Occurrence and Antibiotic Resistance Pattern of Gram-Negative Bacteria Isolated from Restaurant Table Surfaces

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A thesis submitted to the Department of Microbiology in

partial fulfillment of the requirements for the degree of

Bachelor of Science in Microbiology

Department of Microbiology

School of Life Sciences

BRAC University

January, 2026

Declaration

It is hereby declared that

1. The thesis submitted titled "Occurrence and Antibiotic Resistance Pattern of Pathogenic Bacteria Isolated from Restaurant Table Surfaces in Dhaka Metropolitan Area" is our original work while completing our degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

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In Spring, 2025 it has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology in Spring, 2025.

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Ethics Statement

All specimens are collected at some of the selected locations as per the safety practice and ethical procedures. The study was conducted using the institutional biosafety regulations at BRAC University Life Sciences Laboratory. The ethical principles, which are outlined in the Declaration of Helsinki and its amendments, were observed in compliance with the research protocol wherever necessary. Because there were no human subjects, human biological materials, or personal information as well as animal trials involved in this study, no formal ethical consent of human or animal study was to be adopted.

Abstract

Pathogenic bacteria present on frequently touched surfaces represent a significant public health concern, particularly due to their potential to spread antibiotic-resistant infections. This study aimed to isolate and characterize pathogenic bacteria from restaurant (15 restaurant table surfaces) in Dhaka city and to evaluate their antibiotic susceptibility patterns. A total of *Acinetobacter, Escherichia coli* (N=5), *Salmonella* spp. (N=6), *Shigella* spp. (N=15), *Vibrio* spp. (N=14), *Klebsiella pneumoniae* (N=18), and *Staphylococcus aureus* (N=1) were analyzed against six commonly used antibiotics: Vancomycin, Amoxicillin, Azithromycin, Cefepime, Kanamycin, and Tetracycline. All Gram-negative isolates demonstrated 100% resistance to Vancomycin, indicating its ineffectiveness against these organisms. High resistance to Amoxicillin was observed among *E. coli* (80%), *Salmonella* spp. (83%), *Shigella* spp. (87%), *Vibrio* spp. (64%), and *K. pneumoniae* (83%). In contrast, Kanamycin, Cefepime, and Tetracycline showed comparatively higher effectiveness, particularly against *E. coli*, *Shigella* spp., *Vibrio* spp., and *K. pneumoniae*. *Klebsiella pneumoniae* exhibited the highest sensitivity to Cefepime (84%), while *Shigella* spp. showed notable sensitivity to Kanamycin (80%) and Cefepime (74%). Intermediate susceptibility was most prominent for Azithromycin and Cefepime across several isolates, especially in *Salmonella* spp. and *Vibrio* spp. The single *Staphylococcus aureus* isolate showed complete resistance to Vancomycin and Amoxicillin but remained fully sensitive to Azithromycin, Cefepime, and Tetracycline. Although identification and resistance profiling were based on conventional microbiological and phenotypic methods, molecular confirmation using polymerase chain reaction (PCR) targeting species-specific genes and antibiotic resistance determinants would further enhance the accuracy and reliability of pathogen identification. Overall, the findings highlight the presence of multidrug-resistant pathogenic bacteria on restaurant table surfaces, emphasizing the need for improved hygiene practices, molecular surveillance, and continuous monitoring of antibiotic resistance to reduce public health risks.

Keywords: Pathogenic bacteria, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Klebsiella pneumoniae*, *Staphylococcus aureus, Acinetobacter* table surface, antibiotic resistance, antibiotic susceptibility pattern, PCR.

Acknowledgment

First and foremost, we would like to express our gratitude to Almighty Allah for granting us the opportunity to embark on this amazing journey to conduct this research.

We are grateful to the Chairperson of the Department of Mathematics and Natural Sciences and our respected Supervisor, Professor and supervisor Dr. Nadia Sultana Deen, Department of Microbiology, BRAC University, for always appreciating and encouraging us to complete our undergraduate thesis and also for her consistent supervision, assistance, constructive criticism, dedicated involvement, and active participation in pursuing current ideas and never-ending motivation throughout the duration of our research work. We would like to express our heartfelt gratitude to her; without her invaluable cooperation and assistance, this paper would not have been accomplished.

We would like to express our sincere appreciation to all of the laboratory assistants of BRAC University Microbiology & Biotechnology Laboratory including **Mahmudul Hasan** for their continuous support and cooperation in conducting laboratory work to complete our thesis work.

We would like to give our special thanks to our mentor, **Nazifa Tasnim**, for her continuous encouragement and attentive direction which made our journey through this research much smoother. Finally, our warmest gratitude goes to my friend **Aflatun Zaman** for her generous help and company throughout our thesis journey.

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List of Acronyms

µL	Micro-liter
AST	Antibiotic Susceptibility Test
BT	Biochemical Test
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
MAC	MacConkey Agar
MDR	Multidrug-resistant
MHA	Muller Hinton Agar
Mm	Millimeters
NA	Nutrient agar
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
Spp.	Species
SS	Salmonella Shigella Agar
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TCBS	Thiosulfate citrate bile salts sucrose agar
TE	Tris-EDTA
XLD	Xylose Lysine Deoxycholate Agar
ZOI	Zone of Inhibition

Chapter 1

1 Introduction

1.1 Background

Food contact surfaces come in contact with raw, processed, and finished products while processing any food products where the incorrect material selection and design for equipment construction, as well as inappropriate cleaning procedures for installed equipment, can become a gateway to microbes from raw materials or other sources. Food contamination is a situation where some harmful microorganisms, chemicals or foreign materials are added to food making them unsafe to consume. This contamination may occur during any of the food production stages, processing, storage, or handling. The most frequent sources of foodborne diseases as well as food spoilage are microbial contamination, which can be caused by bacteria, viruses, or fungi. The risk of contamination further increases by the surfaces, equipment or food handlers cross-contaminating (Singh et al., 2024). These inappropriate cleaning procedure, uncleaned equipment of cooking, such as - utensils, knives, cooking aprons, refrigerators, microwaves, scissors, mixer, deep fryer, surface of table can become the source of pathogenic organism whose are very harmful for people due to lack of maintaining hygienist and proper cleaning. Nowadays from children to adults many people rely on restaurant food for their daily meat. If people are continuously having contact with these unhygienic restaurant table surfaces and eating unhealthy food then they can be infected by this pathogenic microorganism. That can cause serious health issues and some may lead to death.

In recent years, researchers have identified some pathogenic microorganisms from various sources which are also multi-antibiotic-resistant pathogens (Akter et al., 2024, Melebari, 2023, Bukhari et al., 2021). Akter et al reported that there was the presence of some pathogenic bacteria of *Escherichia coli* (50%), *Staphylococcus aureus* (33.4%), *Pseudomonas aeruginosa* (33.4%), *Vibrio cholerae* (23.4%) on the surface of food area and equipment in many restaurants near Sadarghat launch station in Dhaka (Akter et al., 2024). Furthermore, Melebari reported that he had identified some foodborne- illness causing microorganism of about *Staphylococcus aureus* (43.12%), *Staphylococcus epidermidis* (42.2%), *Salmonella spp* (6.42%), *Klebsiella aerogenes* (4.58%), *Escherichia coli* (2.75%) from the local restaurants in Al-Mandaq City,

Saudi Arabia (Melebari, 2023). Moreover, Bukhari et al found microbial contamination of about *Klebsiella* spp. (18.7%), *Escherichia coli* (17.7%), *Staphylococcus aureus* (4.4%), *Pseudomonas* spp. (1.7%), *Proteus spp*. (0.7%), *Bacillus cereus* (0.7%), *Candida* sp. (0.3%) in restaurants in Makkah City (Bukhari et al., 2021). These pathogenic microorganisms can cause several food-borne diseases like abdominal cramps, food poisoning, hemolytic-uremic syndrome (HUS) , bacterial gastroenteritis, and reactive arthritis. And also, death.

Additionally, Akter et al have also discussed how those pathogenic bacteria were multi-antibiotic resistant. *Pseudomonas aeruginosa* (100%) showed the highest resistance phenomenon to ampicillin and tetracycline, *Vibrio cholerae* (100%) to ampicillin, *Escherichia coli* (80%) to ampicillin and streptomycin, and *Staphylococcus aureus* (100%) to ciprofloxacin. All of the examined isolates of *Pseudomonas aeruginosa* were found to be most sensitive to piperacillin-tazobactam, *Escherichia coli* to imipenem and nitrofurantoin, *Staphylococcus aureus* to gentamicin, amikacin, tetracycline and chloramphenicol, and *Vibrio cholerae* to piperacillin-tazobactam, imipenem and amikacin.

On the other hand, Melebari focused on the fact that among the sample collection from the restaurants the highest number of pathogenic isolates was observed in raw foods, followed by samples collected from the tools. Of the bacterial counts, *Staphylococcus aureus* contributed 43.12%, followed by *Staphylococcus epidermidis* (42.2%), *Salmonella spp.* (6.42%), and *Klebsiella aerogenes* (4.58%). The frequency of *Escherichia coli* occurrence was low (2.75%) in all the samples collected from the restaurants.

Also, Kusumaningrum studied that some non-spore-forming bacteria might be able to withstand dry conditions on surfaces for an extensive period of time. In this study the survival of *Salmonella enteritidis*, *Staphylococcus aureus* and *Campylobacter jejuni* on stainless steel surfaces at different initial levels was determined at room temperature. The transfer rates of these pathogens from kitchen sponges to stainless steel surfaces and from these surfaces to foods were also investigated (Kusumaningrum, 2003). Then Hosen & Afrose, analyzed higher percentages of microbial contamination were observed in street side food premises (76.92% for gram-positive bacteria, 69.23% for gram-negative bacteria (Hosen & Afrose, 2019).

1.2 Epidemiology & Impact of Foodborne Illness

Foodborne diseases are caused by the consumption of food with bacteria and their toxins, viruses, parasites, or chemical substances where each year, approximately one in ten individuals worldwide becomes ill due to the consumption of contaminated food, resulting in more than 420,000 deaths, mainly affecting children under the age of five years old (Rossi et al., 2025).

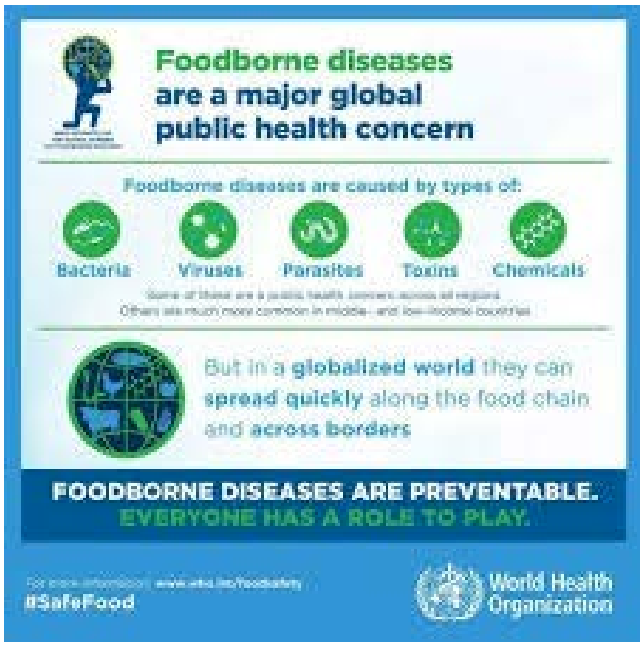


Figure 1.1: Over 200 foodborne diseases are caused by eating contaminated food (WHO,2023). The World Health Organization found that over 200 diseases are caused by eating contaminated food with bacteria resulting in human health risks (WHO,2023).

Foodborne pathogens are causing a great number of diseases with significant effects on human health and economy. The characteristics of the most common pathogenic bacteria (*Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Cronobacter sakazakii*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp. and *Yersinia enterocolitica*), viruses (Hepatitis A and Noroviruses) and parasites (*Cyclospora cayetanensis*, *Toxoplasma gondii* and *Trichinella spiralis*) (Bintsis, 2017). The illnesses of food are ordinarily intended and malicious come out by bacteria, parasites, viruses, or chemicals when swelled by humans or animals through spoiled

nutrients and liquid and almost yearly, 0.1% get hurt when taking polluted food around the world, leading towards 420,000 passing away resulting on loss of 33,000,000 healthy lives per year according to Disability Adjusted Life Years (DALYs) also infected by diarrhea reckoning more than 50% of diseases caused by food, and its leading to death about 2,000,000 at a year, (Almaary, 2023).

Escherichia coli (*E. coli*) are mostly harmless bacteria that live in the intestines of people and animals and contribute to intestinal health. However, eating food contaminated with certain types of *E. coli* can cause mild to severe gastrointestinal illness. Some types of pathogenic (illness-causing) *E. coli*, such as Shiga toxin-producing *E. coli* (STEC), can be life-threatening. Different types of *E. coli* tend to contaminate different types of foods of pathogenic *E. coli* have included leafy greens, sprouts, raw milk and cheeses, and raw beef and poultry (Office of the Commissioner, 2020).

Salmonella is a genus of Gram-negative bacteria that is responsible for numerous food poisoning outbreaks worldwide. *Salmonella* enterica which is an increasing global public health concern for humans and animals is antimicrobial resistance by *Salmonella* species worldwide. *Salmonella* infections can be lethal; conditioned with an increased prevalence of multi-drug resistant (MDR) strains in the future (Billah & Rahman, 2024). Salmonella is considered the major zoonotic and foodborne pathogen responsible for human infections. It includes the serovars causing typhoid fever (*S. typhi* and *S. paratyphi*) and the non-typhoidal salmonella (NTS) serovars (*S. enteritidis* and *S. typhimurium*), causing enteric infections known as “Salmonellosis” (Zizza et al., 2024).

Klebsiella pneumoniae (K. pneumoniae) is an opportunistic bacterium found in various microbiological niches such as soil; the skin, intestines, and feces of mammals; and food. *K. pneumoniae* has been documented to cause bacteremia, pneumonia, and urinary tract infection ; the gastrointestinal carriage of *K. pneumoniae* has been said to be a predisposing factor for liver abscess , and hypervirulent *K. pneumoniae* strains have emerged as a predominant cause of pyogenic liver abscess in Asia. Although *K. pneumoniae* is more commonly associated with nosocomial infections, food has also been reported to be a possible transmission vector. *K. pneumoniae* has been isolated from raw meat, raw vegetables, fruit juice, and ready-to-eat (RTE) food. Several studies on *Klebsiella pneumoniae* in food have also reported its worrying

resistance to antibiotics, on several occasions citing foodborne *Klebsiella pneumoniae* as resistant to three or more antibiotic classes (multidrug resistance) (Hartantyo et al., 2020).

Shigella, a highly virulent pathogen that causes bacterial dysentery, is one of the leading causes of diarrheal disease that contributes significantly to the burden worldwide (Zaidi & Estrada-García, 2014). Foods are most commonly contaminated with *Shigella* by an infected food handler who practices poor personal hygiene. *Shigella* is acid resistant, salt tolerant, and can survive at infective levels in many types of foods such as fruits and vegetables, low pH foods, prepared foods, and foods held in modified atmosphere or vacuum packaging. Survival is often increased when food is held at refrigerated temperatures (Warren et al., 2006). The global burden of shigellosis remains considerable. In a large prospective case-control study in Africa and Asia, *Shigella* was isolated from 17%, 66% and 78%, respectively, of infants, toddlers and children with moderate-to-severe dysentery and was among the top four pathogens associated with moderate-to-severe diarrhea (Zaidi & Estrada-García, 2014).

Foodborne illness caused by pathogenic *Vibrio* is generally associated with the consumption of raw or undercooked seafood. Fish and other seafood can be contaminated with Vibrio species, natural inhabitants of the marine, estuarine, and freshwater environment. Pathogenic Vibrios of major public health concerns are *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*. Common symptoms of foodborne *Vibrio* infection include watery diarrhea, stomach cramping, nausea, vomiting, fever, and chills (Dutta et al., 2021).

A recent study conducted in Spanish hospitals identified *E. coli* as the most frequent pathogen accounting for more than 40% of bloodstream infection episodes, and a global analysis of adult *E coli* bacteremia incidence in high-income countries estimated an incidence rate of 48 per 100 000 person-years and a case-fatality rate (CFR) of 12.4% (Doua et al., 2023). In Bangladesh, seizures have been documented in 8% of children with *Shigella dysenteriae* infections (all serotypes) and 5% of *Shigella flexneri* infections, Hemolytic uremic syndrome (HUS) occurs in about 8-13% of dysenteric patients infected with SD1, with a case-fatality rate of 36%. It may occur, albeit rarely, with *S. flexneri* infections. SD1 HUS typically develops one week or more after the onset of diarrhea. In contrast to *E. coli* O157:H7, it occurs after a course of bloody diarrhea with fever, almost exclusively in young children. Composed of the triad of hemolytic anemia,

thrombocytopenia and renal insufficiency, primarily due to the action of Shiga toxin. For example, 36% of children hospitalized at our center for *Shigella* gastroenteritis had a fever greater than 38.5° C, compared to only 7% of young infants in a rural community in Yucatan, Mexico (Zaidi & Estrada-García, 2014). In 2022, there were 65,208 confirmed cases of salmonellosis reported, an increase from 60,169 cases in 2021. In the EU, 62.3% of salmonellosis cases were acquired, a decrease of 10.5% compared to 2021. In addition, 5.0% of cases were linked to travel outside the EU, while 32.7% had unknown origin (Zizza et al., 2024).

Recent studies showed that the prevalence of *Klebsiella* colonization ranges from 18.8 to 87.7% in Asia and 5 to 35% in Western countries. In the non-hospital settings, the carrier rate of *Klebsiella* in fecal samples ranges from 5 to 38%, while the carrier rates in the nasopharynx range from 1 to 6% (Chang et al., 2021). In 2017, 1.2 million cholera cases and 5654 fatalities worldwide were reported to the WHO, with Yemen accounting for 84% and 41% of cholera-attributed cases and deaths (Deen et al., 2019).

Table 1.1: Studies of Restaurant Isolates with Their Antimicrobial Susceptibility Characteristics.

Route of Transmission	***Bacteria***	**Types of Disease**	**Test Performed**	**Antibiotic Resistance Spectrum of the Isolated Bacteria**	**Reference**
Kitchen sink, stove knob, cutting board, refrigerator handle, phone handle, etc.	*E. coli*	Food-borne illness	BT, AST, PCR	1.CPD (10 µg) 2.NIT (300 µg)	(Rossi *et al*., 2023)
Cooked and sold food samples	*K. pneumoniae*	Bacterial infection	BT, AST	1.AMC (20/10 µg) 2.CRO (30 µg) 3.CTX (30 µg) 4.CAZ (30 µg) 5.TIM (75/10 µg)	(Dutta et al., 2021)
Carcass and cutting board of butcher shops	*E. coli O157*	Severe diarrhea	Slide agglutination test, AST	1.AMX (10 µg) 2.C (30 µg) 3..STR (10 µg)	(Hartantyo et al., 2020)
Local restaurant tables, fast food restaurants, hospital canteens, and	*E. coli, K. pneumoniae, Staphylococcus aureus, Salmonella spp.*	Foodborne illness	BT, AST	Most of the isolates showed a greater degree of susceptibility towards 1.SXT (25 µg)	(Hosen & Afrose, 2019)

academic institutions tabletops				2.TE (30 µg) and were resistant to 1.CRO(30µg)	
Tables of the dining hall	*S. aureus, E. coli, K. pneumonia* and *Shigella* spp.	Food poisoning	BT, AST, gram staining,	2.GEN (10 µg) and resistant to most other antibiotics.	(Doua et al., 2023)
Restaurant menus	*E. coli, Staphylococcus aureus*	Foodborne illnesses	BT	N/A	(Zizza et al., 2024)
Food contact surfaces of hotels and restaurants	*E. coli, S. aureus*	Foodborne illnesses	BT	N/A	(Almaary, 2023)
Food contact surfaces like cutting boards and the whole area of small utensils like Knives of restaurants	*K. pneumoniae, E. coli, Pseudomonas spp. Proteus spp., Bacillus cereus, and Candida spp.*	Food-borne illnesses	Vitek 2 compact test	N/A	(Singh et al., 2024)
Raw food, Cooked food, Preparation area, tools, hands, fridge, and cashier in restaurants	*E. coli, K. aerogenes Salmonella spp., Shigella spp., S. aureus, and S. epidermidis*	foodborne infections	BT, gram staining	N/A	(Warren et al., 2006)
Meat and Swab Samples of Various Contact Surfaces	*E. coli* and *E. coli* O157:H7	foodborne disease	BT, AST test by Kirby-Bauer disc diffusion method	Higher resistance against 1.AMP (10 µg)and 2.E(15µg) With the prevalence of (97.1%) and (92.6%) and (91.2%) and (88.9%), respectively. They were susceptible to 1.CTX(30 µg) 2.CRO(30 µg) 3.CAZ(30 µg) 4.CIP(5 µg) 5.GEN(10 µg) 6.K(30 µg) 7.STR(10 µg)	(Deen et al., 2019)

Water, food, and restaurant surface in a buffet-style restaurant	*E. coli (STEC) O111:NM*	hemolytic uremic syndrome	PCR, pulsed-field gel electrophoresis (PFGE)	N/A	(Chang et al., 2021)
Tables and sink surface	*S. aureus, E. coli, Proteus, K. pneumoniae*	Food poisoning	BT, AST	N/A	(Warren et al., 2006)
Restaurant plates, spoons, chopping boards, and table	*E. coli, Salmonella spp., and S. aureus*	Causing early-onset Sepsis in very-low-birth-weight infants	BT, AST	1.AMX(10 µg) and 2.CIP(5 µg) (46.2 %) and 3.(SPX, 5 µg) (53.8 %) were resistant.	(Zaidi & Estrada-García, 2014

1.3 Hypothesis and Objective

The table surface of restaurants can be a source of pathogenic bacteria. The pathogenic bacteria can transfer through physical contact, using uncleaned equipment and having the food by a cook who is not using proper precautions before preparing the food. These bacteria can be resistant to antibiotics and can spread antibiotic resistance. For that, having the food from the restaurant on a daily basis can cause severe foodborne illnesses that can affect the health badly and also can lead the people to the dead (Hartantyo et al., 2020).

1.4 Aim of Study

The aim of this study was to isolate selected Gram-negative bacteria from restaurant table surfaces and to apply PCR for molecular detection and confirmation of foodborne pathogens.

1. To isolate seven targeted bacteria-*Escherichia coli, Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Klebsiella pneumoniae,* and *Acinetobacter spp.* from table surfaces of restaurants.
2. To identify the isolated bacteria by Polymerase Chain Reaction (PCR).
3. To examine the antibiotic resistance profiles of the identified bacteria.

Chapter 2

2 Method and Materials

2.1 Workflow

During the research some steps were followed and these are shown below using a flowchart.

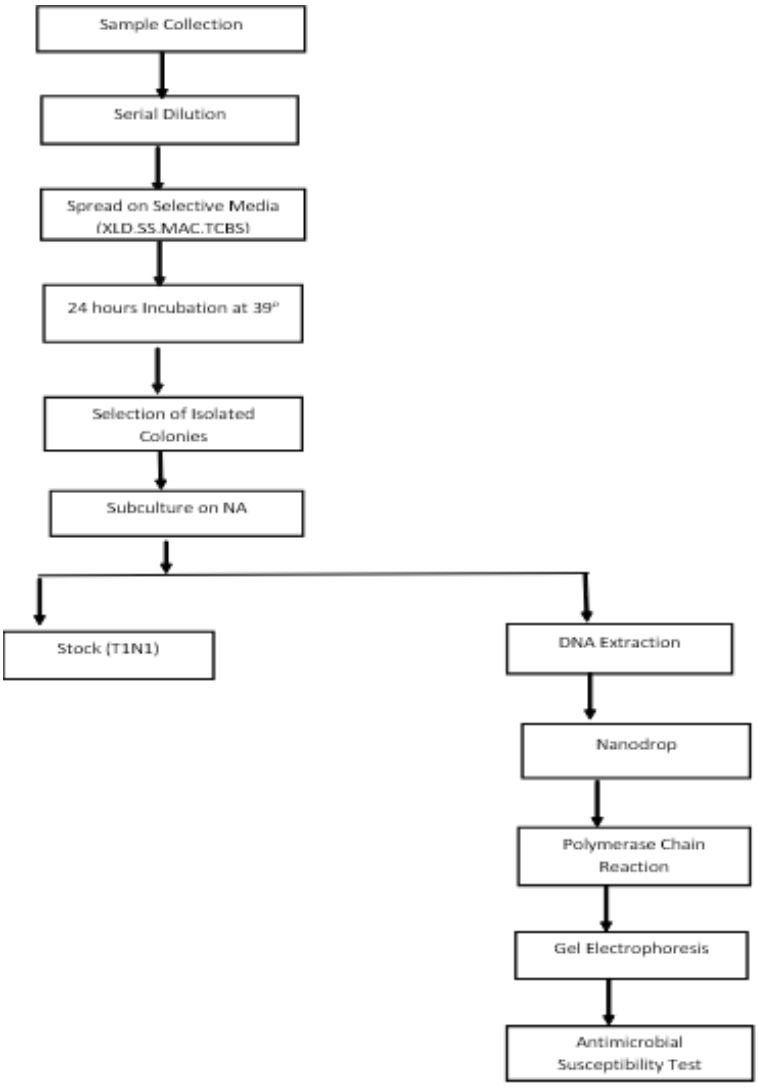


Figure 2.1: Workflow diagram of Isolated Organisms from Restaurant Table Surfaces. To perform this research study, this flow chart was followed by serial dilution, spreading on selective media, AST, DNA extraction, Nanodrop, PCR and Gel electrophoresis.

2.2 Media, solutions and Reagents

Nutrient agar (NA), MacConkey agar (MAC), *Salmonella Shigella* agar (SS), Thiosulfate-Citrate- Bile-Salts-Sucrose agar (TCBS), Xylose Lysine Deoxycholate agar (XLD), Eosine Methylene Blue agar (EMB), *Klebsiella pneumoniae* carbapenemase(KPC), Sodium Chloride (NaCl) were purchased from Mark Chemicals and HI Media Laboratories Pvt. Ltd, India.

2.3 Sample Collection Site

For this project, fifteen different local restaurants within Dhaka City, mostly around BRAC University, Merul Badda and Aftab Nagar were selected. During the period of May 2025 to December 2025. The samples were collected from the table surfaces of these restaurants.

2.4 Sample Collection

A cotton swab was dipped in a 10 ml sodium chloride (NaCl) solution. After that, the swab was used to wipe a 3*3 square foot area on the table, moving from the center to the left and right. After swabbing, the cotton swab was placed in the test tube and the sample was taken to the lab for further analysis within 24 hours.

Table 2.1 Sample Collection Sites

Location Name
Chill House, Merul Badda
Food Velly, Merul Badda
Zafran Kacchi House, Merul Badda
Petuk Naki, Merul Badda
Taiking Thai, Merul badda
AZ Resturant and Lodge, Merul Badda
SALT café and Restuarant
Khichuris Badda, Uttar Badda
Khabar Bari Restora, Aftabnagar
Noon Kabab, Aftabnagar
Khabar Ghor Hotel and Resturant, Aftabnagar

These locations were selected to observe the pathogenic bacterial isolation from the table surfaces.

All the samples were transported to the laboratory immediately after collection to get the exact integrity. All the samples were sealed and labeled properly for correct identification.

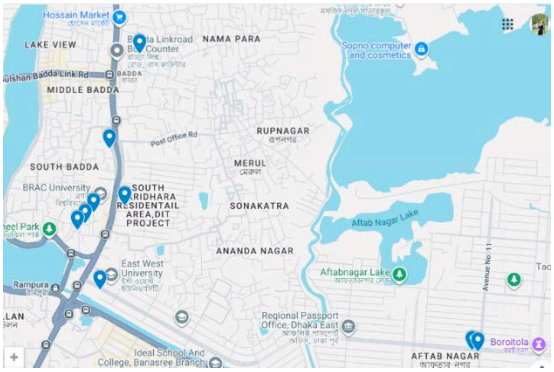


Figure 2.2: Sample Collection Locations

2.5 Isolation and Identification

Bacterial assessment was conducted using the total plate count (TPC) method. NA was utilized to determine the overall microorganism count, while MAC, SS, XLD, and TCBS agar were employed for selective identification purposes. Additionally, KPC agar was used to differentiate between *E. coli* and *Klebsiella.* These growth media selectively suppress the development of gram-positive bacteria and only allow gram-negative bacteria to grow.

2.6 Spread Plate Technique

The spread plate technique is used to isolate microorganisms, mainly bacteria from the samples. (Dahal, 2025) For this technique, selective media such as TCBS, MAC, XLD, NA, and SS were prepared, and serial dilution was done up to 10^{-4} serial dilutions. Then, 100 µL of the diluted sample was spread in the selective media by using a sterile glass rod. After that, the plates were incubated in an inverted position for 24hours at 37^0C. Lastly, the plates were observed to see the bacterial growth and the number of colonies were calculated. For further analysis, selected colonies were streaked into NA plates. (Dahal, 2025)

2.7 Streaking of the Selected Isolated Colonies on NA Media

The streak plate technique is used to isolate and purify a bacterial colony. After growing on selective media, single isolated colonies were selected and streaked into NA plates within a

laminar air flow. After this all the culture plates were incubated for 24 hours at 37°C. After incubation, bacterial growth was observed on NA plates and used for further analysis. (Dahal, 2025b)

2.8 Antibiotic Susceptibility Test

The Kirby-Bauer disk diffusion test is the most commonly used method to determine antibiotic resistance or susceptibility in various bacteria. (Bayot & Bragg, 2024). This test utilizes specialized Mueller Hinton Agar (MHA) due to its higher diffusion rate compared to standard growth media. MHA also contains starch, which acts as an energy source and absorbs toxins produced by bacteria, preventing interference with medications. (Bayot & Bragg, 2024).

Microorganisms are grouped into three categories based on their zone diameter values: resistant (R), intermediate (I), or susceptible (S). The values are measured in millimeters. The Clinical and Laboratory Standards Institute (CLSI) categorizes these to align with the guidelines (Bayot & Bragg, 2024).

These cultures were incubated for 24 hours at 37°C. After getting pure cultures, a small amount (around one loop full) of each bacterium was taken and mixed in a tube. The tube contains 10 ml of 0.9% NaCl. The turbidity of the tubes was adjusted to match the McFarland 0.5, thereby standardizing the size of the inoculums. Then, an autoclaved cotton swab was dipped and the swab was used to evenly distribute the bacteria across the entire surface of MHA. Therefore, around seven antibiotic discs were placed to the surface of MHA media by using sterile forceps. Then, the plates were incubated at 37°C for 24 hours and the zone of inhibition (ZOI) was measured by the guidelines of CLSI. ZOI is the clear zone around the antibiotic disc.

2.9 DNA Extraction

DNA was extracted by a double centrifuge method. Though the boiling method is more popular and easier, for this research a double centrifuge method was performed.

For this method, 200 µL of TE buffer was taken in 1.5 ml micro-centrifuge tubes. Using the loop inoculation method, a loop-full isolated colony was inoculated inside the TE buffer. Then, the tubes were centrifuged at 13000 revolutions per minute for 10 minutes. After that, the

supernatant was discarded and 200µL of TE buffer was taken and resuspended carefully. To lyse or release the DNA, resuspended cells were boiled for 10 minutes at 99°C. After that, it was centrifuged at 13,000 revolutions per minute for 10 minutes at room temperature. Lastly, the pellet was discarded, and the supernatant containing extracted DNA was stored at -20°C temperature for further analysis.

2.10 Measurement in Nanodrop

Thermo Scientific Nanodrop spectrophotometer enables fast, accurate quantification of DNA, RNA, and proteins using just 1–2 µL of sample. They deliver results in seconds to streamline lab workflows. The "Nanodrop" Thermofisher scientific machine (Country: USA and Model: microvolume UV-Vis spectrophotometers) provides high accuracy for advanced applications, while the Nanodrop Eight increases throughput with eight-sample measurement. The Nanodrop Lite Plus offers a compact, easy-to-use option for routine analyses. (Erk, 2025).

After measuring the concentration of the sample, the concentration is adjusted by dilution for PCR. Adjusting is important because very high concentration can lead to poor results, non-specific binding etc. Proper concentration ensures a clear result.

2.11 Polymerase Chain Reaction (PCR)

The concept of PCR is with the help of DNA polymerase to generate a new strand of DNA that is complementary to the provided template strand. Since DNA polymerase can only add a nucleotide onto a 3'-OH that is already there, it requires a primer against which it can add the first nucleotide. The particular sequence will be copied in multiple copies. Polymerase Chain Reaction is an effective laboratory method that can accurately make millions of copies of a specific DNA fragment, it copies DNA to study, diagnose or forensically purpose etc. Early detection of genetic conditions, pathogen or abnormal cells, and formation of forensic profiles, it is important to detect infectious diseases early, as well as identify genetic profiles. (No, 2013)

Steps and Application

- **Denaturation:** The sample of DNA is heated (approximately 95 degree C) in order to separate the two-stranded DNA strands to individual strands. The high temperature

breaks the double strands and separates them into single strands by breaking the hydrogen bonds. These single strands then act as a template for primer binding. (Khehra et al., 2025)

- **Annealing:** Short DNA primers (synthetic fragments) are added and the temperature is reduced (around 50-65 degree C) so that the primers bind to the particular target DNA sequences. The exact temperature depends on the primer melting temperature. Proper annealing ensures that the primer has binded specifically. (Khehra et al., 2025)
- **Extension:** The DNA polymerase (usually Taq polymerase) copies the existing strands of DNA (which are the primers) to form new strands at an opportune temperature (approximately 72 degree Celsius). The enzyme adds dNTPs and the extension depends on the length of the target DNA. The step results in the formation of double strand DNA. (Khehra et al., 2025)

Multiple PCR were performed to identify the presumptive bacteria isolated from selective media.

Table 2.2: List of Primers

Bacteria Name	**Forward Primer**	**Reverse Primer**	**Primer Name**	**Amplicons Size**
Acinetobacter Baumannii	5'-CATAAACTGAGATAACTGGCTTGAACC -3'	5'-CGTCGACTTCACCTTTACCGTTAC -3'	bla-OXA-51	353bp
Vibrio cholerae	5'-AAATCAGGCTCGGGCCCT-3'	5'-GCAATTTTGTCGACCGG -3' (Kim et al., 2015)	VC C634002F VC C634002R	154bp
E. coli	5'GACCTCGGTTTAGTTCACAGA3'	5'CACCACGCTGACGCTGACCA3' (Punom et al., 2020)	16s rDNA	575bp
K.pneumoniae	5' CTTGTCTCTCATGGCCGCTGG 3'	5' ACGGAACGTGGTATCGCCGAT 3'(van der Zwaluw et al., 2015)	Diguanylate-cyclase	130bp

2.11.1 Identification of *Acinetobacter* through PCR

Table 2.3: Reaction mixture for a 13 µL reaction volume (*Acinetobacter*)

Components	**Volume**
Primer Concentration	10 picomol
Master mix, 2X	12.5 µL
Forward Primer	1 µL
Reverse Primer	1 µL
Nuclease Free Water	8.5 µL
DNA template	2 µL

Thermocycling conditions were initial denaturation at 94°C for t minutes followed by 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55°C and 1 min extension at 72°C. The final extension was carried out at 72°C for 10 minutes.

2.11.2 Identification of *Vibrio* through PCR

Table 2.4: Reaction mixture for a 25 µL reaction volume (*Vibrio)*

Components	**Volume**
Primer Concentration	10 picomol
Master mix, 2X	12.5µL
Forward Primer	2.5 µL
Reverse Primer	2.5 µL
Nuclease Free Water	2.5 µL
DNA template	5 µL

Thermocycling conditions were initial denaturation at 94°C for three minutes followed by 25 cycles of 30 seconds denaturation at 94 °C, 30 seconds annealing at 60°C and 30 seconds extension at 72°C. The final extension was carried out at 72°C for 10 minutes.

2.11.3 Identification of *E. coli* through PCR

Table 2.5: Reaction mixture for a 13 µL reaction volume (*E. coli*)

Components	**Volume**
Primer Concentration	2.5 picomol
Master mix, 2X	6.5 µL
Forward Primer	0.25 µL
Reverse Primer	0.25 µL
Nuclease Free Water	4 µL
DNA template	2 µL

Thermocycling conditions were initial denaturation at 94°C for three minutes followed by 30 cycles of 1min denaturation at 94 °C, 1 min annealing at 55°C and 1 min extension at 72°C. The final extension was carried out at 72°C for 5 minutes.

2.11.4 Identification of *Klebsiella pneumonia* through PCR

Table 2.6: Reaction mixture for a 13 µL reaction volume (*Klebsiella)*

Components	**Volume**
Primer Concentration	10 picomol
Master mix, 2X	6.5 µL
Forward Primer	1 µL
Reverse Primer	1 µL
Nuclease Free Water	2.5 µL
DNA template	2 µL

Thermocycling conditions were initial denaturation at 94°C for 4 minutes followed by 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55°C and 1.5 min extension at 72°C. The final extension was carried out at 72°C for 10 minutes.

2.11.5 Identification of *Shigella* through PCR

Table 2.7: Reaction mixture for a 13 µL reaction volume (*Shigella)*

Components	**Volume**
Primer Concentration	10 picomol
Master mix, 2X	6.5 µL
Forward Primer	1 µL
Reverse Primer	1 µL
Nuclease Free Water	2.5 µL
DNA template	2 µL

Thermocycling conditions were initial denaturation at 95°C for 5 minutes followed by 35 cycles of 30 seconds denaturation at 95 °C, 30 seconds annealing at 60°C and 1 min extension at 72°C. The final extension was carried out at 72°C for 3 minutes.

Chapter 3

3. Results

3.1. Total bacterial count from the samples

In the tests, the total number of bacteria isolated from seven distinct samples was counted using nutrient agar (NA). The samples' NA spread plates were used to count the colony forming units (CFU) (Figure 3.1). All samples, including R6, R7, R8, R9, R10, R11, R12, R13, R14, and R14 had CFU counts of, 2.78 × 10^6, 2.12×10^6, 2.25×10^6, 2.5235×10^6, 2.958 ×10^6, 2.06×10^6 and 0 CFU/sample, respectively.

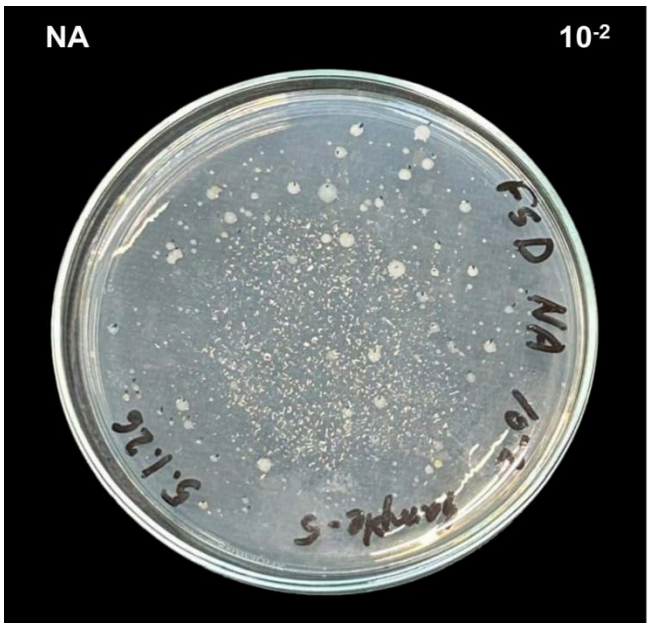


Figure 3.1: Total Aerobic counts on NA. Samples were diluted and inoculated on NA using spread plate procedures. After a 24-hour incubation period, each sample's CFU was counted to look for separate colonies. Spread plating of the original sample of 1 in 100 dilution.

Table 3.1: Total Aerobic Count

Sample ID No.	Media	Dilution	CFU Per Replicate	Average CFU per Sample
R6	NA	10^{-2}	2.78×10^{6}	2.78×10^{6}
R7	NA	Direct 10^{-2}	0 0	0
R8	NA	Direct 10^{-2}	0 0	0
R9	NA	Direct 10^{-1} 10^{-2}	4.5×10^{5} 5.12×10^{5} 5.4×10^{6}	2.12×10^{6}
R10	NA	Direct 10^{-2}	3×10^{5} 4.2×10^{6}	2.25×10^{6}
R11	NA	Direct 10^{-2}	0 0	0
R12	NA	Direct 10^{-2}	5.67×10^{5} 4.48×10^{6}	2.5235×10^{6}
R13	NA	Direct 10^{-2}	3.36×10^{5} 5.58×10^{6}	2.958×10^{6}
R14	NA	Direct 10^{-2}	4.5×10^{5} 3.67×10^{6}	2.06×10^{6}
R15	NA	Direct 10^{-2}	0 0	0

3.2. Presence of targeted bacteria in the samples

Using samples from fifteen distinct local restaurants-R1, R2, R3, R4, R5, R6, R7, R8, R9, R10, R11, R12, R13, R14, and R15 we sought to identify seven different types of bacteria, including *E. coli, Salmonella spp., Shigella spp., K. pneumoniae, and V. cholerae, V. para, S. aureus, Acinetobacter*. The presumptive identification revealed the presence of *Shigella spp., V. cholerae, V. para,* and *K. pneumoniae,* in R6, R9, R10. Additionally, *Salmonella* spp. were undetectable in R6 and prevalent in R7, R8, R9, R10, R11, R12, R13, R14 and R15. Moreover, R9 has *E. coli* , while other restaurants do not. R1, R2, R3, R4, R5, R6, R9, R10, R15 contained *K. pneumoniae;* the other samples did not. Furthermore, R7, R8 do not have any bacteria. And *S. aureus* was only present in R14 along with that *Acinetobacter* was present in only one restaurant sample.

3.3. Presumptive Bacterial Identification on Selective Media

In order to isolate *E. coli, Salmonella spp., Shigella spp., K. pneumoniae, and V. cholerae, V. para, S. aureus, Acinetobacter* seven samples were taken from neighborhood eateries close to BRAC University in Merul Badda, Dhaka. The spread plate technique was used on various selective media, such as MAC, TCBS, XLD, and SS, to find the targeted bacteria, and colony morphology was examined after a 24-hour incubation at 37°C.

3.3.1 Presumptive Identification of *E. coli*:

Presumptive identification of *E. coli* was performed based on their colony morphology and biochemical characteristics on the selective media. On the MAC agar, *E. coli* showed red colonies and appeared flat yellow colonies on the XLD agar due to lactose fermentation.

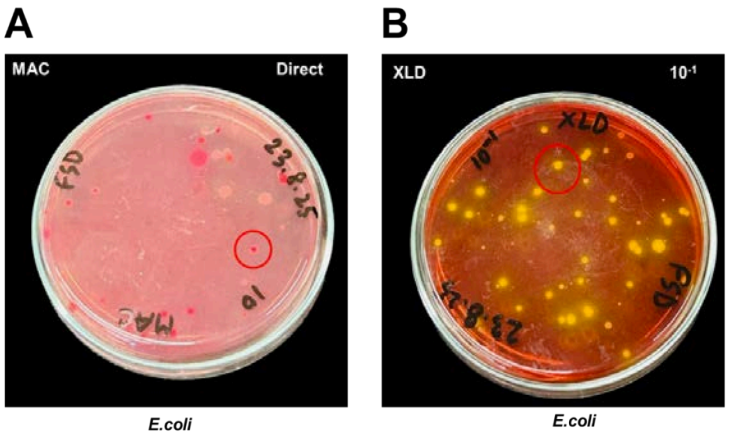


Figure 3.2: *E. coli* observed on MAC and XLD agar plates. Undiluted suspension prepared from the samples were inoculated on the selective media by the spread plate technique and incubated for 24 hours. Growth characteristics were observed on a) MAC agar, b) XLD agar.

3.3.2 Presumptive identification of *K. pneumoniae spp.*:

Presumptive identification of *K. pneumoniae* was performed based on their colony morphology and biochemical characteristics in the selective media. On the MAC agar, *K. pneumoniae* showed pink colonies.

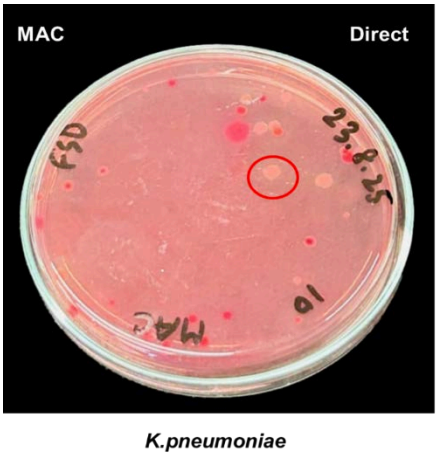


Figure 3.3: *K. pneumoniae* observed on MAC and XLD agar plates. Diluted suspension prepared from the samples were inoculated on the selective media by the spread plate technique and incubated for 24 hours. Growth characteristics were observed on MAC agar.

3.3.3 Presumptive Identification of *Vibrio cholerae* spp.:

Presumptive identification of *Vibrio cholerae* was performed based on their colony morphology and biochemical characteristics on the selective media. On the TCBS agar, *Vibrio cholerae* showed yellow colonies due to sucrose fermentation. Because of fermentation Vibrio produces acid and turns the colonies yellow.

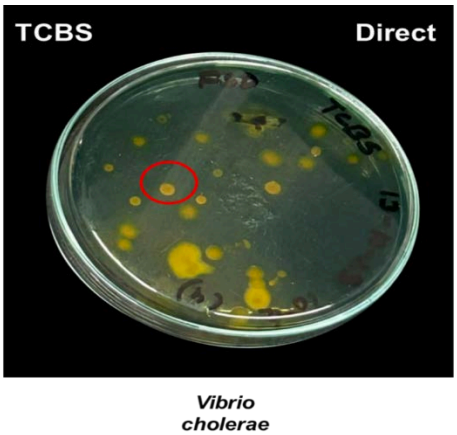


Figure 3.4: *Vibrio cholerae* observed on TCBS agar plate. Undiluted suspension prepared from the samples were inoculated on the selective media by the spread plate technique and incubated for 24 hours. Growth characteristics were observed as yellow colonies.

3.3.4 Presumptive identification of *Shigella* spp.:

In SS, it appeared yellow and did not ferment lactose. These colonies typically exhibit non-lactose fermenting characteristics, appearing colorless or transparent on SS agar.

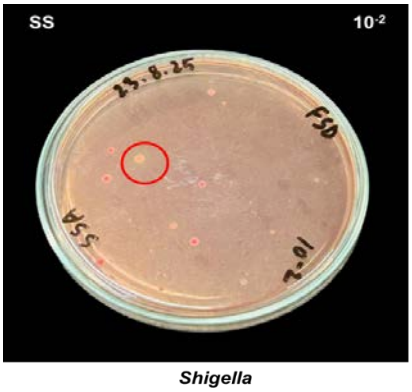
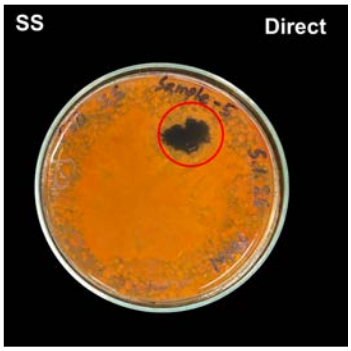


Figure 3.5: *Shigella* spp. observed on SS agar plates. Following an incubation period of 24 hours, growth characteristics were observed SS agar.

3.3.5 Presumptive Identification of Salmonella:

In SS, it appears colorless and does not ferment lactose. These colonies typically exhibit non-lactose fermenting characteristics, appearing colorless or transparent but black centered colonies on SS agar. The black center is due to H2S production.



Salmonella spp.

Figure 3.6: *Salmonella* spp. observed on SS agar plates. Following an incubation period of 24 hours, growth characteristics were observed SS agar.

Table 3.2: Presence of targeted bacteria in restaurant sample

	Types of bacteria	*E. coli*	*Shigella spp.*	*Salmonella spp.*	*V. cholerae*	*V. parahemolyticus*	*K. pneumoniae*	*Acinetobacter*	*S. aureus*	*Unknown*
Sample number	R1	-	+	-	+	-	+	-	-	-
	R2	+	+	+	-	-	+	+	-	-
	R3	-	+	+	+	-	+	-	-	-
	R4	+	+	+	+	-	+	-	-	-
	R5	+	+	+	+	-	+	-	-	-
	R6	-	+	-	+	+	+	-	-	-
	R7	-	-	-	-	-	-	-	-	-
	R8	-	-	-	-	-	-	-	-	-
	R9	+	+	-	+	-	+	-	-	-
	R10	-	-	-	+	-	+	-	-	+
	R11	-	+	-	-	-	-	-	-	-
	R12	-	-	-	-	-	-	-	-	+
	R13	-	-	-	-	-	-	-	-	+
	R14	-	+	-	+	-	-	-	+	-
	R15	-	-	-	+	-	+	-	-	-

Note: (+) Present & (-) Absent.

3.4 Antibiotic Susceptibility Test

In this study, a total of 37 bacterial isolates were subjected to antibiotic susceptibility testing. The test was carried out using 12 different antibiotic agents. The standard Kirby–Bauer disc diffusion technique was employed on Mueller–Hinton Agar (MHA) plates. Following an incubation period of 18–24 hours, the diameters of the inhibition zones were carefully measured. The susceptibility patterns of the isolates were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. This analysis allowed for the classification of isolates as sensitive, intermediate, or resistant to the tested antibiotics.

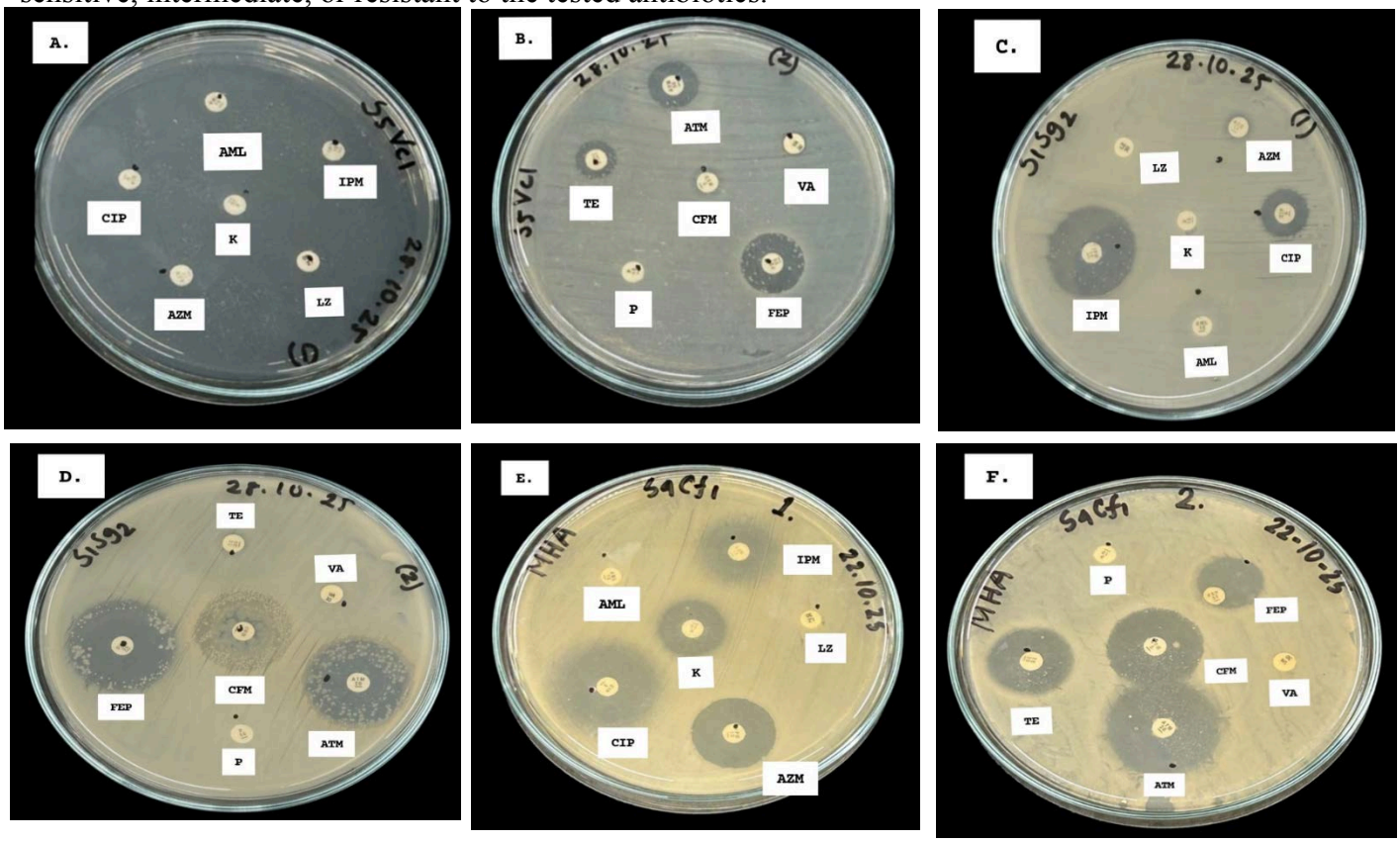


Figure 3.7: MHA plates after incubation for AST. Following the incubation, zones of inhibition surrounding antibiotic disks were observed on the MHA plates a, b, c, d, e and f, indicating bacterial sensitivity to the tested antibiotics.

Most of the isolates were resistant to (LZ), (AML), (P), (VA). Antibiotics (CIP), (ATM), (CFM) were found to be the most effective against all bacteria. Beside this, many bacteria were multidrug resistant.

3.4.1 Antibiotic resistance profile of *E. coli*:

Firstly, *Escherichia coli* (N=5) In the 5 isolates of *Escherichia coli*, there was a 100% resistance rate to Vancomycin (VA) and an 80% resistance rate to Amoxicillin (AML), while Tetracycline (TE) showed 20% resistance and all other antibiotics showed 0% resistance. The bacteria were 100% sensitive to Kanamycin (K), and 80% sensitive to Azithromycin (AZM), Cefepime (FEP), and Tetracycline (TE), with 20% sensitivity to Amoxicillin (AML). Intermediate results were limited to 20% for both Azithromycin (AZM) and Cefepime (FEP), with no intermediate response for the remaining drugs.

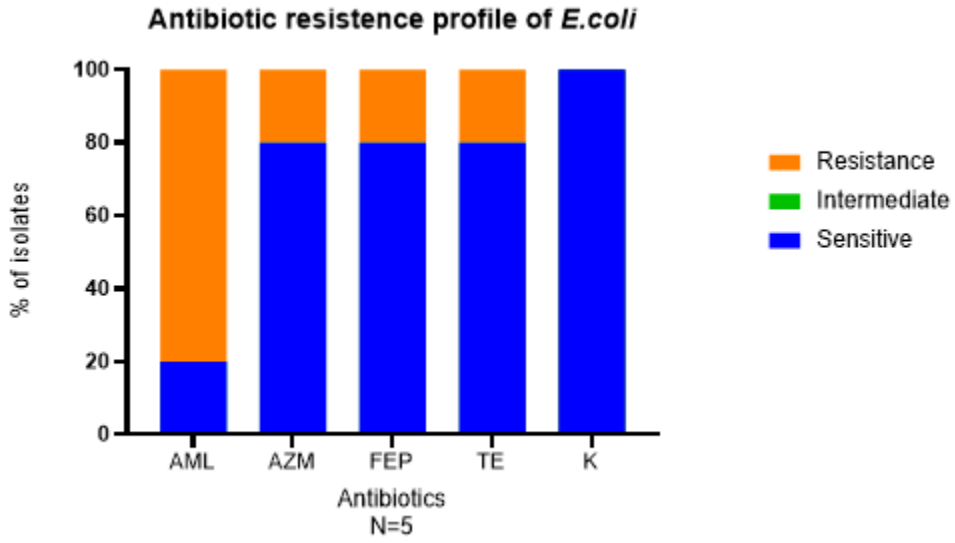


Figure 3.8: Antibiotic resistance pattern observed in *E. coli.* 100% isolates of *E. coli* showed highly vulnerable to Kanamycin, showing 100% Sensitivity. It also shows a strong response to Azithromycin, Cefepime, and Tetracycline, with 80% Sensitivity for each. However, it is completely immune to Vancomycin (100% Resistant) and faces significant challenges with Amoxicillin, which has an 80% Resistance rate.

3.4.2 Antibiotic resistance profile of *Salmonella* spp.:

Salmonella (N=6) The 6 *Salmonella* isolates were 100% resistant to Vancomycin (VA), 83% resistant to Amoxicillin (AML), and 67% resistant to Cefepime (FEP), while Azithromycin (AZM) showed 33% resistance and Kanamycin (K) and Tetracycline (TE) both showed 17%. Kanamycin (K) was the most effective with 83% sensitivity, followed by Tetracycline (TE) at 66%, Cefepime (FEP) at 33%, and 17% for both Amoxicillin (AML) and Azithromycin (AZM). A high intermediate rate of 50% was seen for Azithromycin (AZM), while Tetracycline (TE) showed 17% intermediate susceptibility.

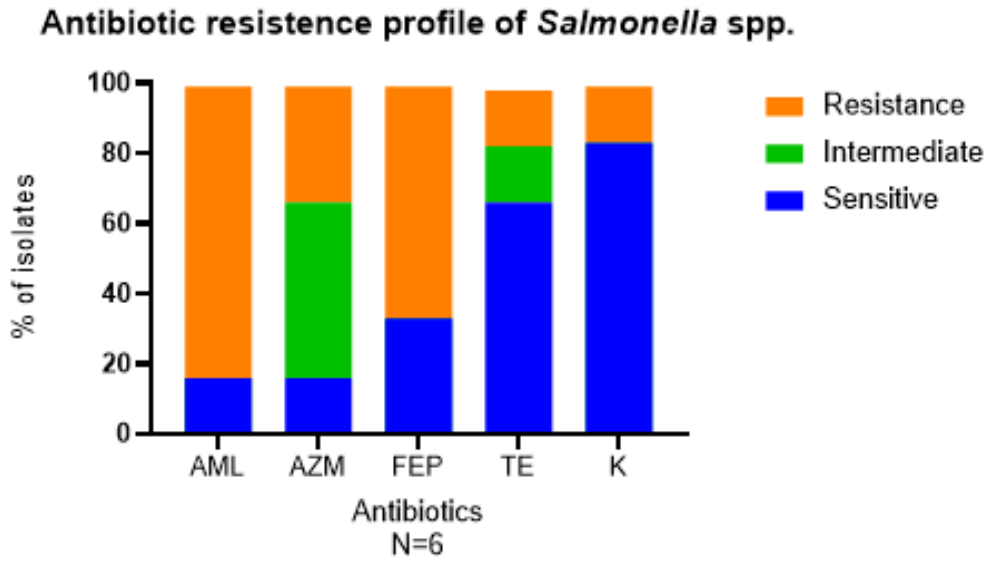


Figure 3.9: Antibiotic resistance pattern observed in *Salmonella* spp. 100% isolates of *Salmonella* spp. appears most treatable with Kanamycin (~83% Sensitive) and Tetracycline (~66% Sensitive). It is completely immune to Vancomycin (100% Resistant) and shows high resistance to Amoxicillin (~83% Resistant).

3.4.3 Antibiotic resistance profile of *Shigella* spp.*:*

Shigella (N=15) For the 15 *Shigella* isolates, Vancomycin (VA) showed 100% resistance, Amoxicillin (AML) showed 87%, and Azithromycin (AZM) showed 67%, while Tetracycline (TE) at 27%, Kanamycin (K) at 20%, and Cefepime (FEP) at 13% had lower resistance rates. The isolates were highly sensitive to Kanamycin (K) at 80%, Cefepime (FEP) at 74%, and Tetracycline (TE) at 67%, but much less so to Azithromycin (AZM) at 20% and Amoxicillin (AML) at 13%. Intermediate susceptibility was recorded for Azithromycin (AZM) and Cefepime (FEP) at 13% each, and Tetracycline (TE) at 6%.

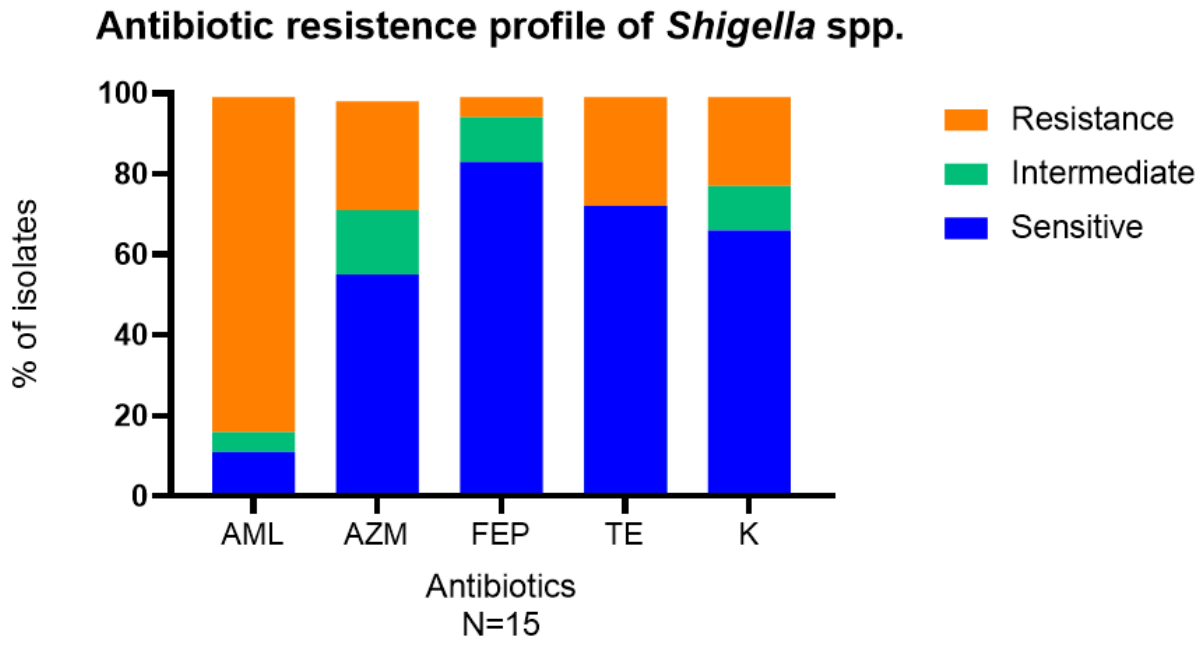


Figure 3.10: Antibiotic resistance pattern observed in *Shigella* spp. 100% isolates of *Shigella* spp. The group displays high sensitivity to Kanamycin (80% Sensitive) and Cefepime (~72% Sensitive). The biggest concern here is Vancomycin, which shows 100% Resistance, followed by Amoxicillin, which faces ~88% Resistance.

3.4.4 Antibiotic resistance profile of *V. cholerae:*

Vibrio (N=14) The 14 isolates of *Vibrio* were 100% resistant to Vancomycin (VA), followed by 64% resistance to Amoxicillin (AML), 50% to Azithromycin (AZM), and 35% to both Kanamycin (K) and Tetracycline (TE), with Cefepime (FEP) showing the lowest resistance at 15%. Sensitivity was highest for Tetracycline (TE) at 65%, followed by 50% for Azithromycin (AZM), Cefepime (FEP), and Kanamycin (K), while 29% were sensitive to Amoxicillin (AML). Cefepime (FEP) showed a significant intermediate rate of 35%, with Kanamycin (K) at 15% and Amoxicillin (AML) at 7%.

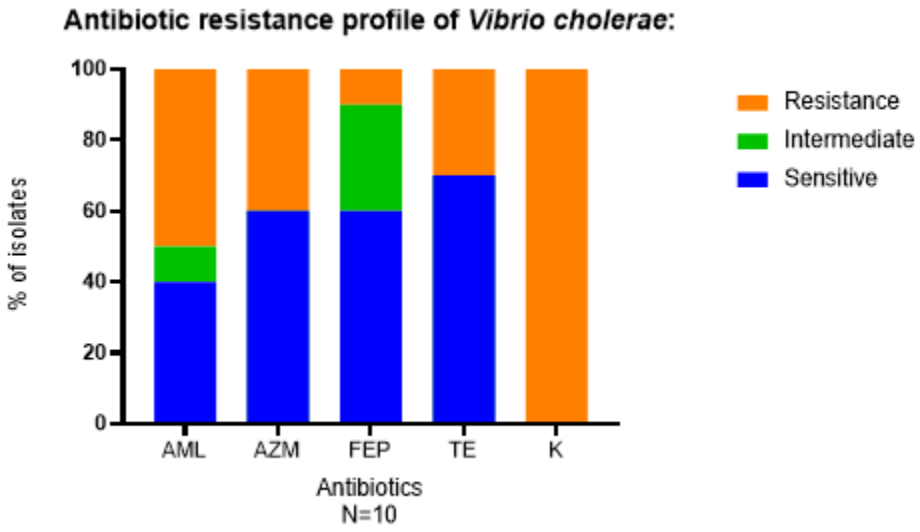


Figure 3.11: Antibiotic resistance pattern observed in *V. Cholerae.* This bacterium shows a mixed response depending on the drug. It is most vulnerable to Tetracycline (70% Sensitive) and Azithromycin (60% Sensitive). However, it shows a dangerous level of resistance to Vancomycin, which is 100% Resistant, and significant resistance to Amoxicillin (50% Resistant).

3.4.5 Antibiotic resistance profile of *K. pneumoniae*

Klebsiella pneumoniae (N=18) For the 18 isolates of *Klebsiella pneumoniae*, the bacteria showed the highest resistance toward Vancomycin (VA) at approximately 94% and Amoxicillin (AML) at 83%, while resistance to Azithromycin (AZM) and Tetracycline (TE) both stood at 27%. Kanamycin (K) showed a 22% resistance rate, and Cefepime (FEP) was the most effective with only 5% resistance. Regarding sensitivity, Cefepime (FEP) performed best at 84%, followed by Tetracycline (TE) at 73%, Kanamycin (K) at 67%, Azithromycin (AZM) at 57%, and Amoxicillin (AML) at 12%, while no isolates were sensitive to Vancomycin (VA). Intermediate susceptibility was noted for Azithromycin (AZM) at 16%, Cefepime (FEP) at 11%, Kanamycin (K) at 11%, Vancomycin (VA) at 6%, and Amoxicillin (AML) at 5%.

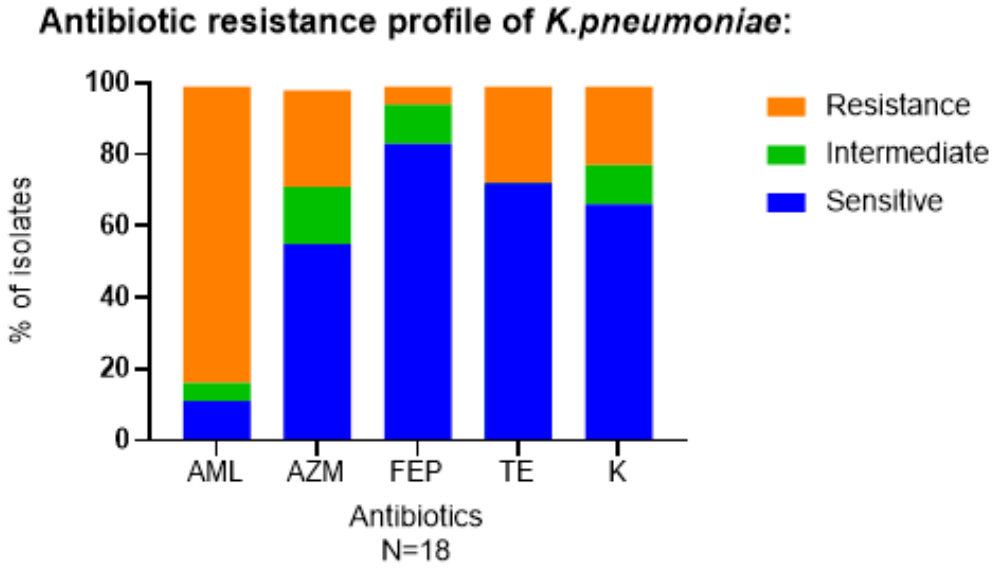


Figure 3.12: Antibiotic resistance pattern observed in *K. pneumoniae.* 100% isolates of *K. pneumoniae,* showed this is most effectively treated with Cefepime, exhibiting approximately 85% Sensitivity. It also shows relatively high sensitivity to Tetracycline (~72%) and Kanamycin (~68%). Consequently, it is nearly entirely resistant to Vancomycin (~95% Resistance) and Amoxicillin (~85% Resistance), with very small percentages of intermediate or sensitive isolates for those drugs.

3.4.6 Antibiotic resistance profile of *S. aureus*

Staphylococcus aureus (N=1) The single isolate of *Staphylococcus aureus* was 100% resistant to Amoxicillin (AML) and Vancomycin (VA), with 0% resistance to the other four antibiotics. It was 100% sensitive to Azithromycin (AZM), Cefepime (FEP), and Tetracycline (TE), but showed 0% sensitivity to Kanamycin (K), Amoxicillin (AML), and Vancomycin (VA). Notably, this bacterium showed a 100% intermediate response specifically to Kanamycin (K).

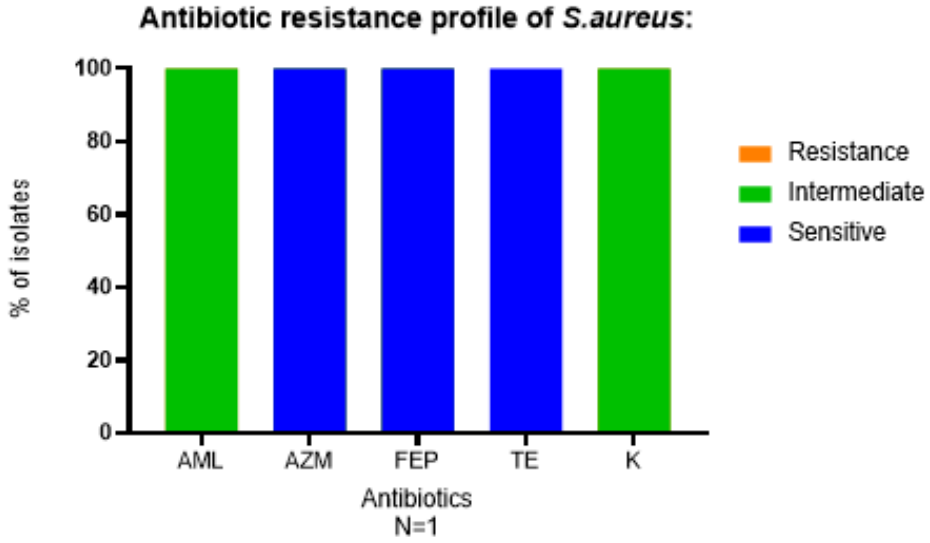


Figure 3.13: Antibiotic resistance pattern observed in *S. aureus.* 100% isolates of *S. aureus,* showed based on this single isolate, the profile is quite polarized. The bacteria are 100% Sensitive to Azithromycin, Cefepime, and Tetracycline. Conversely, it is 100% Resistant to Vancomycin and shows 100% Intermediate levels (not fully sensitive or resistant) for Amoxicillin and Kanamycin.

3.4.7 Antibiotic resistance profile of *Vibrio parahaemolyticus*

***Vibrio parahaemolyticus* (N=4)** For the 4 isolates of *Vibrio parahaemolyticus*, there was a 100% resistance rate to Amoxicillin (AML) and Vancomycin (VA), while resistance to Azithromycin (AZM) and Cefepime (FEP) both stood at approximately 75%, and Tetracycline (TE) showed 50% resistance. Kanamycin (K) was the most effective antibiotic with 100% sensitivity, followed by Tetracycline (TE) at 50%, and Azithromycin (AZM) and Cefepime (FEP) at approximately 25% sensitivity each, while no isolates were sensitive to Amoxicillin (AML) or Vancomycin (VA). Notably, intermediate susceptibility was only recorded for Cefepime (FEP) at approximately 50%, with all other antibiotics showing 0% intermediate response.

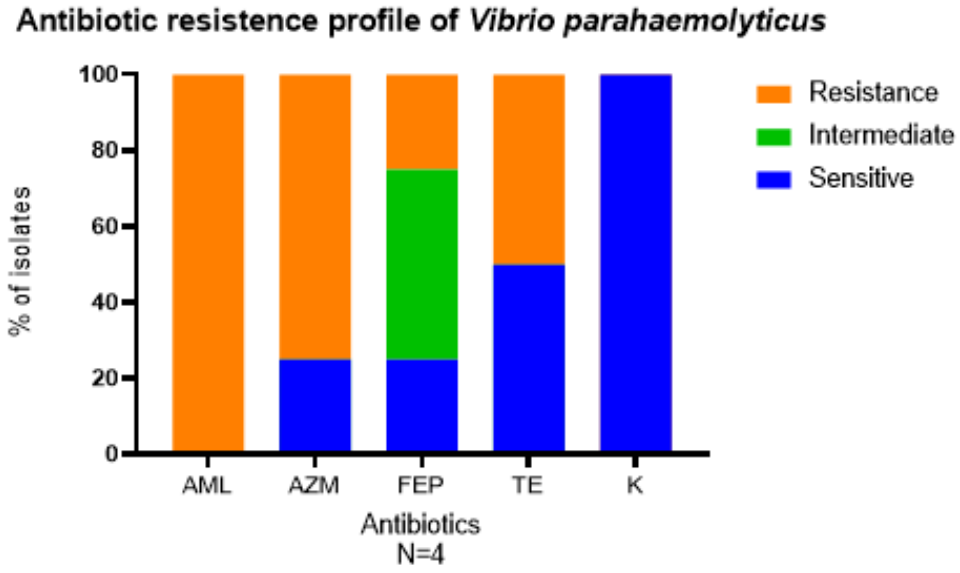


Figure 3.14: Antibiotic resistance pattern observed in *Vibrio parahaemolyticus.* 100% isolates of *V. parahaemolyticus,* showed this bacterium is 100% Sensitive to Kanamycin, but also 100% Resistant to Amoxicillin and Vancomycin. It shows high levels of uncertainty with Cefepime, which has a 50% Intermediate rating. B

3.5 PCR and gel electrophoresis:

To confirm the presumptive bacteria, multiple PCR were performed. The results are shown below.

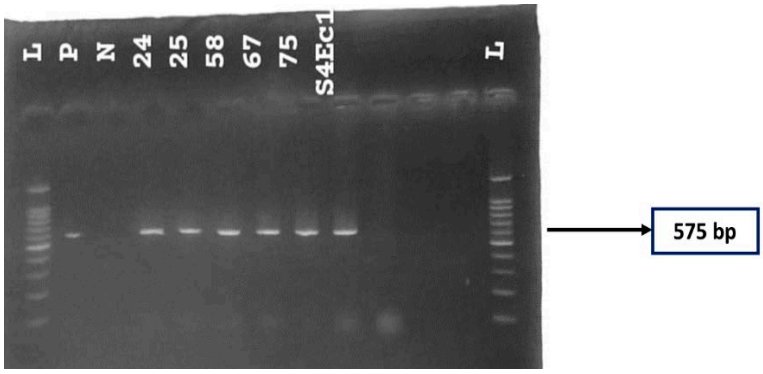


Figure 3.15: PCR confirmation of *Escherichia coli* using Gel electrophoresis. L= ladder, P= Positive Control, N= Negative Control

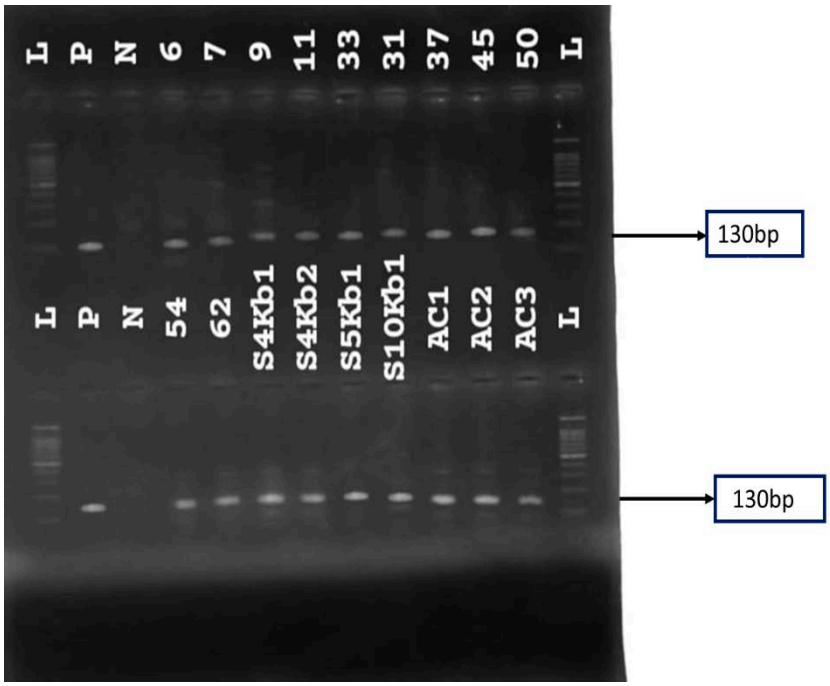


Figure 3.16: PCR confirmation of *Klebsiella pneumoniae* using Gel electrophoresis. L= ladder, P= Positive Control, N= Negative Control

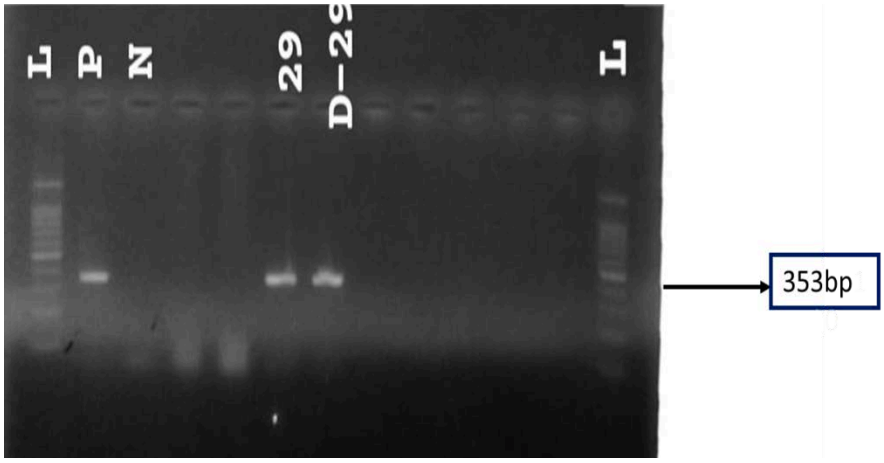


Figure 3.17: PCR confirmation of *Acinetobacter baumannii* using Gel electrophoresis. L= ladder, P= Positive Control, N= Negative Control

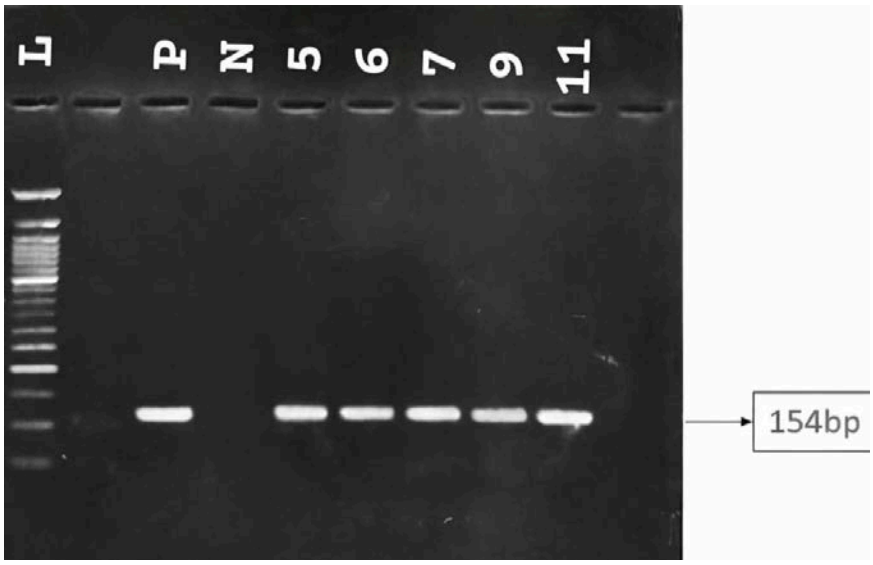


Figure 3.18 : PCR confirmation of *Vibrio cholerae* using Gel electrophoresis. L= ladder, P= Positive Control, N= Negative Control

Chapter 4

4. Discussion

The main objective of this study was to accurately identify and characterize various clinically and environmentally important bacterial isolates and to evaluate their antibiotic susceptibility patterns. Accurate identification of bacteria is crucial, as it informs both clinical treatment and public health interventions, particularly in the context of rising multidrug resistance. To achieve this, selective and differential media were employed to support presumptive identification: *Klebsiella pneumoniae* and *Escherichia coli* were grown on MacConkey agar, producing pink colonies due to lactose fermentation, *Shigella* and *Salmonella* were cultured on Xylose Lysine Deoxycholate (XLD) agar, yielding colorless or red colonies (with black centers for *Salmonella* due to hydrogen sulfide production), *Vibrio* spp. were isolated on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, producing yellow or green colonies depending on sucrose fermentation, and *Staphylococcus aureus* was identified on Mannitol Salt Agar (MSA) by the formation of yellow colonies resulting from mannitol fermentation. Following phenotypic characterization, the isolates were subjected to antibiotic susceptibility testing using commonly prescribed antibiotics, including vancomycin (VA), amoxicillin (AML), cefepime (FEP), tetracycline (TE), azithromycin (AZM), and kanamycin (K). This allowed for the assessment of sensitive, intermediate, and resistant responses among Gram-positive and Gram-negative bacteria, highlighting multidrug resistance trends. Molecular confirmation using PCR, conducted with 1.5% agar concentration at 10 mV, provided additional validation of bacterial identities, ensuring the reliability of the observed phenotypic patterns. By integrating media characteristics, colony morphology, antibiotic responses, and molecular confirmation, this study aimed to provide a comprehensive understanding of the resistance profiles and similarities among enteric and environmental bacteria, which is critical for informing empirical treatment strategies and monitoring antimicrobial resistance in both clinical and environmental settings (Shama et al., 2024).

So here, *Klebsiella pneumoniae* showed high sensitivity to cefepime (FEP, 84%) and tetracycline (TE, 73%), while displaying substantial resistance to vancomycin (VA, 94%) and amoxicillin (AML, 83%). Moderate sensitivity was observed for kanamycin (K, 67%) and azithromycin (AZM, 57%). On selective media, *K. pneumoniae* preferentially grew on MacConkey agar as

pink mucoid colonies due to lactose fermentation, which facilitated initial presumptive identification. This isolate was also confirmed through PCR using 1.5% agar concentration at 10 mV, providing molecular validation of its identity. The AST profile aligns with reports that *K. pneumoniae* commonly exhibits multidrug resistance, especially against beta-lactams and glycopeptides, reinforcing its role as a significant nosocomial pathogen.

Escherichia coli demonstrated near-complete sensitivity to kanamycin (100%), and high sensitivity to AZM, FEP, and TE (80% each), but showed resistance to VA (100%) and AML (80%). Intermediate resistance was minimal, indicating a relatively straightforward susceptibility profile. *E. coli* typically forms smooth, pink colonies on MacConkey agar due to lactose fermentation, aiding its presumptive identification. PCR confirmation under the same conditions (1.5% agar, 10 mV) validated these phenotypic findings. The similarity of *E. coli* resistance patterns to those of *Shigella* and *Salmonella* suggests shared mechanisms of resistance within Gram-negative enteric bacteria, particularly against VA and AML.

Vibrio spp. displayed moderate sensitivity to TE (65%) and AZM (50%), while resistance was highest for VA (100%) and AML (64%). On selective Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, *Vibrio* formed yellow or green colonies depending on sucrose fermentation, which assisted presumptive identification. PCR conducted with 1.5% agar at 10 mV confirmed the genus-level identity. The resistance pattern reflects *Vibrio*'s environmental adaptability and highlights the potential for multidrug resistance even in non-clinical isolates, particularly in water sources.

Staphylococcus aureus, a Gram-positive bacterium, presented a contrasting profile compared to the Gram-negative species. It showed high sensitivity to AZM, FEP, and TE (100%) but complete resistance to AML and VA (100%), indicating a distinct resistance mechanism. On Mannitol Salt Agar (MSA), *S. aureus* formed yellow colonies due to mannitol fermentation, providing a clear visual distinction from other bacteria. PCR validation using 1.5% agar at 10 mV further confirmed the identity. The distinct AST profile emphasizes the divergence between Gram-positive and Gram-negative resistance trends, which is critical for targeted antimicrobial therapy.

Shigella spp. exhibited high resistance to VA (100%) and AML (87%), with moderate sensitivity to kanamycin (80%), FEP (74%), and TE (67%). On Xylose Lysine Deoxycholate (XLD) agar, *Shigella* formed colorless colonies, reflecting its non-lactose fermenting nature. PCR-based presumptive identification with 1.5% agar and 10 mV ensured accurate genus-level classification. The similarity of *Shigella*'s resistance pattern to *E. coli* and *Salmonella* underscores the potential for shared resistance mechanisms among enteric pathogens, which is a significant concern for both clinical treatment and public health surveillance.

Salmonella spp. showed a mixed susceptibility profile, with high resistance to VA (100%) and AML (83%) and moderate sensitivity to K (83%) and TE (66%). On XLD agar, colonies appeared red with black centers due to hydrogen sulfide production, aiding visual differentiation from *Shigella*. PCR confirmation with 1.5% agar and 10 mV reinforced the phenotypic identification. The strong similarity between *Salmonella*, *E. coli*, and *Shigella* resistance patterns further highlights the conserved multidrug resistance traits among Gram-negative enteric bacteria, particularly against VA and AML.

Overall, the similarity ratio analysis demonstrated that Gram-negative enteric bacteria (*E. coli*, *Shigella*, and *Salmonella*) share highly comparable resistance trends, especially against vancomycin and amoxicillin. In contrast, the Gram-positive *S. aureus* displayed a distinct susceptibility profile, reflecting inherent differences in cell wall structure and antibiotic target sites. The integration of selective media characteristics, colony color observation, and PCR-based presumptive identification using standardized agar concentration and voltage provided robust confirmation of bacterial identities, reinforcing the reliability of both phenotypic and genotypic approaches in antimicrobial resistance studies.

Strength of Our Study

The greatest strength of this study is that it will facilitate the better health and food safety of urban centres like Dhaka City due to the recognition of the restaurant table surfaces as neglected sources of bacterial contamination. This research will be valuable in supporting the claim that often-contacted dining places can form significant pathways of transmission of food-borne diseases through the isolation and characterization of multidrug-resistant (MDR) bacteria on

such surfaces. Molecular techniques, especially Polymerase Chain Reaction (PCR) will improve the accuracy and reliability of pathogen identification alone as compared to traditional methods, which aid in early detection and effective diagnosis of the infectious agent. Also, the patterns of antibiotic susceptibility analysis were not only supplying additional information on the global war on antimicrobial resistance (AMR) through the identification of locally ineffective antibiotics and new trends in resistance. On the whole, the results provide a scientific foundation on how to maximize hygiene measures, reinforce molecular surveillance, inform antibiotic stewardship initiatives, and help policy makers implement specific strategies to minimize the transmission of antibiotic-resistant diseases within the community.

Limitations of Our Study

While this study provided valuable insights into bacterial contamination on restaurant surfaces in Merul Badda and Aftab Nagar Dhaka, it was constrained by a relatively small sampling area, which may not fully represent the diversity of bacterial populations across the city. Although PCR was performed to support bacterial identification, the study did not include quantitative analysis, such as bacterial load measurement, which could provide a clearer understanding of contamination levels. Additionally, environmental factors like humidity, cleaning frequency, and surface material were not systematically recorded, limiting the ability to correlate bacterial presence with hygiene practices. Incorporating these aspects, along with more advanced phenotypic or genomic analyses in future studies, would strengthen the depth and applicability of the findings.

Conclusion

This study effectively identified and characterized six bacterial isolates—*Klebsiella pneumoniae*, *Escherichia coli*, *Shigella*, *Salmonella*, *Vibrio*, and *Staphylococcus aureus*—using selective media, colony morphology, antibiotic susceptibility testing, and PCR confirmation. MacConkey, XLD, TCBS, and MSA media enabled presumptive identification based on color and fermentation patterns, while PCR ensured accurate validation. Antibiotic susceptibility testing revealed multidrug resistance among Gram-negative enteric bacteria, particularly to vancomycin and amoxicillin, whereas *S. aureus* showed a distinct resistance profile. Similarity analysis highlighted shared resistance trends in enteric bacteria, emphasizing conserved resistance

mechanisms. Overall, combining phenotypic and molecular methods provided a comprehensive understanding of bacterial identities and resistance patterns, which is crucial for guiding treatment and monitoring antimicrobial resistance.

Chapter 5

5. References

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Appendices

Appendix- I

Media compositions

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

Nutrient Agar

Component	**Amount (g/L)**
Peptone	5.0
Sodium chloride	5.0
Beef extract	3.0
Agar	15.0
Final pH	7.0

Xylose-Lysine-Deoxycholate Agar

Component	**Amount (g/L)**
Yeast extract	3.00
L-lysine	5.00

Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.00
Sodium deoxycholate	2.50
Sodium thiosulfate	6.80
Ferric ammonium	0.80
Phenol red	0.08
Agar	15.00

MacConkey Agar

Component	**Amount(g/L)**
Peptic digest of animal tissue	1.5
Casein enzymatic hydrolysate	1.5

Pancreatic digest of gelatin	17.00
Lactose	10.00
Bile salts	1.50
Crystal Violate	0.001
Neutral red	0.03
Agar	15.00

Mueller Hinton Agar

Component	**Amount (g/L)**
Beef, infusion from	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH	(at25°C) 7.3±0.1

Thiosulfate Citrate Bile Salt Sucrose Agar

Component	Amount (g/L)
Proteose peptone	10.0
Yeast extract	5.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Bile	8.0
Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar	15.0

***Salmonella Shigella* Agar**

Component	Amount (g/L)
Lactose	10.0
Bile salts No. 3	8.5

Sodium citrate	8.5
Sodium thiosulfate	8.5
Beef extract	5.0
Proteose peptone	5.0
Ferric Citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	13.5

Appendix – II

Instrument	Manufacturer
Weighing Machine	Adamequipment, UK
Incubator	SAARC
Laminar Flow Hood	SAARC
Autoclave Machine	SAARC
Sterilizer	Labtech, Singapore
Shaking Incubator, Model: WIS-20R	Daihan Scientific Companies, Korea
Spectrophotometer, UV mini–1240	Shimadzu Corporation, Australia
NanoDrop2000Spectrophotometer	Thermo Scientific, USA
Microscope	A. Krüssoptronic, Germany
UV Transilluminator, Model:MD-20	WealtecCorp, USA
-20°CFreezer	Siemens, Germany
magnetic stirrer, Model: JSHS-180	JSR, Korea
Vortex Machine	VWR International
Microwave Oven, Model:MH6548SR	LG, China
pH Meter: pHepTester	Hanna Instruments, Romania
Micropipette	Eppendorf, Germany
Disposable Micropipette tips	Eppendorf, Ireland
Refrigerator(4^{O}C) Model:0636	Samsung
Conductivity meter(digital)	CD-4302
Membrane filter unit	Mo-a.s-2.0Dynair