerobic microorganisms (Sapkota, 2022). It is widely employed to distinguish catalase-positive bacteria, such as Staphylococcus species, from catalase-negative bacteria, such as Streptococcus species.

Catalase Test Procedure:

Using Sterile Loop/Stick, Pick a Colony (18–24 hrs Old)
↓
Place the Sample on a Clean Microscope Slide
↓
Add 1 Drop of 3% Hydrogen Peroxide (H_2O_2)
↓
Observe for Bubble Formation (O_2 Release)
↓
Are Bubbles Produced Within a Few Seconds?
↙ ↘
Yes No
↓ ↓
Catalase Positive Catalase Negative
↓
Record Results

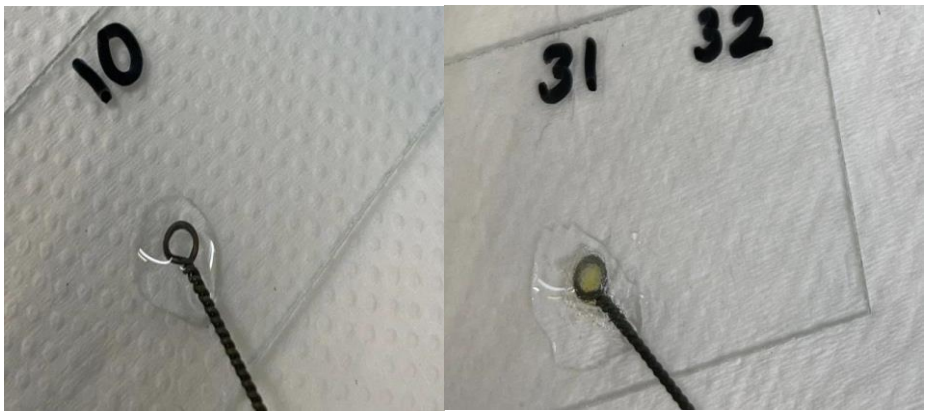

Figure 16: Catalase Test

Triple Sugar Iron (TSI) Test

A bacterial test called the Triple Sugar Iron (TSI) test gets its name from its ability to determine an organism's ability to ferment sugars and generate hydrogen sulfide.

Procedure:

Touch the top of a colony that has been well isolated with a straight inoculation needle.

↓

To inoculate TSI, first stab through the middle of the medium to the tube's bottom, and then streak the agar slant's surface.

↓

For 18 to 24 hours, incubate the tube in ambient air at 37°C with the cap loosely on.

Results:

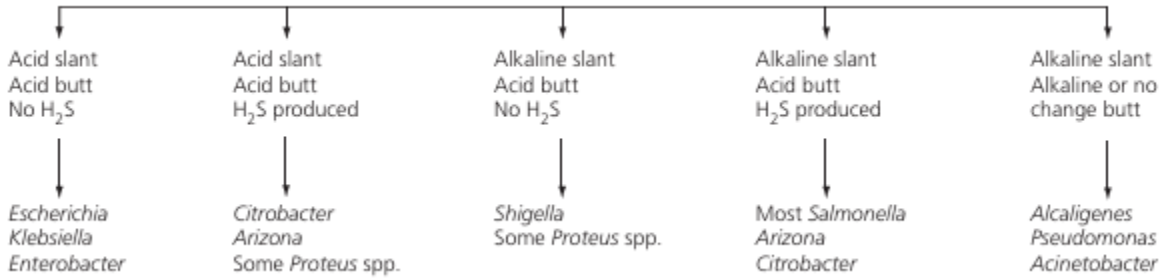

Figure 17: TSI result interpretation

Cappuccino, J. G., & Welsh, C. (2019).

Microbiology : a laboratory manual. Pearson Education South Asia Pte Ltd.

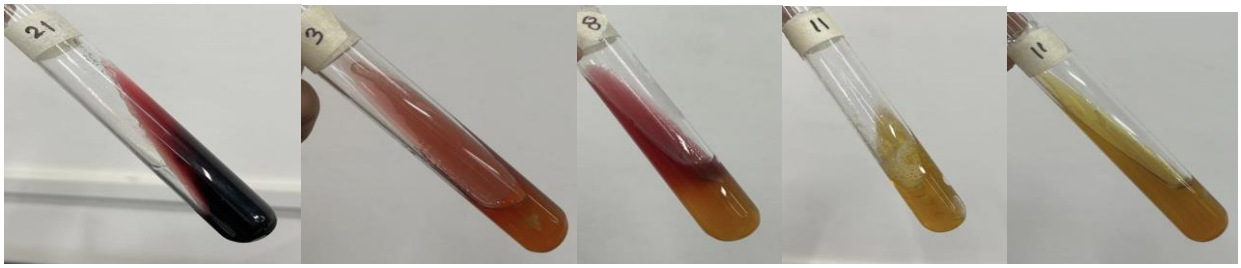

Figure 18:TSI Result

MIU Test

The motility indole urease test, or MIU test, is used for three tests: one for bacterial motility, one for indole production, and one for urea hydrolysis. (MIU Test: Uses, Principle, Composition, Procedure, Result Interpretation, 2021)

Method of Testing

Using an inoculating needle, stab a single, well-isolated colony in the medium, leaving a third of the colony at the tube's bottom.

As a test tube, use a cotton plug that fits loosely.

Incubate for 18 to 24 hours at 37°C.

Results

Check the tube for changes in the medium's color, growth, and motility. Only measure the motility and urease reaction to check for indole production. A positive urease test results yellow-orange to pink-red color shift. A negative reaction is indicated by no change in color. Test of motility Clouding of the medium or growth extension from the inoculating line are indicators of a good reaction. Restricting the growth to the inoculating line results in a negative reaction. Indole test results are positive. If Kovac's reagent is added to a test tube and a pink-red color ring forms, note the reaction as Indole Positive; if not, note it as Indole Negative.

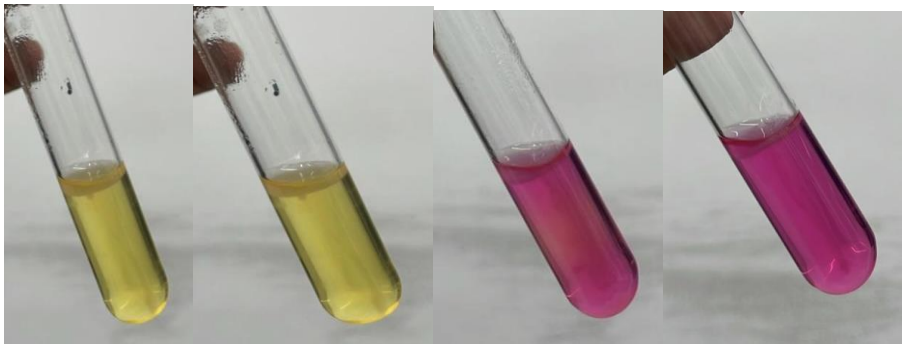

Figure 19: MIU Test result

2.7 Data Analysis

For data analysis google sheet was used

2.8 Molecular Detection of Bacterial Isolates

After the initial morphological characterization based on colony appearance from the growth of selective media, molecular identification techniques were employed to confirm the identity of isolates. Molecular detection provides greater specificity by targeting bacterial DNA or specific genes, which ensures accurate classification of isolates (Gupta, 2019). To carry out this process, genomic DNA was extracted from selected bacterial colonies, followed by polymerase chain reaction (PCR) and agarose gel electrophoresis.

Flowchart of molecular detection process:

Bacterial Isolation

↓

DNA Extraction from Isolates

↓

PCR Preparation

↓

PCR Amplification

↓

Agarose Gel Electrophoresis

↓

Visualization under UV Light

↓

Result Interpretation

DNA extraction

All the selected isolates from the selective agar were cultured on Nutrient Agar plates to ensure pure growth. Then 1 loopful of the bacterial culture was transferred into a sterile microcentrifuge tube along with 150µL of TE (Tris-EDTA) buffer and vortexed for 15-30 seconds. TE buffer facilitates cell lysis. The mixture was subjected to heat treatment on a heat pad at 95°C for 20 minutes, which lyses the bacterial cells and releases genomic DNA. Immediately after the heat shock, the samples were placed in a cold centrifugation machine, which was performed at 10,000 rpm for 10 minutes at 4°C to separate cellular debris from the lysate. 120µL of the clear supernatant, which contained the extracted DNA, was carefully collected and stored at –20 °C until further use in PCR amplification (Zaman et al., 2024). This extracted DNA served as the template for the molecular identification of bacterial species.

Pure Colony (from Nutrient Agar)

↓

Take 150 µL TE Buffer

↓

Take 1 loopful of colony → Vortex 15–20 seconds

↓

Heat shock at 90°C for 20 min

↓

Cold shock→ Centrifuge at 10,000 rpm for 10 min at 4°C

↓

Collect supernatant (DNA extract) → Store at –20°C

PCR

The polymerase chain reaction (PCR) is a laboratory nucleic acid amplification technique used to denature and renature short segments of DNA using DNA polymerase I enzyme, an isolate from Thermus aquaticus, known as Taq polymerase (Nimrat Khehra et al., 2023). For this study samples on a monthly basis from above mentioned areas, and PCR was frequently employed in order to detect *Vibrio spp., Staphylococcus aureus, Salmonella spp., Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella spp., and Escherichia coli.* The PCR assay was done in PCR tubes with appropriate PCR master mix, forward primer, reverse primer, and Nuclease-free water (NFW) for the specific organism and gene. Careful pipetting and spinning were applied to ensure thorough mixing and prevent bubble formation. The PDR was run in an Applied Biosystems (Thermo Fisher) thermal cycler using the following modified program. The detailed information on the targeted gene, organisms, primer sequences, PCR thermal cycler annealing temperature, and amplicon sizes is discussed in **Table 2.**

Table 2: Primer name, Sequence, Product size, PCR condition, PCR mix, Reference

Name of Bacteria	Primer Name	Sequence	Product Size	PCR conditions	References
Staphylococcus aureus	*Nuc*-F	GCGATTGATGGTGG ATACGGT	279	Initial Denaturation: 94°C, 4 minutes Denaturation: 94°C, 1 minute Annealing: 55°C, 45 seconds	(Akanbi et al., 2017)

	Nuc-R	AGCCAAGCCTTGAC GAACTAAAGC		Elongation: 72℃, 45 seconds Final Elongation: 72℃, 10 minutes Cycle: 32	
Klebsiella pneumoniae	Pf	ATT TGA AGA GGT TGC AAA CGA T	260	Initial Denaturation: 94℃, 10 minutes Denaturation: 94℃, 30 seconds Annealing: 57℃, 20 seconds Elongation: 72℃, 20 seconds	(Osman et al., 2020)
	Pr2 (R)	CCG AAG ATG TTT CAC TTC TGA TT			
	Pr1 (R)	TTC ACT CTG AAG TTT TCT TGT GTT C	130	Final Elongation: 72℃, 10 minutes Cycle: 35	(Osman et al., 2020)
Pseudomonas aeruginosa	*PA-SS* F	GGGGGATCTTCGGA CCTCA	956	Initial Denaturation: 95℃, 5 minutes Denaturation: 94℃, 30 seconds Annealing: 58℃, 30 seconds Elongation: 72℃, 35 seconds Final Elongation: 72℃, 2 minutes Cycle: 30	(Spilker et al., 2004)
	PA-SS R	TCCTTAGAGTGCCC ACCCG			
Escherichia coli	ECO-F	GAC CTC GGT TTA GTT CAC AGA	585	Initial Denaturation: 95℃, 5 minutes Denaturation: 94℃, 45 seconds Annealing: 45℃, 45 seconds Elongation: 45℃, 1 minute Final Elongation: 72℃, 5 minutes Cycle: 35	Moawad et al. (2017)
	ECO-R	CAC ACG CTG ACG CTG ACC A			

Salmonella spp.	*Inv-A* F	CGG TGG TTT TAA GCG TAC TCT T	796	Initial Denaturation: 94℃, 1 minutes Denaturation: 94℃, 1 minute Annealing: 58.3℃, 30 seconds Elongation: 72℃, 30 seconds Final Elongation: 72℃, 7 minutes Cycle: 35	(Farahani et al., 2018)
	Inv-A R	CGA ATA TGC TCC ACA AGG TTA			
Shigella spp.	GF	TCCGTCATGCTGGA TGAACGATGT	159	Initial Denaturation: 95℃, 5 minutes Denaturation: 95℃, 30 seconds Annealing: 55℃, 1 minute Elongation: 72℃, 30 seconds Final Elongation: 72℃, 7 minutes Cycle: 30	(Ranjbar et al., 2014)
	GR	ACAGTTCAGGATTG CCCGAGACACA			
Vibrio spp.	VG R, VG F	F 5'-TGCAGATAATTCAC GCCCAG-3'	689	"Initial Denaturation: 94℃, 5 minutes Denaturation: 94℃, 30 seconds Annealing: 60℃, 30 seconds Elongation: 72℃, 30 seconds Final Elongation: 72℃, 10 minutes Cycle: 25	(Kim et al., 2015)
		R 5'-ACCCGCTGGACGCC AT-3'			

Agarose Gel Preparation & Gel Electrophoresis

The agarose gel was prepared using agarose powder. The agarose powder was taken in various quantities depending on the gel concentration required for proper visualization of the PCR products. Agarose powder, along with required distilled water and working solution of Tris-acetic acid (TAE) buffer, was taken into a conical flask and then dissolved using heat. Afterwards, 4µl of Ethidium Bromide (EtBr) was added to the solution to visualize the subject base pairs (bps) under UV light. Afterwards, adding the EtBr the solution was mixed and poured into the Gel apparatus. And combs were placed and then waited for the gel to dry. After the gel was dried, the comb was pulled out, and the TAE buffer running solution was poured. Afterwards, the gel electrophoresis was run for 40 minutes at 100 volts.

2.9 Antibiotic Susceptibility Testing (AST)

To evaluate how sensitive or resistant the bacterial isolates were to selected antibiotics, AST was performed. Through this method, a clear idea of the ability of an antibiotic to inhibit bacterial growth can be determined. Although there are several techniques for performing AST, such as broth dilution, Etest, and other automated systems, the Kirby-Bauer disk diffusion method was chosen for this study as it is simple, time-efficient, and widely used (Gajic et al., 2022).

To proceed with the disk diffusion method, Mueller-Hinton agar (MHA) plates were used as a standard medium. A bacterial suspension was prepared by picking a loopful of colonies from pure bacterial cultures and mixing them in 0.9% saline solution. The turbidity of the suspension was then adjusted to match the 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/mL. This step ensured that all plates received a uniform bacterial load (Humphries et al., 2021).

The prepared suspension was evenly spread on the surface of the MHA plate using a sterile cotton swab by swabbing in different directions. After allowing the surface to dry for a few minutes, antibiotic disks were placed carefully on the agar using a sterile forcep. The plates were then incubated at 37 °C for 24 hours.

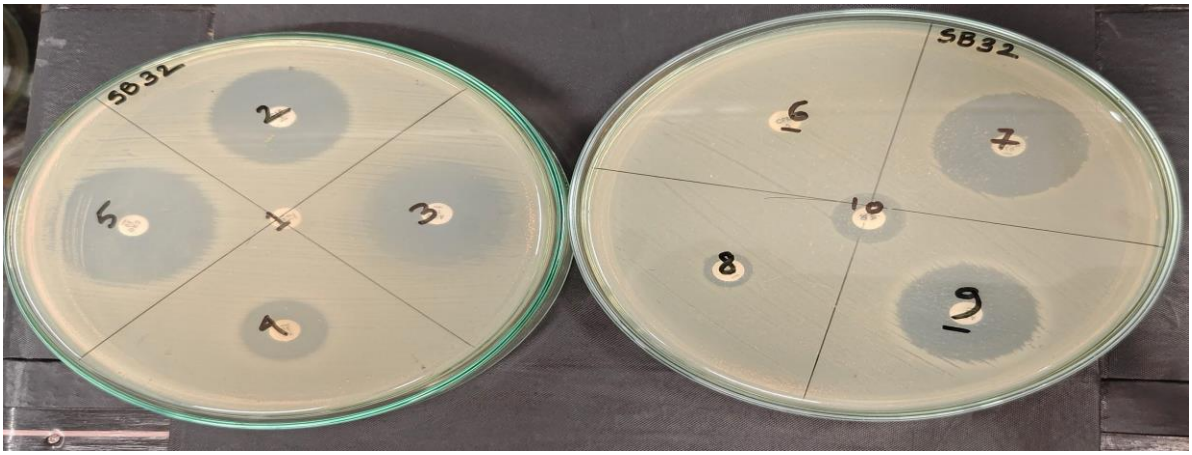

Figure 20: Kirby-Bauer Test on MHA agar

After the incubation period, the zones of inhibition formed around the antibiotic disks were measured in millimeters. These measurements were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines to determine whether the isolates were Sensitive (S), Intermediate (I), or Resistant (R) to each antibiotic tested. This method helped identify the resistance profile of the bacterial isolates and provided useful insights into their antimicrobial susceptibility.

Table 4: Table: The following chart is a list of antibiotics that were used to analyses the sensitivity or resistance activity of bacteria (HiMedia, Mumbai, India):

Antibiotic name	Antibiotic symbol	Disc content (µg)
Amikacin	**AK**	**30**
Amoxicillin	**AMX**	**30**
Amoxyclav	**AMC**	**30**
Azithromycin	**AZM**	**15**
Cefixime	**CFM**	**5**
Ceftriaxone	**CTR**	**30**
Ciprofloxacin	**CIP**	**5**
Meropenem	**MRP**	**10**
Tetracycline	**TE**	**30**
Vancomycin	**VA**	**30**

Chapter 3:

Results

3.1 Bacterial Isolation

A diverse range of bacterial isolates was possible from the collected housefly *(Musca domestica)* samples using various selective and differential media. From 7 different selected locations, 53 houseflies were collected, which led to isolating 320 bacterial colonies. Colony selection was performed presumptively based on morphological appearance and phenotypic characteristics specific to each medium. For instance, TCBS agar is highly selective for isolating *Vibrio* species due to its unique components.

Figure 21: Culture plate TCBS

Similarly, Colonies showing greenish pigment and fluorescence under UV light were presumptively identified as *P. aeruginosa.*

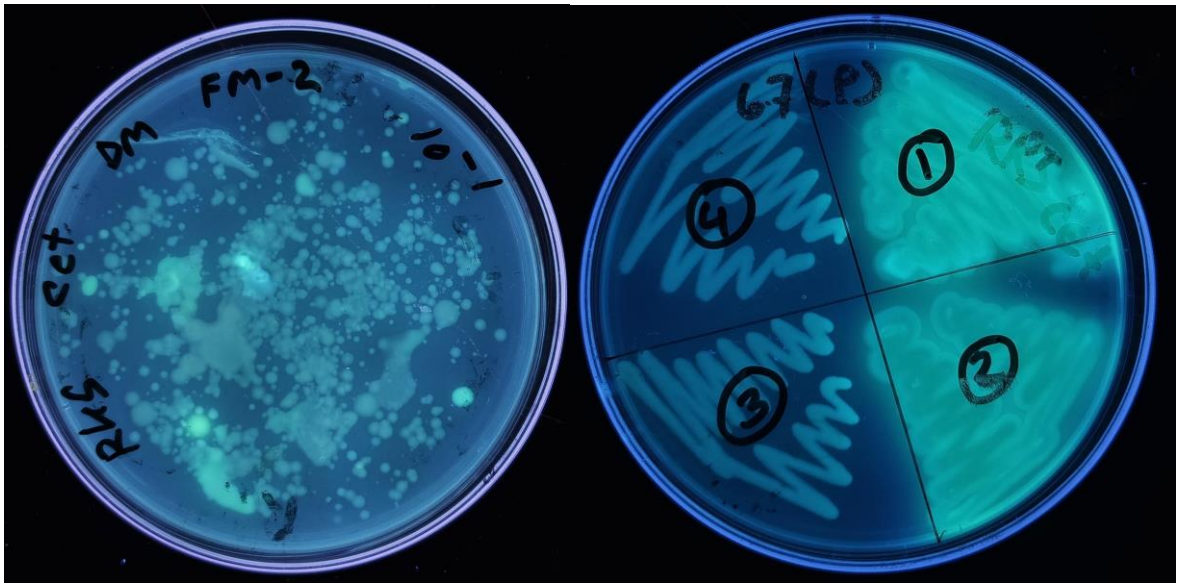

Figure 22: Culture plate of Cetrimide

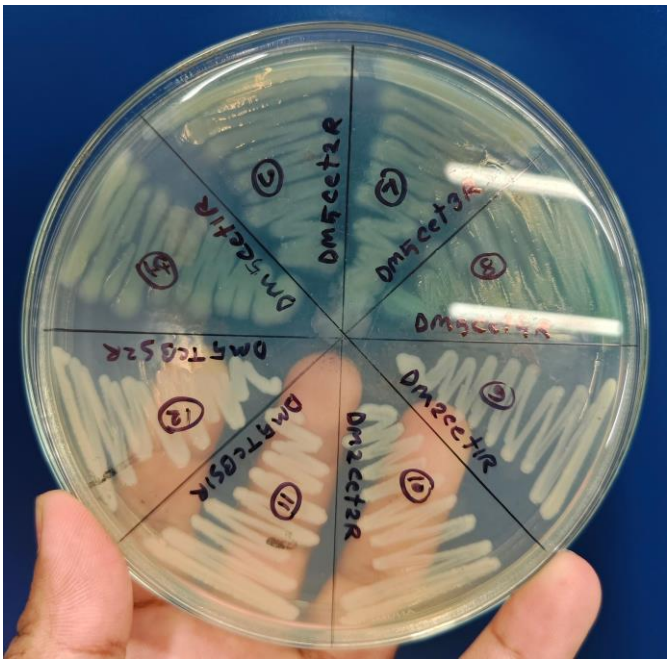

Figure 23: Sub-culture from cetrimide plate

On MacConkey agar, mucoid pink to yellow colonies were selected as presumptive *Klebsiella pneumoniae*, which is known to ferment lactose and produce mucoid like colonies.

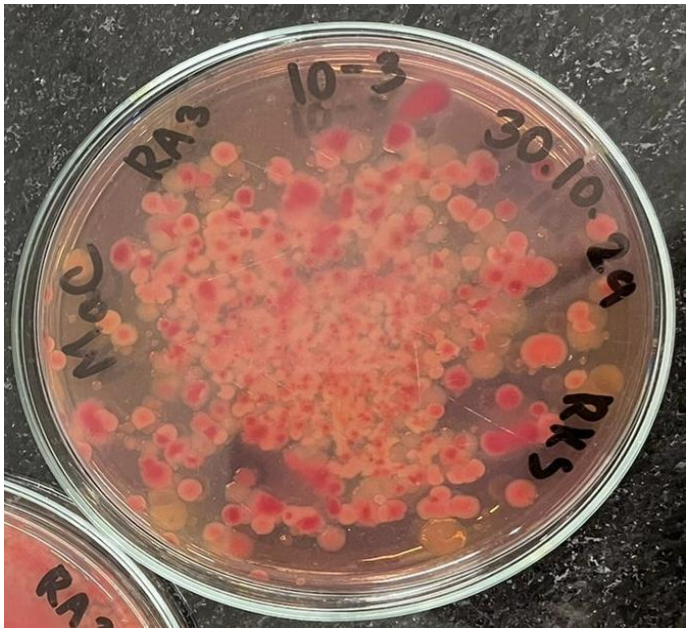

Figure 24: Culture Plate of MAC

Golden yellow colonies on MSA suggested the presence of *Staphylococcus aureus* as it ferments mannitol, turning the medium yellow.

Figure 25: Culture Plate MSA

Colonies on XLD agar that appeared red with black centers were considered *Salmonella* due to their production of hydrogen sulfide.

Figure 26: Culture plate of XLD

Based on these presumptive characteristics, the most frequently isolated bacteria across the samples were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholerae,* and *Staphylococcus aureus*. The distribution and number of isolates from each location are summarized in the following table.

Table 5: Presumptive identification of isolates based on location

Sample Collection Area	Sample Number Per Location	Total Isolate Number	Suspected Organisms
Ramna	2	19	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella*
Shahbag	6	22	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella*
Khilgaon	9	48	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella*
Paltan	9	47	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella*
Motijheel	9	44	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella, Pseudomonas aeruginosa*
Jatrabari	9	72	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella, Pseudomonas aeruginosa*
Dhanmondi	9	69	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio spp., Salmonella, Shigella, Pseudomonas aeruginosa*

3.2 Biochemical Test

The study had 53 house flies and from that 355 bacterial samples were isolated. Furthermore, from that only, 113 isolates underwent 5 biochemical tests. Such as Gram's Staining, Oxidase test, Catalase test, Triple Sugar Iron (TSI) test, Motility Indole Urea (MIU) test

The detailed biochemical test according to the sample is shown on fig:

Bacteriological Assesment of Commonfly

AREA 1		
Zone: Ramna		
Total Sample 2		
Area: Residential Area		
Sample Code: RM1, RM2		
Media: NA,MSA,MAC,XLD,TCBS,CETRIMIDE		

DNA EXTRACTION SERIAL	SERIAL	ORGANISM CODE	PCR	GRAM STAIN	Colony Shape	CATALASE	OXIDASE	TSI			DNA EXTRACTION	CULTURE STOCK	MIU	
								Butt/slant	*H2S Production*	*Gas Production*			*Urease*	*Motile*
1	1	RM2MSA1R	☑	positive	Cocci	☑	☐	yellow	☐	☐	☑	☑	positive	☐
2	2	RM2MSA2R	☑	positive	Diplococci	☑	☐	yellow	☐	☐	☑	☑	positive	☐
3	3	RM1XLD11	☑	negative	Coccobacilli	☐	☑	red	☑	☐	☑	☑	negative	☑
4	4	RM1XLD12	☑	negative	Coccobacilli	☐	☐	yellow	☐	☐	☑	☑	negative	☐
5	5	RM1TCBS11	☑	negative	rod	☑	☑	red	☐	☑	☑	☑	negative	☑
6	6	RM1TCBS12	☑	negative	rod	☑	☑	red	☐	☑	☑	☑	negative	☑
7	7	RM1MAC22	☑	negative	rod	☑	☐	yellow	☐	☑	☑	☑	positive	☐
8	8	RM1TCBS1R	☑	negative	rod	☑	☑	red	☐	☑	☑	☑	negative	☑
9	9	RM1MAC12	☑	negative	rod	☑	☐	yellow	☐	☑	☑	☑	positive	☐
10	10	RM1MAC11	☑	negative	rod	☑	☐	red	☐	☑	☑	☑	positive	☐
11	11	RM1MSA21	☑	positive	Cocci	☐	☑	yellow	☐	☑	☑	☑	positive	☑
12	12	RM1NA11	☑	positive	Cocci	☑	☐	yellow	☐	☐	☑	☑	negative	☐
13	13	RM2NA1R	☑	negative	Diplococci	☐	☑	yellow	☐	☑	☑	☑	positive	☑
14	14	RM1NA21	☑	positive	Diplococci	☑	☐	red	☐	☐	☑	☑	positive	☐
15	15	RM2NA2R	☑	positive	Cocci	☑	☐	yellow	☐	☐	☑	☑	positive	☐
16	16	RM1MSA12	☑	positive	Cocci	☐	☑	yellow	☐	☐	☑	☑	negative	☐
17	17	RM1MSA11	☑	positive	Cocci	☑	☐	yellow	☐	☐	☑	☑	negative	☑
18	18	RM2NA3R	☑	positive	rod	☑	☐	red	☐	☐	☑	☑	positive	☐
19	19	RM1NA31	☑	positive	Rod	☑	☐	yellow	☐	☐	☑	☑	positive	☐
20	20	RM1NA32	☑	positive	Rod	☑	☐	yellow	☐	☐	☑	☑	negative	☐
21	21	RM1NA12	☑	positive	Coccobacilli	☑	☐	red	☑	☐	☑	☑	positive	☐
22	22	RM1MAC13	☑	negative	rod	☑	☐	yellow	☐	☑	☑	☑	positive	☐
23	23	RM2XLD1R	☑	negative	rod	☑	☐	yellow	☑	☐	☑	☑	positive	☐
24	24	RM1NA22	☑	negative	rod	☑	☑	yellow	☑	☐	☑	☑	positive	☐
25	25	RM1XLD21	☑	negative	Rod	☑	☐	red	☐	☐	☑	☑	Negative	☐
26	26	RM1XLD41	☑	negative	Rod	☐	☑	yellow	☐	☑	☑	☑	positive	☐
27	27	RM2TCBS1R	☑	negative	rod	☑	☑	red	☐	☑	☑	☑	negative	☑
28	28	RM1XLD31	☑	negative	Coccobacilli	☑	☑	red	☐	☐	☑	☑	negative	☐

Figure 27: Excel Sheet of different area

Gram's stain

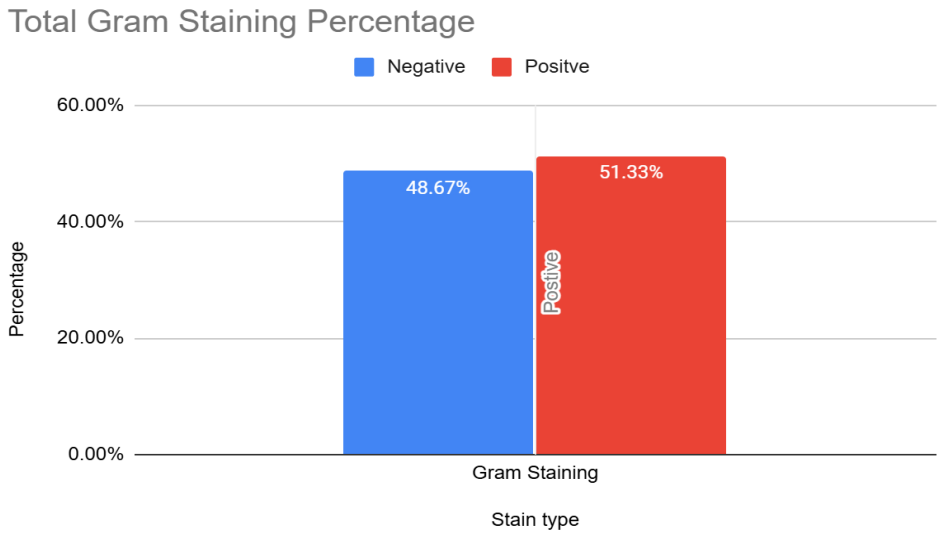

Figure 28: Total Gram Stain Percentage

The bar data shows that around 48.67% of all isolates were gram-negative bacteria. Similarly, the percentage of gram-positive bacteria was 51.337%

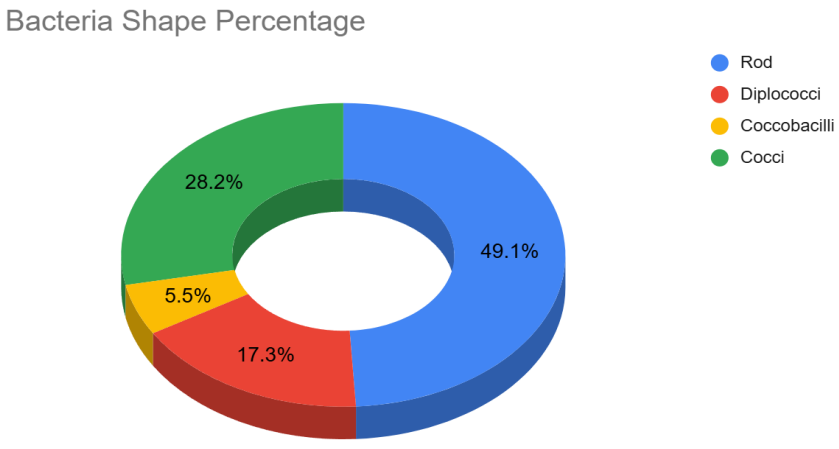

Figure 29: Bacterial Shape Percentage

The pie chart shows that among all gram-negative and gram-positive bacteria were 49.1% rod shape, 28.67% cocci shape, 17.3% diplococci and 5.5% coccobacilli shaped bacteria. The majority of the samples were rod shaped.

Catalase

From the 113-catalase test 71 was found to be positive and 43 was found to be negative.

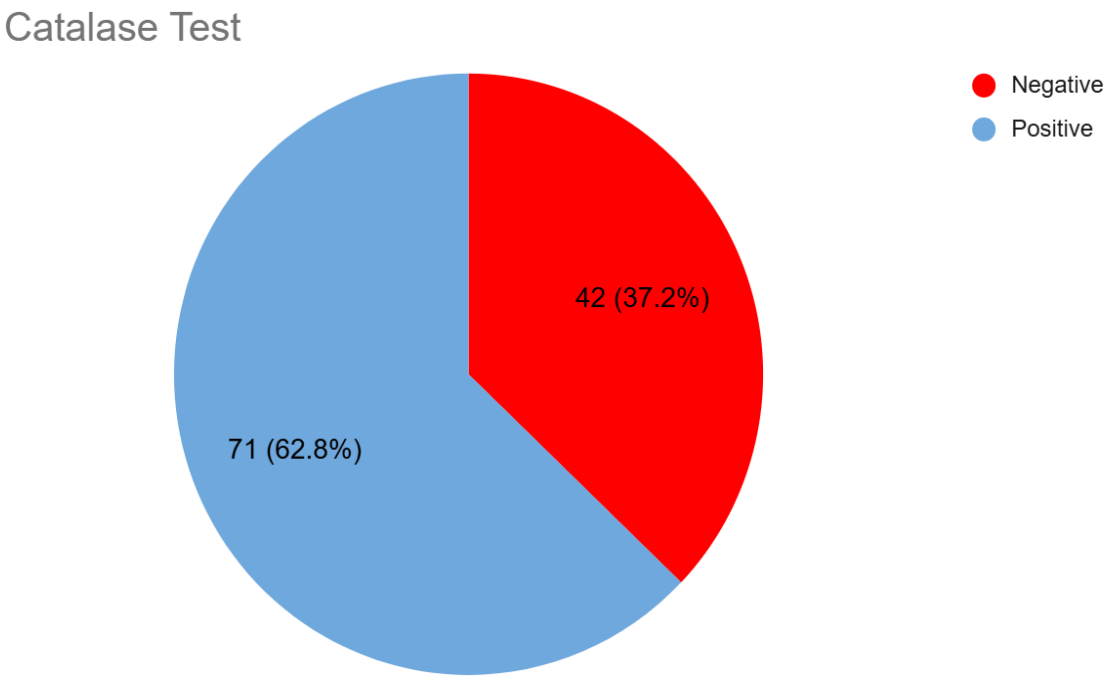

Figure 30: Result of Catalase Test

The pie chart shows that, in this catalase test 62.8% were found to be positive out of 113 samples and similarly, 37.2% were negative results found. This result showed that the majority of the samples were catalase positive, which was 62.8% (71).

Oxidase Test

From the 113-oxidase test 37 was found to be positive and 76 was found to be negative.

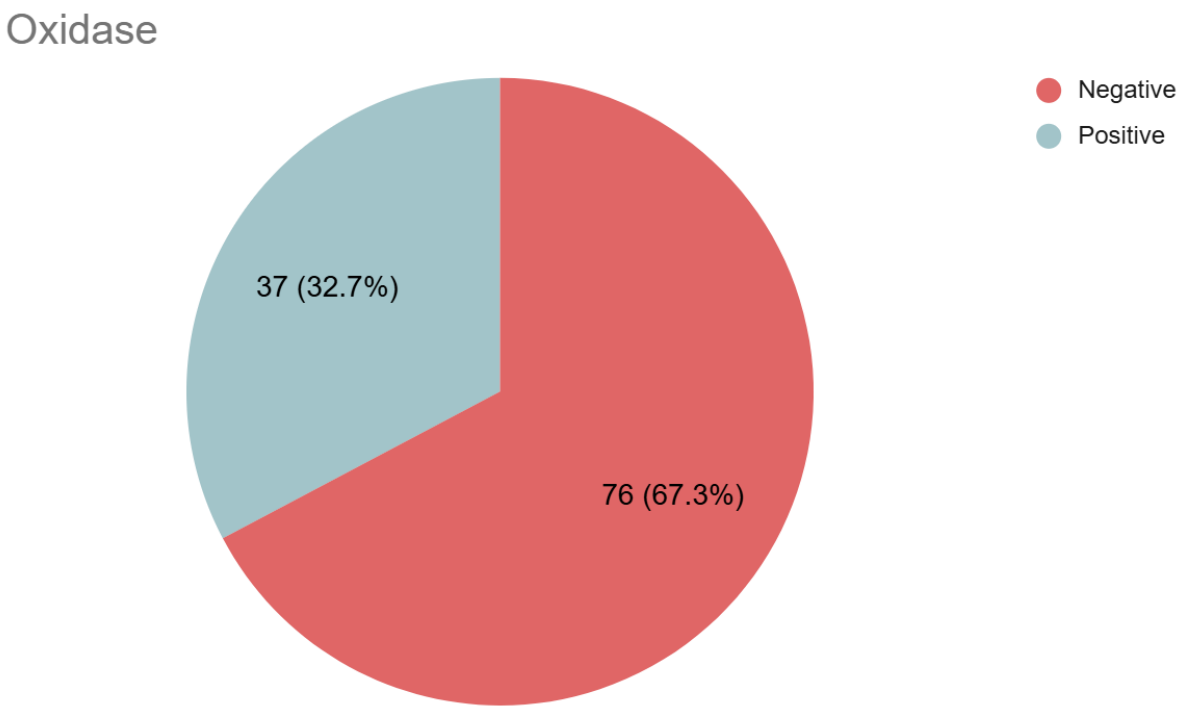

Figure 31: Result of Oxidase Test

The pie chart shows that, in this oxidase test 32.74% were found to be positive out of 113 samples and similarly 67.26% were negative results found. This result showed that the majority of the samples were oxidase negative., which was 76 (67.3%).

TSI

Result Intepretation	Isolate Number
Yellow Butt/Slant, H2S Production, Gas Production	0
Yellow Butt/Slant, No H2S Production, Gas Production	18
Yellow Butt/Slant, H2S Production, No Gas Production	2
Yellow Butt/Slant, No H2S Production, No Gas Production	46
Red Butt/Slant, H2S Production, Gas Production	0
Red Butt/Slant, No H2S Production, Gas Production	8
Red Butt/Slant, H2S Production, No Gas Production	10
Red Butt/Slant, No H2S Production, No Gas Production	17
Yellow/Red, Butt/Slant, H2S Production, Gas Production	0
Yellow/ Red, Butt/Slant, No H2S Production, Gas Production	0
Yellow/Red, Butt/Slant, H2S Production, No Gas Production	0
Yellow/Red, Butt/Slant, No H2S Production, No Gas Production	11

Figure 32: Result of TSI

The majority of isolates showed Yellow Butt/Slant with H_2S and Gas production, indicating typical glucose, lactose/sucrose fermenters with sulfur and gas production (47 isolates).

Some isolates had Red Butt/Slant or lacked gas/H_2S, representing non-fermenters or partial fermenters. The variation reflects the presence of diverse enteric bacteria with different metabolic capabilities.

MIU

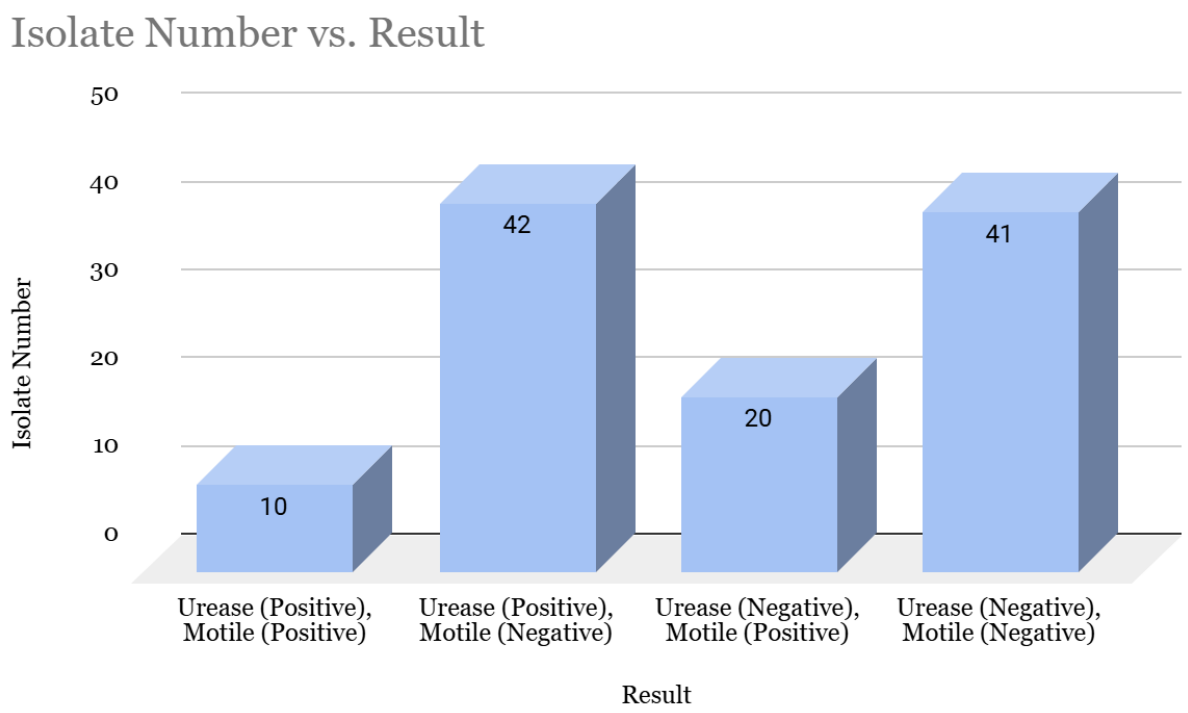

Figure 33: MIU result

Most isolates were either:

Urease Positive, Motility Negative (42 isolates),

Urease Negative, Motility Negative (41 isolates)

indicating a high presence of non-motile organisms, both urease producers and non-producers. A smaller number were motile and urease positive (10) or motile and urease negative (20), suggesting limited motile species in the sample pool.

3.3 Molecular Detection

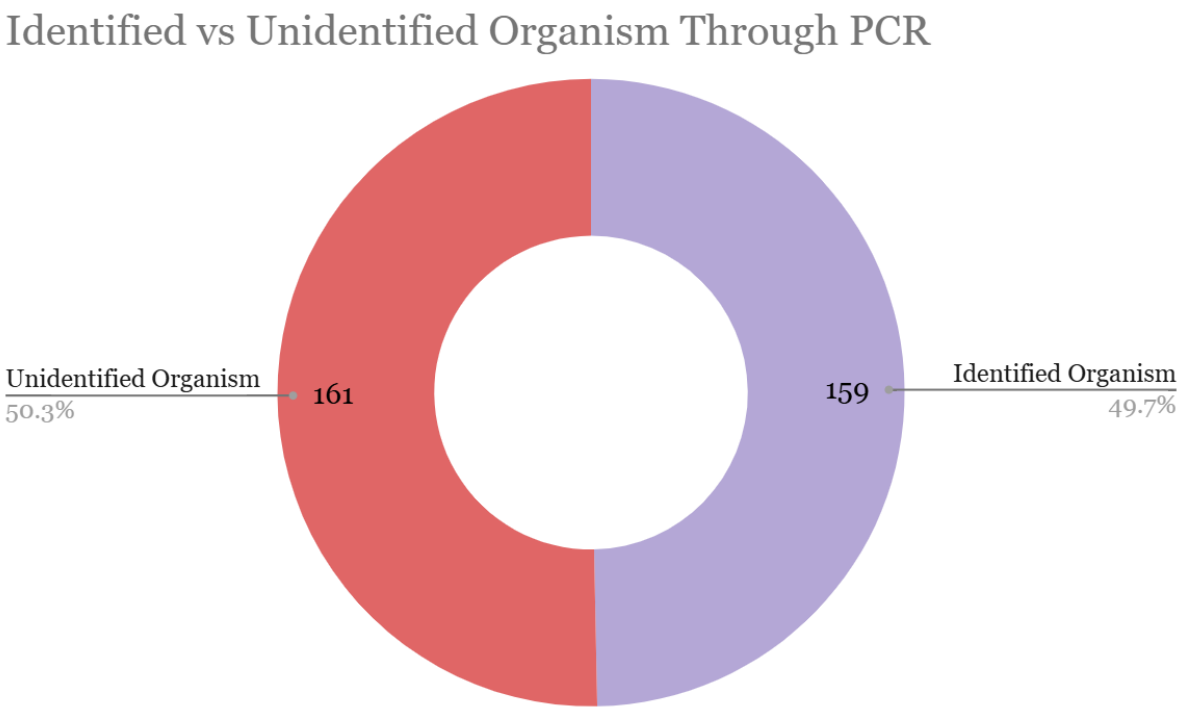

Figure 34: Identified vs Unidentified Organisms

The analysis of PCR diagnostics revealed a total of 320 PCR tests conducted. Among these, 159 samples (49.7%) successfully identified organisms, whereas 161 samples (50.3%) remained unidentified. This indicates that nearly half of the PCR tests failed to detect specific pathogens, highlighting the need for improved diagnostic sensitivity or better sample quality.

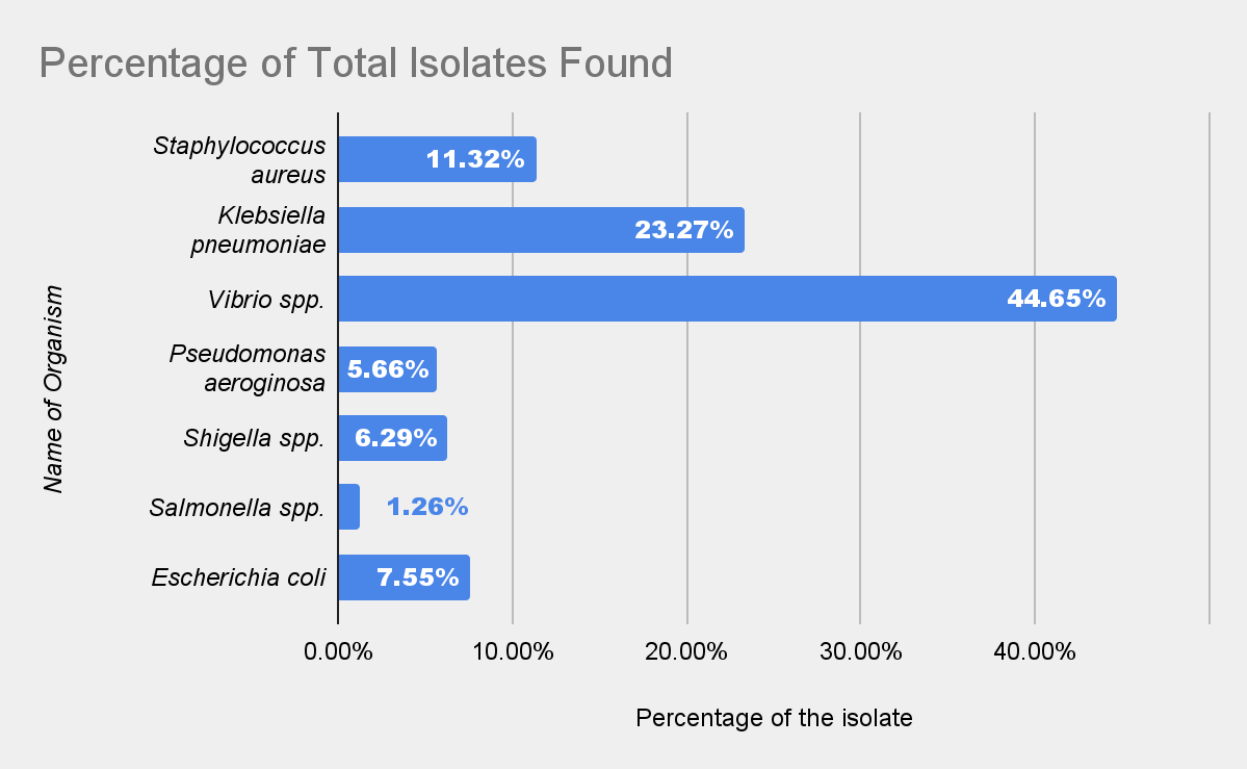

Figure 35: Percentage of total Isolate Found

Among the identified organisms, the most prevalent isolate was *Vibrio spp.*, accounting for 44.65% of total positive detections. This was followed by *Klebsiella pneumoniae* (23.27%), *Staphylococcus aureus* (11.32%), and *Escherichia coli* (7.55%). Other detected pathogens included *Shigella spp*. (6.29%), *Pseudomonas aeruginosa* (5.66%), and *Salmonella spp.* (1.26%)

Identification of *Vibrio spp.*

During the bacterial isolation process, a total of 89 isolated colonies were suspected for *Vibrio species* from the TCBS agar plate. For confirmation of these colonies, a PCR reaction was conducted using VG F, VG R primer sets. The PCR protocol included a total reaction volume of 13µl with an annealing temperature set at 60 °C for 25 cycles. For positive confirmation, the target band size was 689bp.

Subsequently, with a 50 bp DNA ladder and appropriate positive and negative controls were included during the gel electrophoresis run for 40 minutes at 100 V. After electrophoresis, UV visualization revealed that 71 suspected isolates produced bands aligning with the 689 bp marker and the positive control out of 89 isolates. This run confirmed that these 71 confirmed isolates of *Vibrio genus*.

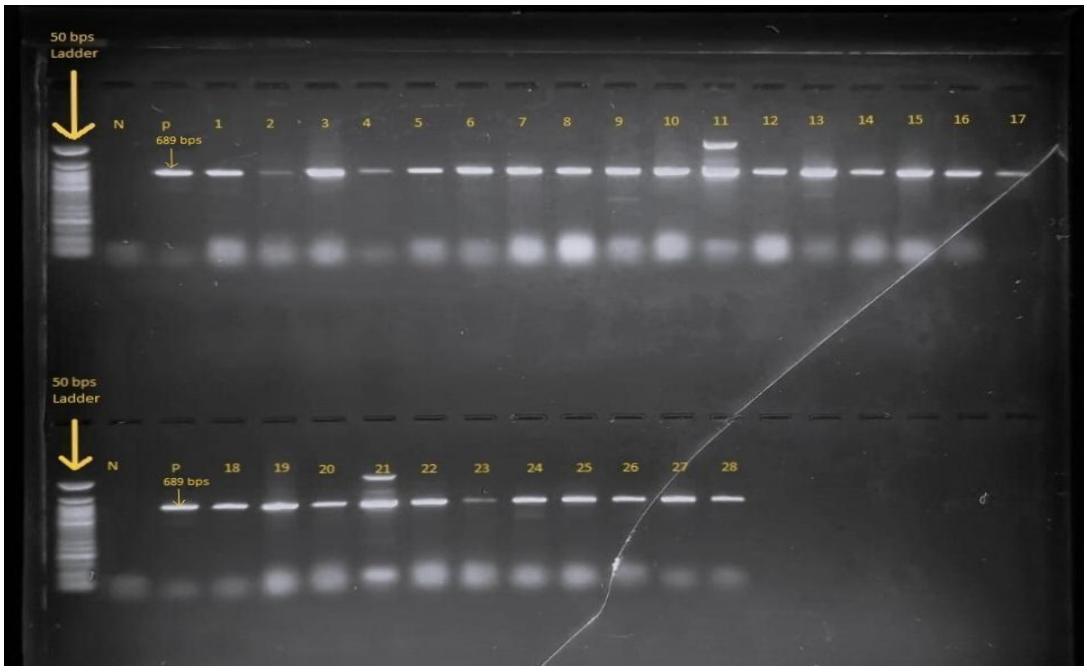

Figure 36:PCR result of Vibrio spp.

Identification of *Pseudomonas aeruginosa*

Again, PCR was conducted to confirm the suspected *Pseudomonas aeruginosa* isolates. From the Cetrimide plates, almost 62 isolates were suspected as *Pseudomonas spp*. For the final confirmation of having *Pseudomonas aeruginosa*, the PCR was run directly using *PA-SS* primer sets with an annealing temperature at 58ºC for 30 cycles. When the PCR reaction was completed, the gel electrophoresis must be run using a 50bp DNA ladder to compare the base pair size of the products. The expected band size for *Pseudomonas aeruginosa* was 956 bp. Following electrophoresis, UV visualization revealed that almost 12 suspected isolates produced bands aligning with the 956 bp marker and the positive control out of 62 isolates. This run confirmed that these 12 confirmed isolates of *Pseudomonas aeruginosa*.

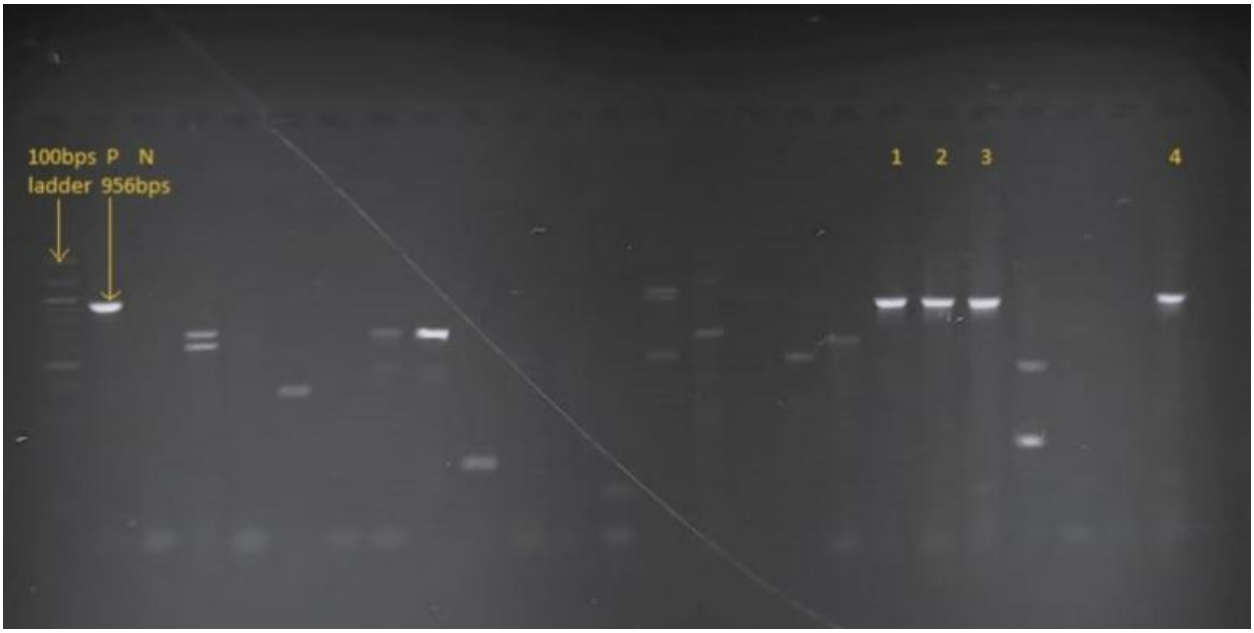

Figure 37: Gel Electrophoresis of P. aeruginosa

Identification of *Staphylococcus aureus*

Similarly, the PCR method was again conducted for the final confirmation of having Staphylococcus aureus. The PCR was run directly using *Nuc* F, *Nuc* R primer sets with an annealing temperature of 72ºC for 32 cycles. After completing the PCR, gel electrophoresis was conducted to visualize and confirm the identity of the isolates by identifying the target DNA. The band size was determined to be 279 base pairs, as indicated by the primer properties. Among 56 samples, there were 18 confirmed isolates of *Staphylococcus aureus*.

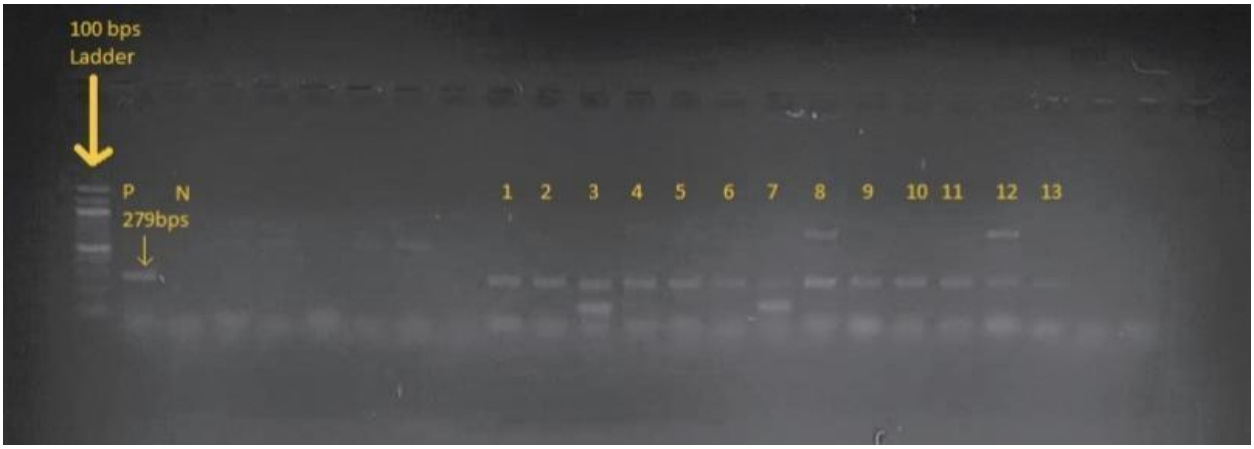

Figure 38: Gel Electrophoresis of Staphylococcus aureus

Identification of *Shigella Spp.* and *Salmonella spp.*

During the sampling process, an XLD agar plate was used to detect *Shigella Spp.* and *Salmonella Spp.* Among all the isolates, 31 isolates were suspected for *Shigella Spp.* and *Salmonella Spp.* During the PCR reaction, the annealing temperature was maintained at 55ºC for 30 cycles for *Shigella Spp.* confirmation. The volume of the mixture was 13µl and the GF, GR primer sets were mixed in it. The main purpose of this reaction was to have an amplicon size of 159bp. This band size confirms the presence of *Shigella Spp.* in the sample. Gel electrophoresis was performed at 100V for 40 minutes. In addition, a 50bps ladder along with controls were used during the gel run. After the gel run, 10 positive bands for *Shigella Spp.* were observed under UV light among 31 suspected.

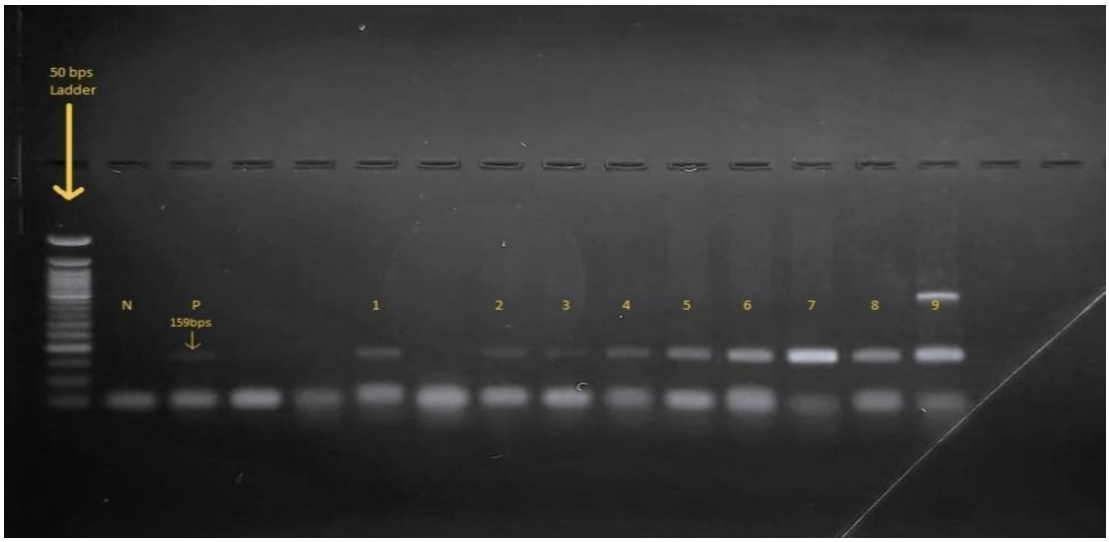

Figure 39: Gel Electrophoresis of Shigella Spp

On the other hand, the annealing temperature was maintained at 58.3ºC for 35 cycles for *Salmonella spp.* The volume of the mixture was 13µl and *Inv-A* R, *Inv-A* F primer sets were mixed in it. The main purpose of this reaction was to have an amplicon size of 796bp. This band size confirms the presence of *Salmonella Spp.* in the sample. Gel electrophoresis was performed at 100V for 40 minutes. In addition, a 50bps ladder, along with controls were used during the gel run. After the gel run, 2 positive bands for *Salmonella Spp.* were observed under UV light among 31 suspected.

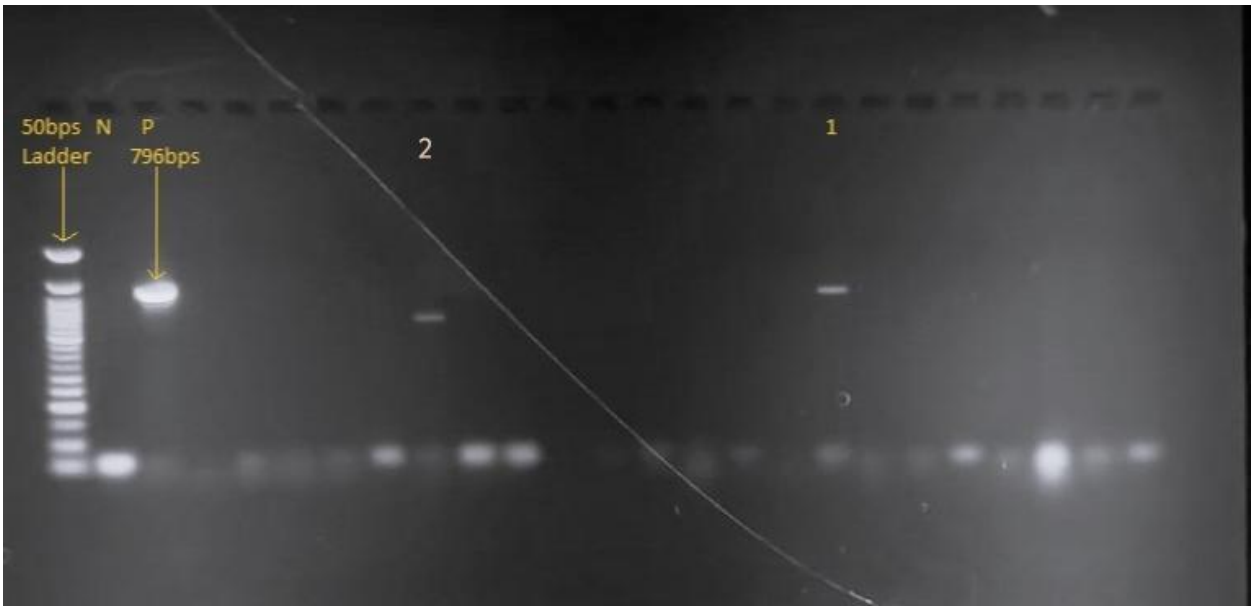

Figure 40: Salmonella spp.

Identification of *Klebsiella pneumoniae* and *Escherichia coli*

During the sampling process, a Macconkey agar plate was used to detect *Klebsiella pneumoniae* and *Escherichia coli*. Among all the isolates, 81 isolates were suspected of *Klebsiella pneumoniae* and *Escherichia coli.*

The isolation of *Klebsiella pneumoniae* was given for PCR to assure the confirmation of this bacteria using *Pr1 R, Pr2 F* primer sets. The annealing temperature of this was 57ºC for 35 cycles. The expected base pair size for this specific primer sequence was 133. The mixture volume was 13µl which included 1µl of forward and reverse primers in each.

After the gel ran in 100V with 1.5% agarose in it, the gel was visualized under UV. However, it showed some positive band size according to ladder and positive control. Though it contained 81 suspected isolates, 37 isolates confirmed the band size of 133bp. Therefore, these 37 positive band size isolates had confirmed identification of *Klebsiella pneumoniae.*

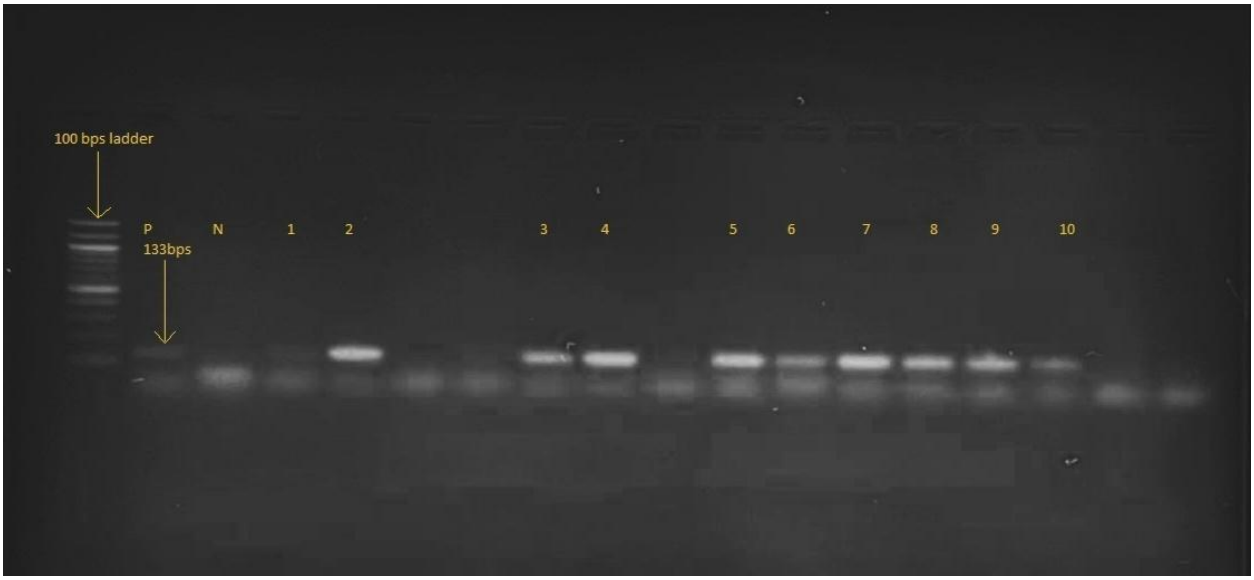

Figure 41: Gel Electrophoresis of Klebsiella pneumoniae

The isolation of *Escherichia coli* was given for PCR to assure the confirmation of this bacteria using Eco R, Eco F primer sets. The annealing temperature of this was 45ºC for 35 cycles. The expected base pair size for this specific primer sequence was 585bp. The mixture volume was 13µl which included 1µl of forward and reverse primers in each.

After the gel ran in 100V with 1.5% agarose in it, the gel was visualized under UV. However, it showed some positive band size according to ladder and positive control. Though it contained 12 isolates, it confirmed the band size of 585bp. Therefore, these 12 positive band size isolates had confirmed identification of *Escherichia coli.*

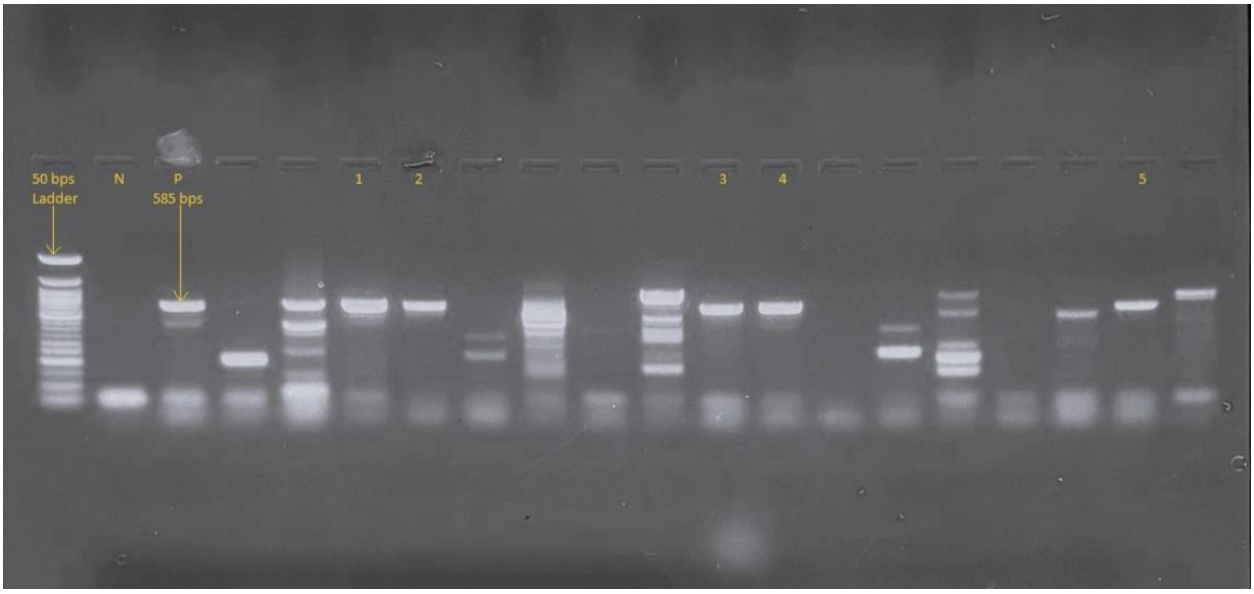

Figure 42: Gel Electrophoresis of Escherichia coli

3.4 Distribution of Bacterial isolates in different locations

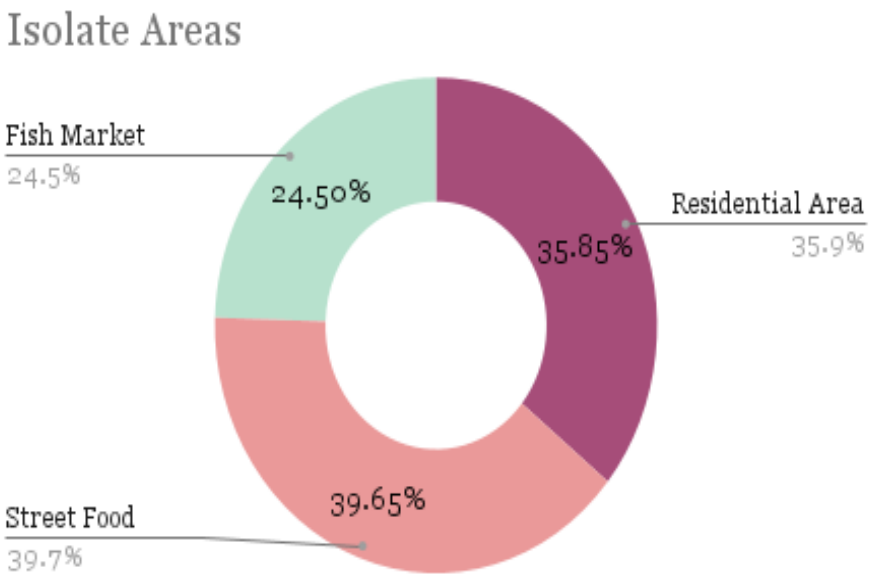

Figure 43: Percentage of Isolates according to area

The pie chart shows that from a total of 159 isolated colonies the Street Food (63) area is the most contaminated source among the sampled areas, which was 39.7%. Then followed closely by Residential Areas (57) the second largest contributor to the isolate count was 35.9%. Lastly, the Fish Market area (39) although the lowest among the three, it still represents a notable proportion of 24.5%.

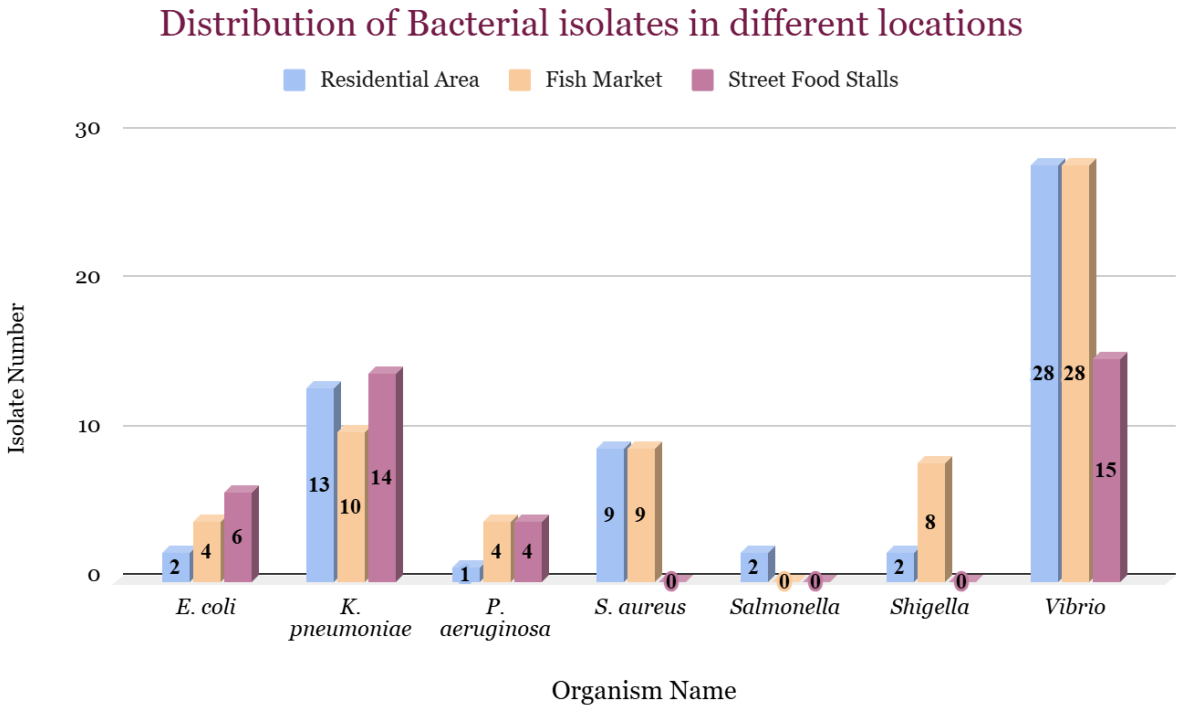

Figure 44: Isolates found in Different Areas

Table 6: Isolate percentage and number according to area

Area/Organism	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*Salmonella spp.*	*Shigella spp.*	*Vibrio spp.*
Residential Area	2 (1.26%)	13 (8.18%)	1 (0.63%)	9 (5.66%)	2 (1.26%)	2 (1.26%)	28 (17.61%)

Fish Market	4 (2.52%)	10 (6.29%)	4 (2.52%)	9 (5.66%)	0 (0%)	8 (5.03%)	28 (17.61%)
Street food	6 (3.77%)	14 (8.81%)	4 (2.52%)	0 (0%)	0 (0%)	0 (0%)	15 (9.43%)
Total	12 (7.55%)	37 (23.27%)	9 (5.66%)	18 (11.32%)	2 (1.26%)	10 (6.29%)	71 (44.65%)

The distribution of bacterial isolates from three different areas: Residential area, Street food area and fish market shows variation of bacterial growth among different organisms.

3.5 Seasonal Trend

From October-February it was observed that, when the temperature was low isolate number and identified organism through PCR were low compared to the warmer season. In the colder season there were 136 bacterial colony isolations, and among them, almost 42.65% isolates were identified. However, in warmer season from March to July there were 184 isolates and from those, almost 55% of summer season isolates were identified. This shows that seasonal variation affects bacterial growth.

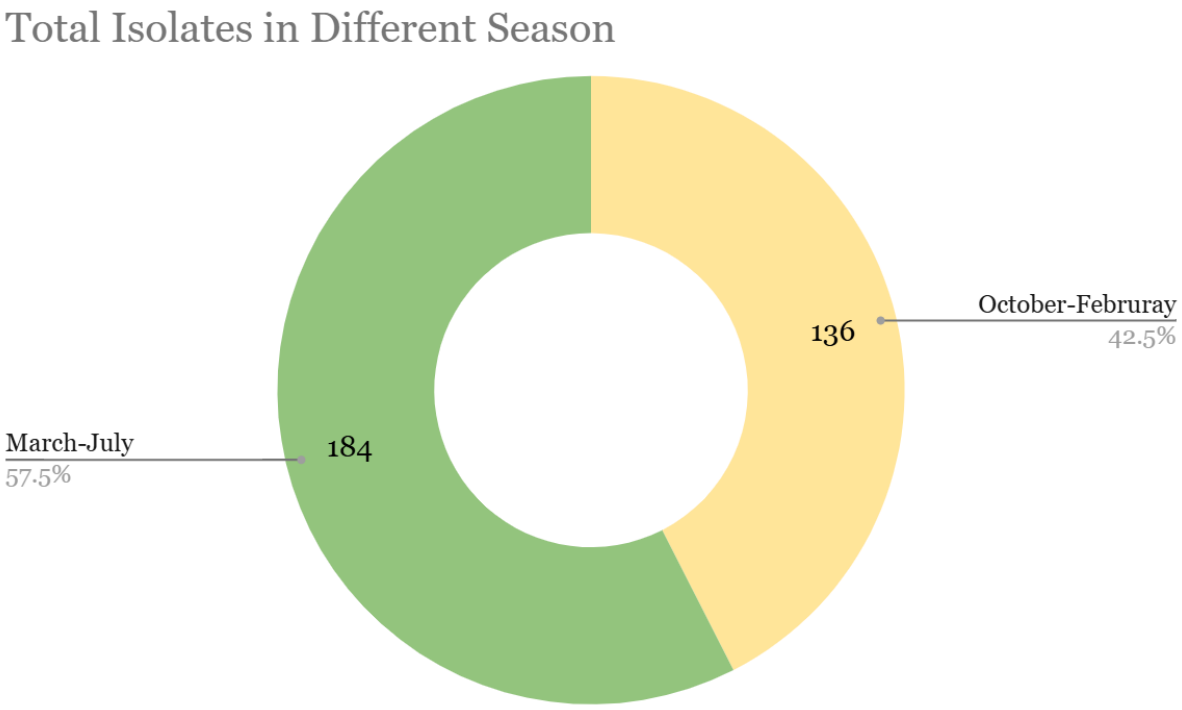

Figure 45: Isolates found in Different Seasons

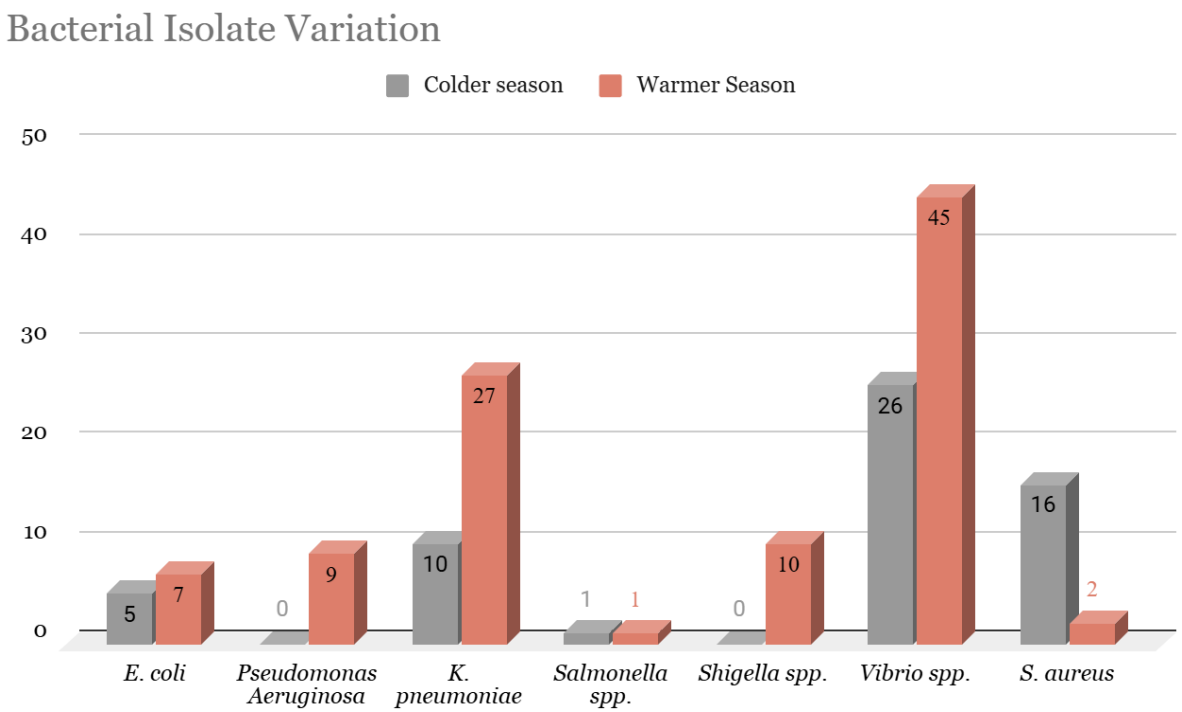

Figure 46: Isolate Variation in Different Seasons

Almost all isolates that have been confirmed through PCR showed that their growth was optimal in the warmer season compared to the colder season. Few bacteria like *Pseudomonas aeruginosa, Shigella* showed no growth (0) at low temperature, whereas *Vibrio spp*. Maintained its growth in every season 45 isolates in warmer and 26 isolates in colder season. Only two isolates of *Salmonella* can not be enough to use in the data, yet it found in both seasons (1 and 1). *E. coli* and *K. pneumoniae* also showed higher growth in warmer temperatures than in lower temperatures 27 and 7 isolates respectively. However, *S. aureus* showed a different result, where its growth showed 16 isolates from winter and only 2 isolates from summer.

3.6 Assessment of Antibiotic Susceptibility Test

Following the molecular confirmation of 159 isolates through PCR antibiotic susceptibility test was carried out using the Kirby-Bauer disk diffusion method. The resistance patterns of *Escherichia coli* isolates were analyzed and presented graphically below.

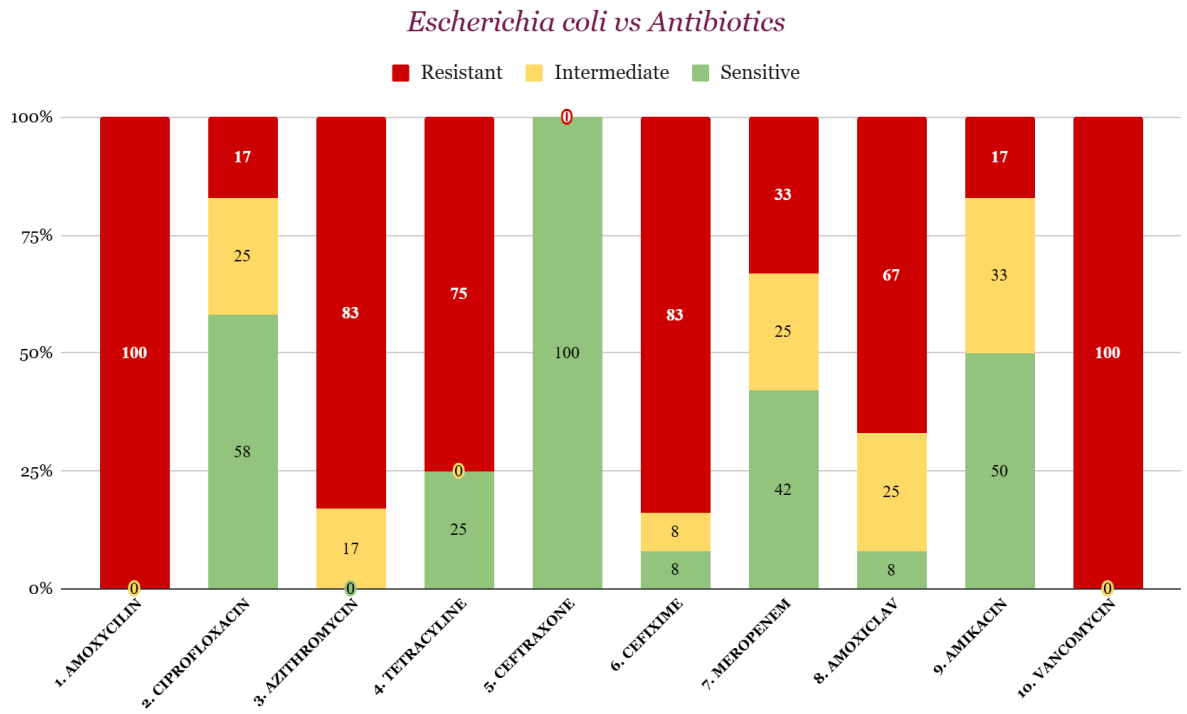

Figure 48: E. coli vs Antibiotics

The results revealed that *E. coli* exhibited 100% resistance to both amoxicillin and vancomycin. This outcome is expected, as these antibiotics primarily target gram-positive organisms, whereas *E. coli* is a gram-negative bacterium, making them ineffective. In the case of azithromycin, no isolates were found to be sensitive. Approximately 17% of the isolates showed intermediate susceptibility, while a significant 83% displayed complete resistance.

A high level of resistance was also observed for other commonly used antibiotics such as tetracycline (75%), cefixime (83%), and amoxiclav (67%), suggesting a reduced efficacy of these drugs against the tested *E. coli* strains. Among all antibiotics tested, ceftriaxone was the only one that showed 100% susceptibility, indicating its continued effectiveness against *E. coli* in this study. On the other hand, meropenem and amikacin demonstrated moderate susceptibility, suggesting they may still be effective treatment options, though resistance may be gradually emerging.

Now, the antibiotic susceptibility pattern of *Klebsiella pneumoniae* and its graphical representation are shown below

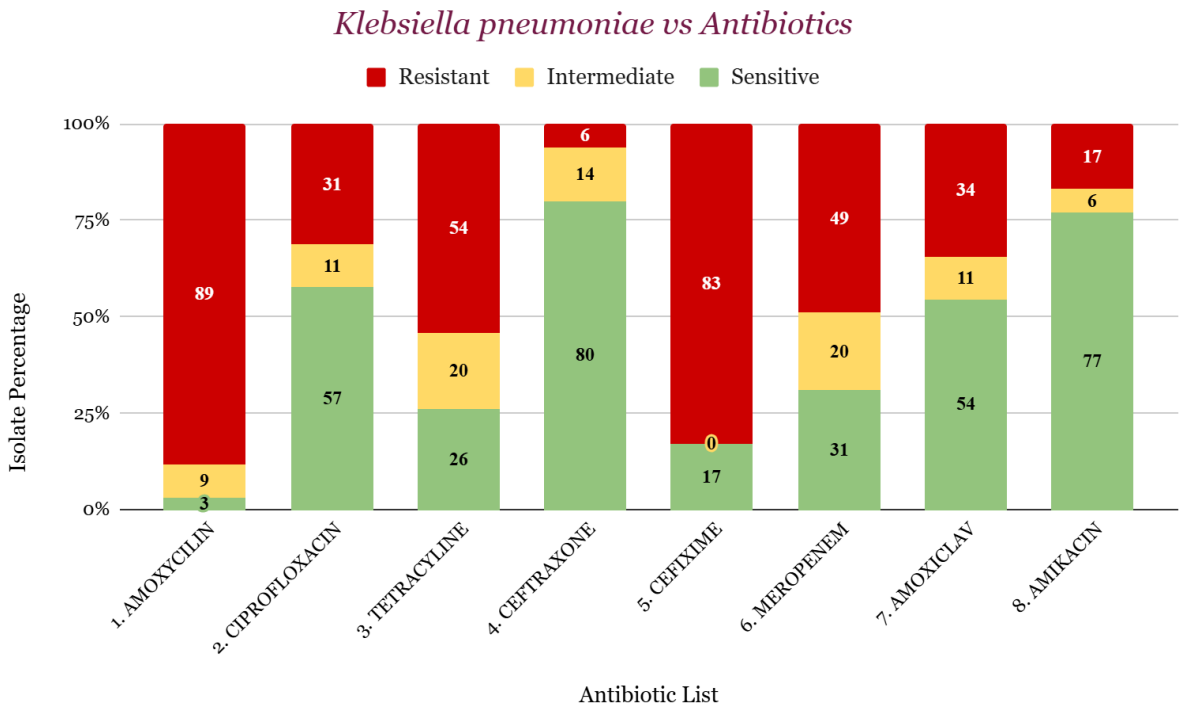

Figure 49: K. Pneumoniae vs antibiotics

Among the antibiotics tested, high resistance was observed against Amoxicillin (89%), Cefixime (83%), and Tetracycline (54%). Meropenem also showed notable resistance at 49%. On the other hand, Ceftraxone and Amikacin showed comparatively lower resistance rates, only 6% and 17%

respectively. All the antibiotics show moderate to intermediate response from 6-20%, indicating growing tolerance. Amoxiclav still demonstrated 54% susceptibility, suggesting partial effectiveness among all tested antibiotics.

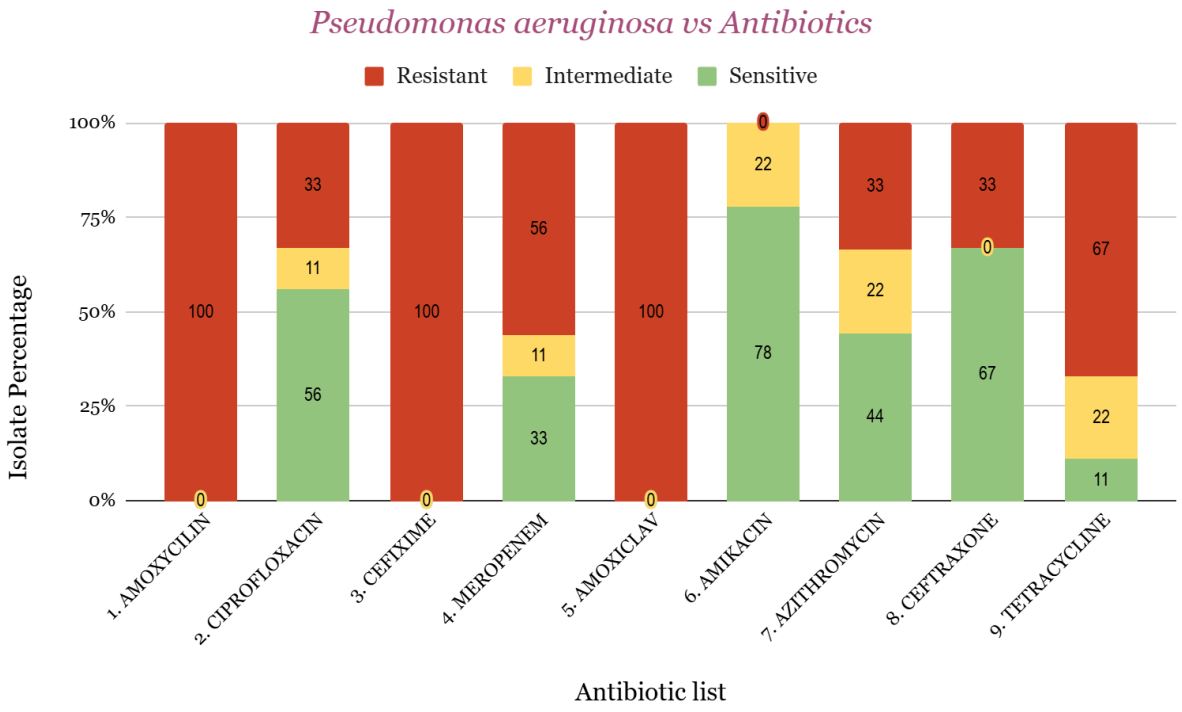

Figure 50. P. aeruginosa vs antibiotics

The antibiotic susceptibility profile of *Pseudomonas aeruginosa* isolates is illustrated in the figure above.

All isolates (100%) were completely resistant to amoxicillin, cefixime, and amoxiclav. This high resistance is expected, amoxicillin, as amoxicillin is primarily effective against gram-positive bacteria, while *P. aeruginosa* is gram-negative. Cefixime and amoxiclav, though broader spectrum, also showed no effectiveness against this species. Tetracycline showed 67% resistance

and only 11% sensitivity against *Pseudomonas,* indicating this antibiotic is soon going to be out of date.

Ciprofloxacin showed moderate activity, with 56% of isolates being sensitive. However, a significant 33% were resistant, and 11% showed intermediate sensitivity. This pattern suggests a potential decline in ciprofloxacin's efficacy, which is critical considering ciprofloxacin is often used to treat infections caused by *Pseudomonas*. Azithromycin and ceftraxone also showed a similar moderate activity where both antibiotics were 33% resistant against the bacteria and showed 44% and 67% sensitivity, respectively.

Meropenem demonstrated mixed results as well. While 33% of isolates were sensitive to this antibiotic, 56% were resistant, and 11% were intermediate. As meropenem is a last-resort antibiotic for multidrug-resistant infections, its reduced effectiveness against *P. aeruginosa* is alarming.

Amikacin was the only antibiotic showing substantial effectiveness where a total of 78% of the isolates were sensitive, 22% were intermediate, and no resistance was observed. This indicates that it still holds therapeutic value against *Pseudomonas aeruginosa* infections. This data shows a concerning level of resistance across most antibiotics tested.

Based on the graphical representation below, *Shigella spp*. demonstrates variable antibiotic susceptibility patterns across the tested antimicrobials.

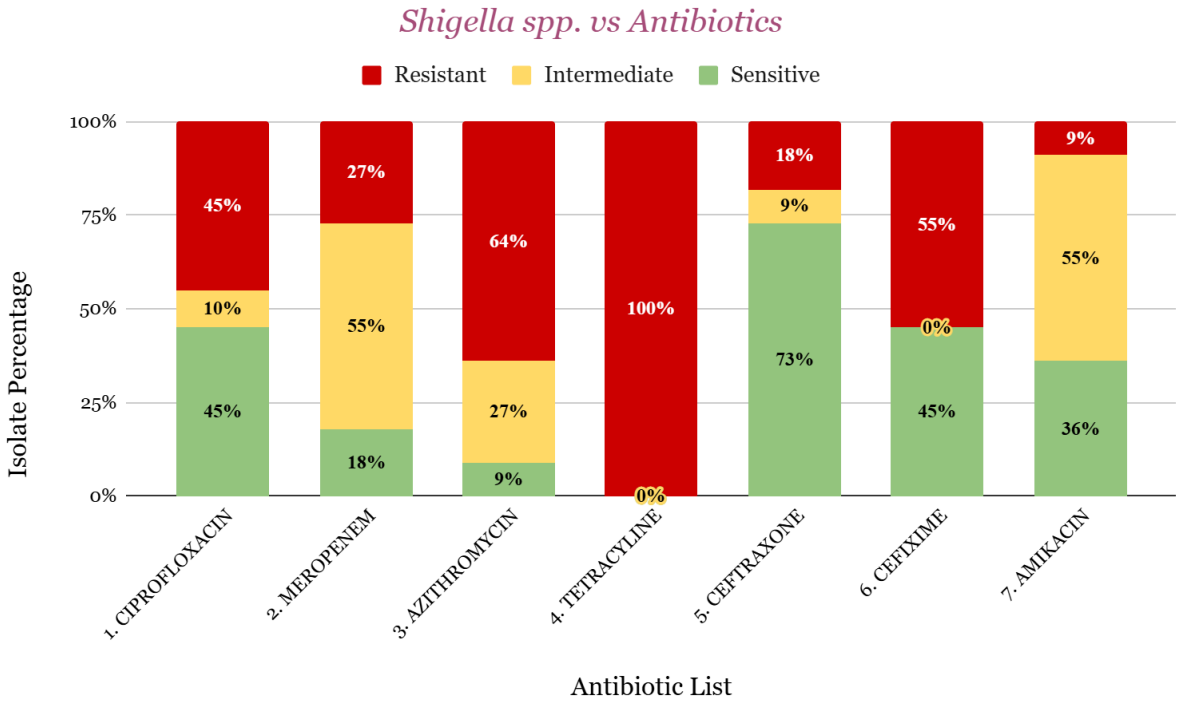

Figure 51:Shigella spp. vs antibiotics

Shigella shows 100% resistance to tetracycline. For ciprofloxacin, *Shigella* exhibits 45% resistance with an equal 45% susceptibility and 10% intermediate response, indicating moderate effectiveness. Meropenem presents a concerning resistance profile with 27% resistant isolates, 55% showing intermediate susceptibility, and only 18% fully sensitive. It clearly suggests that the efficacy of meropenem is being compromised. Azithromycin shows high resistance at 64% with minimal intermediate responses 9% and 27% susceptibility. In contrast, ceftriaxone demonstrates the most promising results with 73% of *Shigella* isolates showing complete susceptibility. Moreover, amikacin and cefixime show moderate results. Both are still sensitive showing 36% and

45% sensitivity against *Shigella,* respectively. However, amikacin raises a concern with 55% intermediate response of *Shigella* isolates, with 9% of resistance, and cefixime with 55% of resistance.

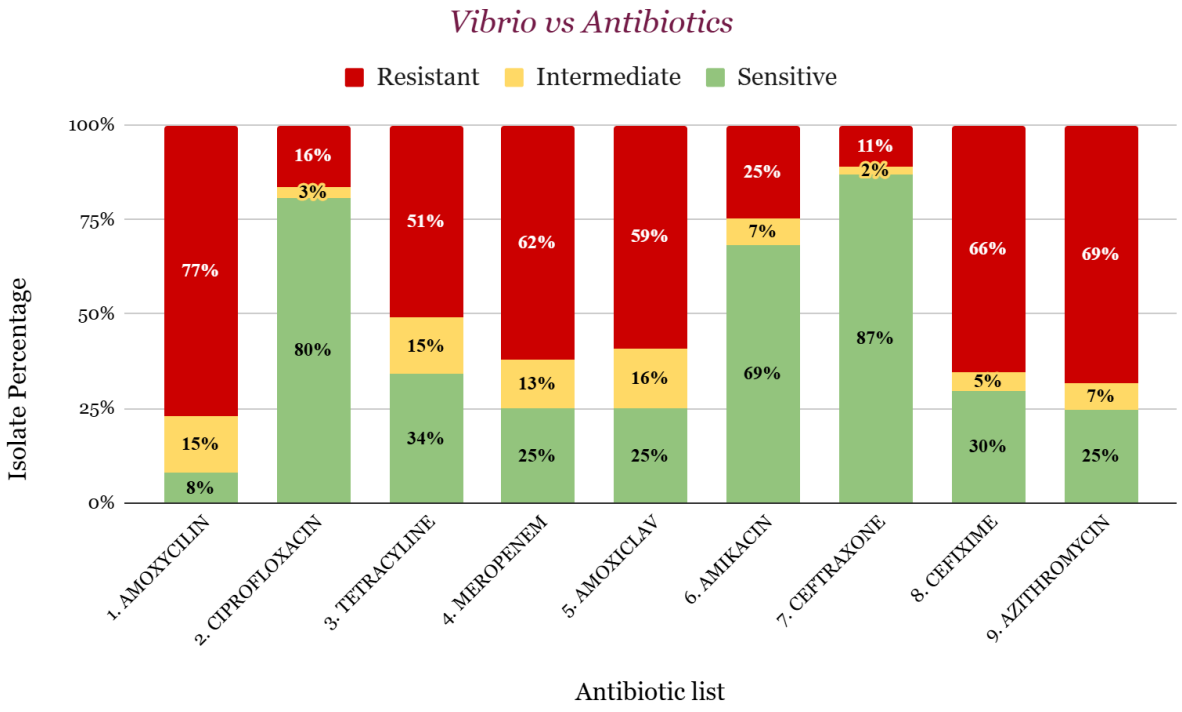

Figure 52: Vibrio vs antibiotics

Based on the graphical representation of *Vibrio* isolates, distinct antibiotic susceptibility patterns can be observed. Amoxicillin shows high resistance at 77% with only 15% susceptibility and 8% intermediate response, indicating poor therapeutic efficacy.

Ciprofloxacin and ceftraxone demonstrate promising results with 80% and 87% of *Vibrio* isolates showing complete susceptibility respectively, only 16% and 11% resistance, and 3% and 2% intermediate response, respectively, making it a highly effective while amikacin shows a favorable

profile with 69% susceptibility. Meropenem shows concerning resistance patterns with 62% resistant isolates, 25% susceptibility, and 13% intermediate response, suggesting limited efficacy.

Amoxiclav and Tetracycline present moderate results with 59% and 51% resistance, 25% and 34% susceptibility, and 16% and 15% intermediate response, respectively. These findings suggest that ciprofloxacin and amikacin represent the most viable therapeutic options for *Vibrio* infections, whereas amoxicillin shows predictably poor efficacy.

Moreover, the organism demonstrates similar 66% and 69% resistance to cefixime and azithromycin, respectively with 5% and 7% of intermediate response, which is alarming for *Vibrio.*

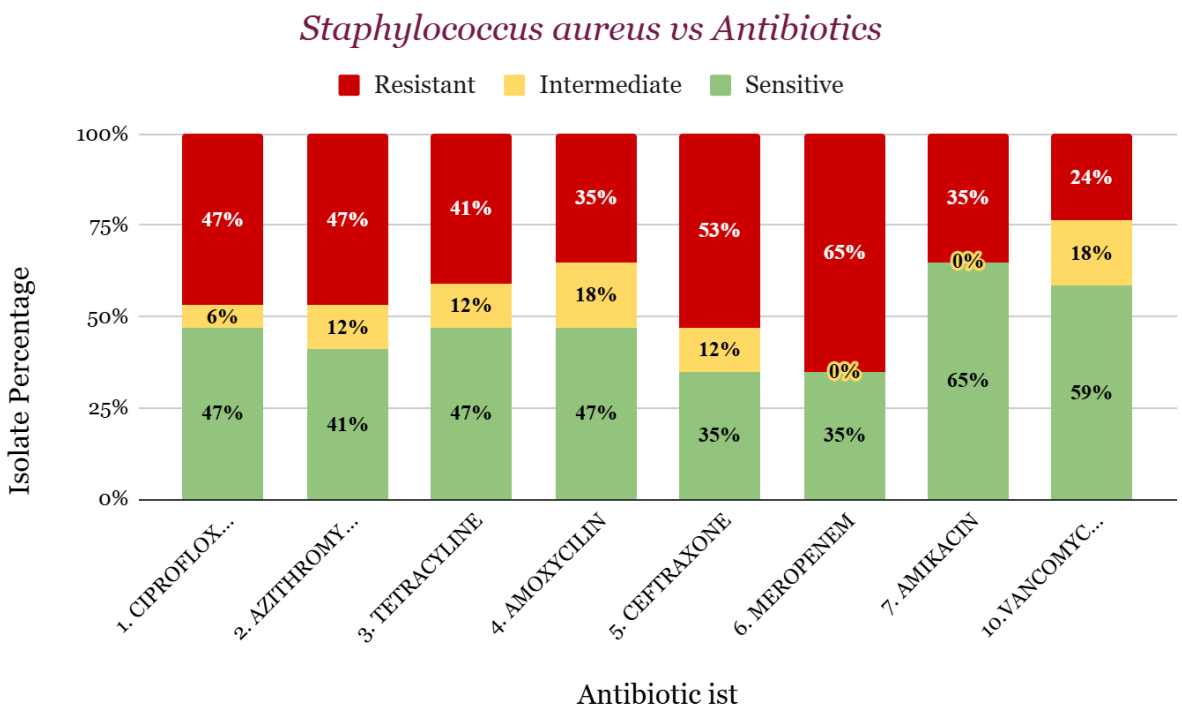

Figure 53: S. aureus vs antibiotics

Based on the graphical representation of *Staphylococcus aureus* isolates, similar antibiotic resistance patterns are observed across the three tested antibiotics. Ciprofloxacin shows 47%

susceptibility and 47% resistance with only 6% intermediate response, indicating moderate effectiveness against *S. aureus.*

Azithromycin and amoxicillin demonstrate 41% and 47% susceptibility, 47% and 35% resistance along with 12% and 18% intermediate response, respectively showing almost similar effectiveness compared to ciprofloxacin. Tetracycline exhibits 47% susceptibility, 41% resistance, and 12% intermediate response, making it a moderate treatment option.

As *Staphylococcus aureus* is a gram positive bacteria and to it vancomycin demonstrated a slightly positive result where it is 59% sensitive, 18% intermediate response along with 24% resistance. Amikacin showed the most promising result, showing no intermediate response but 65% sensitivity and 35% resistance against *S. aureus.*

Almost all antibiotics show high resistance rates of approximately 35-47%, which indicates serious resistance problems in *S. aureus* isolates.

The following graphical representation of *Salmonella* isolate represents results from only a single isolate; therefore, comparative analysis and generalized conclusions cannot be drawn from this limited dataset. However, this individual *Salmonella* organism showed resistance to vancomycin, azithromycin, tetracycline, and meropenem, while demonstrating susceptibility to ciprofloxacin, ceftriaxone, amikacin, and amoxiclav.

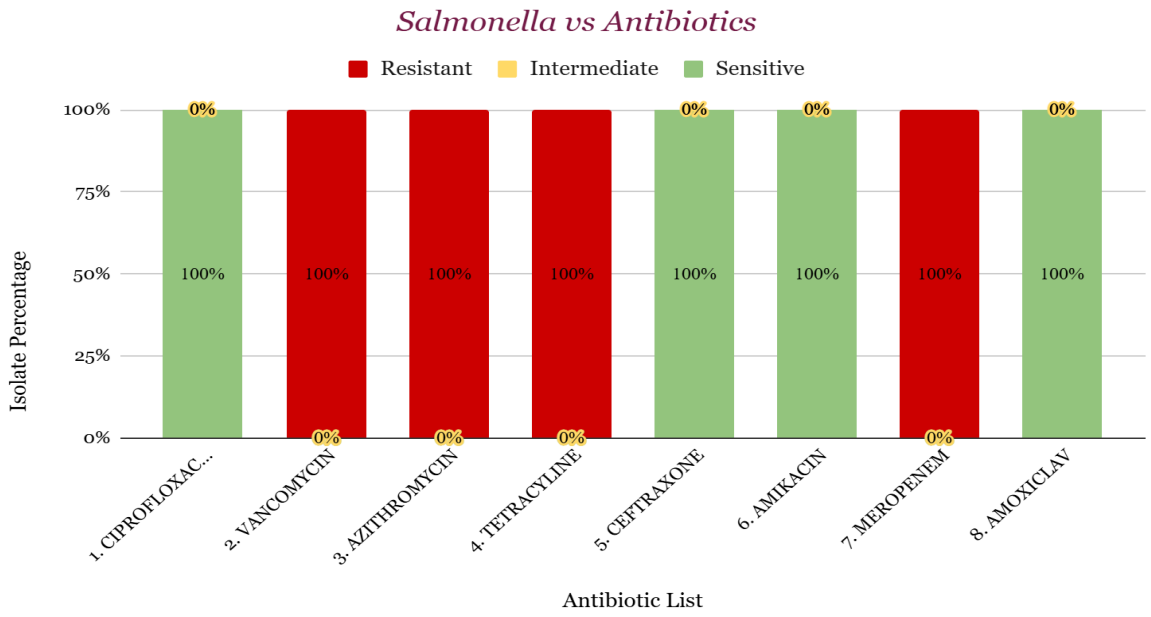

Figure 54: Salmonella vs Antibiotics

The resistance to vancomycin is expected as it primarily targets gram-positive bacteria while *Salmonella* is gram-negative. While these findings provide insight into the antimicrobial profile of this specific organism, larger sample sizes would be necessary to make meaningful epidemiological assessments.

Chapter 3:

Discussion

4.1 Discussion

This research successfully isolated and identified a diverse range of bacterial species from houselies collected across seven major areas of Dhaka city. From 53 collected houseflies 320 bacterial colonies were initially isolated using selective and differential media. 159 isolates were confirmed through PCR analysis at the molecular level. The bacterial species identified includes organisms like *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio spp., Salmonella spp.,* and *Shigella spp.*

The use of various selective media has proved effective in targeted bacterial isolation. MacConkey agar successfully isolated gram negative enteric bacteria while MSA used to isolate *Staphylococcus aureus* based on its salt tolerance and fermentation capabilities (Raymond, 2022). TCBS agar showed high selectivity for *Vibrio* species as its high pH suppresses the competing growth of other intestinal bacteria (Aryal, 2022). Apart from yellow colonies suspected as *Vibrio cholerae* and green colonies suspected as *Vibrio parahaemolyticus*, there has been some growth for black centered colonies in the TCBS agar, which remained unidentified. Furthermore, XLD agar helped differentiate *Salmonella* and *Shigella* species based on their distinct biochemical characteristics.

A notable finding was the seasonal variation observed in bacterial isolation patterns as this study has been carried out in two parts October-February, and March-July. *Pseudomonas aeruginosa* showed complete absence during October-February or winter months but thrived during the summer season in Bangladesh. A study by LaBauve and Wargo (2012) stated that *Pseudomonas aeruginosa* shows satisfactory growth at 37°C. The study also says that this bacteria can also survive a broad range of temperatures from 4°C to 42°C and temperatures highly affect its growth

and virulence, as below 30°C some virulence pathways can stay inactive. This seasonal pattern aligns with characteristics of *Pseudomonas*.

On the other hand *Staphylococcus aureus* exhibited a completely opposite scenario. The optimal temperature for *S. aureus* to grow is between 37°C and 40°C, and it can grow at temperatures as low as 7°C (Missiakas & Schneewind, 2013). But in colder temperatures, almost 16 isolates were identified as *S. aureus* but very minimal growth was exhibited in MSA agar at summertime and there were only 2 isolates during that period of time. Apart from temperature, other technical aspects can be the reason for this mismatch. These observations show consistency with previous studies indicating that environmental temperature and humidity significantly influence bacterial survival and transmission patterns (Bahrndorff et al., 2017). The tropical climate of Bangladesh, with its distinct wet and dry seasons, creates varying conditions that affect both housefly activity and bacterial viability on their external surfaces.

E. coli, K. pneumoniae, and *Vibrio* showed their presence in both warmer and cooler seasons. Indicating their growth in the environment is not limited by temperature and can impose a health risk throughout the year.

In the Residential Area the highest isolate here was *Vibrio spp.* (28), indicating possible water contamination (CDC, 2024). *Klebsiella spp*. (13) and *Staphylococcus aureus* (9) were also notable, suggesting poor hygiene in the household environment (Taonga Mwapasa et al., 2024, Knox et al., 2012). A study done by Nazari et al., 2017 among the all sample found a notable amount of isolates including *E. coli* and *E. coli* is an indicator organism that indicates the presence of fecal coliform in water sample. And *Vibrio spp.* is one of the most renowned fecal coliforms of all.

In Fish Market, the fish market showed a high presence of *Vibrio spp.* (28) and *Shigella spp.* (8), both commonly associated with aquatic or raw food sources (CDC, 2024, Schneider et al., 2019). The presence of *S. aureus* (9) and *Klebsiella peumoniae* (10) indicates unclean handling practices during fish processing (Bujjamma & Padmavathi, 2015). A study Sobur et al., 2019 shows that the fish market of Dhaka city was had around 56.7% of *Salmonella spp.* but this study could not find any isolates of *Salmonella spp.* in the reported fish markets of Dhaka city.

In the Street Food, *Klebsiella peumoniae* (14) and *E. coli* (6) were most common in street food samples, pointing to fecal contamination (Bagley & Seidler, 1977, EPH-DW--Drinking Water--4200, 2025). *Vibrio spp.* (15) and *Pseudomonas aeruginosa* (4) also reflect poor food hygiene and unsafe handling (Health, 2025). A study done by Eshita Shahanaz et al., 2025 shows that mechanically and/or biologically transmitted bacterial pathogens such as *Salmonella enterica, Escherichia coli, Listeria monocytogenes, Klebsiella,* and *Campylobacter spp.*

The high prevalence of multidrug resistant bacteria isolated from houseflies confirms their significant role as mechanical vectors in urban disease transmission. The resistant patterns observed a high rate of resistance to commonly used antibiotics like tetracycline, azithromycin, amoxilin, reflecting the broader antimicrobial resistance crisis in Bangladesh's healthcare system.

The detection of meropenem resistance in *P. aeruginosa and K. pneumoniae* is particularly concerning as this antibiotic represents the last resort carbapenem medicine option for severe infections (Daoud & Dropa, 2023). These studies align with previous studies from Bangladesh showing increasing prevalence of carbapenem-resistant Enterobacteriaceae in clinical settings (Sobur et al., 2019). On the other hand the consistently higher resistance rates (35%-47%) across all antibiotics indicate serious antimicrobial resistance problems in *S. aureus* populations, with no

single antibiotic showing clear superiority. Furthermore, *Shigella* isolates demonstrated 100% resistance to tetracycline and *Pseudomonas aeruginosa* exhibited the resistance profile, with 100% resistance to cefixime, and amoxiclav making the antimicrobial resistance problem most concerning.

The findings of this study align with a previous research done in Dhaka, Bangladesh by Tabassum and Chowdhury (2023) indicating the alarming presence of MDR bacteria in houseflies. Similar to this research observation of high resistance rates in *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *Vibrio*, the study by Tabassum and Chowdhury (2023) reported high resistance to commonly used antibiotics such as amoxicillin, tetracycline, and erythromycin among isolates like *Serratia marcescens*, *Proteus* spp., *Vibrio* spp., *K. pneumoniae*, and *P. aeruginosa*. Notably, their study showed gram positive bacteria were 23.68% resistant, 78.68% sensitive along with 2.64% intermediate response to vancomycin. However, this current study showed higher resistance (24%), much lower sensitivity (59%), and an alarming range of intermediate response (18%) to vancomycin of *S. aureus* isolates.

P. aeruginosa exhibited 100% resistance to amoxiclav, amoxicillin and cefixime in this study. While previous study showed 50%, 80% and 80% resistance to amoxiclav, amoxicillin and cefixime respectively indicating a public health threat. *K. pneumoniae* and *Vibrio spp*. Both showed higher resistance to Amoxicillin, tetracycline, amoxiclav, and amikacin (89%, 54%, 34%, 17%) compared to the previous study, respectively (77%, 51%, 25%, 59%).

If compared with another study by Zuhora et al. (2023) which was conducted in Dinajpur, Bangladesh with the current study's findings, then both reveal a concerning level of antibiotic

resistance among gram-negative bacteria, yet several differences in susceptibility patterns are notable. In this research, *E. coli* showed complete resistance (100%) to amoxicillin, vancomycin, and 83% to azithromycin, with ceftriaxone emerging as the only antibiotic to which all isolates remained fully susceptible (100%). On the other hand, Zuhora et al. observed *E. coli* as 100% sensitive to both ciprofloxacin and azithromycin, which sharply contrasts with the current study where azithromycin resistance reached 83%. For *Pseudomonas aeruginosa*, the current study found absolute resistance to amoxicillin, and cefixime, while amikacin showed strong activity. In contrast, Zuhora et al. reported 100% resistance to ciprofloxacin and gentamicin, but notable sensitivity to amoxicillin, tetracycline, and vancomycin which highlights significant differences. While both studies acknowledge the presence of multidrug resistance of these bacteria together, both studies highlight the ongoing threat posed by antimicrobial resistance.

If compared with global study then a research done by Odetoyin et al. (2020) in Nigeria with the current study, the findings from this comparison also highlight a serious problem of MDR. In both studies, *Pseudomonas aeruginosa* showed 100% resistance to amoxicillin, supporting the strong resistance trend seen in gram negative bacteria. In terms of *E. coli*, both studies found high resistance to amoxicillin/augmentin, with the other study showing 90.3% resistance to augmentin. In the current study, amoxicillin resistance in *E. coli* was 100%, which shows an even more serious resistance issue. However, the other study reported only 3.2% resistance to ciprofloxacin in *E. coli*, whereas the current study noted *E. coli* to have a mixed response to ciprofloxacin, with resistance present 17% along with 35% intermediate response.

Regarding *Klebsiella pneumoniae*, the other study found only 5.9% resistance to ciprofloxacin, suggesting that this antibiotic remains effective. In contrast, the current research found 49%

resistance to meropenem and high resistance to amoxicillin and cefixime, with only 6% resistance to ceftriaxone highlighting a much broader resistance pattern.

If research conducted in Iran by Nazari et al. (2017) and this study is compared then it shows both similarities and differences in resistance patterns for key bacteria. For instance, in the case of *E. coli*, the current research found 100% resistance to amoxicillin and vancomycin, along with high resistance to azithromycin (83%), tetracycline (75%), and cefixime (83%). The hospital housefly study by Nazari et al. (2017) reported lower resistance rates, such as amoxicillin (45.8%), tetracycline (25%), cefotaxime (79.1%), and ciprofloxacin (70.8%).

In the case of *Klebsiella pneumoniae,* the hospital study observed 100% resistance to cefotaxime, 56.2% to ampicillin, and 81.2% to ciprofloxacin, which aligns with the current findings where 89% were resistant to amoxicillin, 83% to cefixime, and moderate resistance to ciprofloxacin and meropenem was noted. Interestingly, the current study found low resistance to ceftriaxone (6%) and amikacin (17%), which could suggest these are still relatively effective options.

For *Pseudomonas aeruginosa,* both studies reported high resistance to beta-lactam antibiotics like amoxicillin/ampicillin and cephalosporins. In the current study, *P. aeruginosa* showed 100% resistance to amoxicillin, cefixime, and amoxiclav, and 56% resistance to meropenem. In contrast, the hospital data found 66.6% resistance to cefotaxime, 43.4% to ciprofloxacin, and low resistance to tetracycline and gentamicin. One of the most positive findings in the current study was 78% sensitivity to amikacin, a drug not covered in the Hamadan University study.

As for *Staphylococcus aureus*, the current study showed almost equal levels of resistance and susceptibility across the three antibiotics tested (ciprofloxacin, azithromycin, tetracycline and they are all around 41–47% resistance). On the other hand, the hospital study found much lower

resistance levels like tetracycline (16%), ampicillin (16%), and ciprofloxacin (60%), suggesting that the *S. aureus* isolates from flies might be under more selective pressure or environmental exposure to antimicrobials.

The presence of these extensively multidrug-resistant bacteria on houseflies poses significant public health risks, especially in overly populated urban areas like Dhaka, where sanitation challenges are extreme. Houseflies can transfer these resistant pathogens from contaminated environments to dining areas, potentially contributing to foodborne illness outbreaks and further spreading of resistance genes by HGT.

4.2 Study Limitations and Future Directions

This study has some limitations that should be addressed in future research. One key limitation is the small sample size, especially for *Salmonella*, where only two isolates were confirmed by PCR. This limited the ability to analyze resistance patterns properly. Future studies should include larger samples collected from various locations and seasons to get more reliable data.

Furthermore, another gap was the lack of testing for virulence genes. While for *S. aureus*, some *Nuc* virulence genes were detected using PCR, other important genes in *E. coli*, *Salmonella*, and *Shigella* were not investigated. Future research should use multiplex PCR to detect a broader range of virulence factors, especially those linked to diarrheagenic and invasive strains.

Long-term research is required to study seasonal changes in microbial populations and to determine how environmental factors such as temperature and humidity affect pathogen levels. Future research should also look into investigating gene transfer across bacteria on the housefly surface and gut, which can reveal more about how resistance spreads.

4.3 Conclusion

This study mainly showed how common houseflies (*Musca domestica*) can carry and spread different types of bacteria. In recent times, houseflies have been seen as possible carriers of both pathogenic and non-pathogenic bacteria. They can spread these microorganisms through their body surfaces. Because flies often land on non-sanitised places like garbage, food waste, street food, and fish markets etc., they can pick up and spread bacteria as a mechanical vector. Some of these bacteria may even carry genes that make them resistant to antibiotics.

In this study, several bacteria were confirmed using PCR, including P*seudomonas aeruginosa, Vibrio* species, *Klebsiella pneumoniae*, *Staphylococcus aureus, Salmonella* species, *Shigella* spp., and *Escherichia coli.* Many of these are known to cause infectious diseases, which can be fatal.

Antibiotic susceptibility testing showed that some of the bacteria were resistant to commonly used antibiotics. This indicates that houseflies might not only spread bacteria but also help in spreading antibiotic-resistant ones. Since the study is still ongoing and some bacteria have not yet been identified, more lab tests will be needed. These will help confirm the remaining bacteria and also help understand how the resistance happens. In the future, this information can help raise awareness and improve hygiene practices, especially in places where food is prepared or sold, to reduce the risk of spreading these harmful bacteria through houseflies.

Chapter 4:

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