Antibiotic Susceptibility Patterns of Pathogenic Bacteria Isolated from the Surface of Tables in Local Restaurants in Dhaka City

By

Tamanna Islam
19326024
Tanjila Toma
19326010

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

Department of Mathematics and Natural Sciences
Brac University
May 2024

Declaration

It is hereby declared that

1. The thesis submitted titled "**Antibiotic Susceptibility Patterns of Pathogenic Bacteria Isolated from the Surface of Tables in Local Restaurants in Dhaka City"** 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 anyother degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's Full name & signature:

Tanjila Toma	**Tamanna Islam**
19326010	**19326024**

Approval

The thesis titled “Antibiotic Susceptibility Patterns of Pathogenic Bacteria Isolated from Surface of Tables of Local Restaurants in Dhaka City” submitted by

1. Tanjila Toma (19326010)
2. Tamanna Islam (19326024)

On Fall, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology in Spring, 2024.

Examining Committee:

Supervisor:
(Member)

_____

Nadia Sultana Deen, PhD

Associate Professor, Microbiology Program

Department of Mathematics and Natural Sciences

Brac University

Program Coordinator:
(Member)

_____

Nadia Sultana Deen, PhD

Associate Professor, Microbiology Program

Department of Mathematics and Natural Sciences

Brac University

Departmental Head:
(Chair)

_____

A F M Yusuf Haider, PhD

Professor and Chairperson,

Department of Mathematics and Natural Sciences

Brac University

Ethics Statement

For the completion of this study, samples from selected areas were collected following all necessary safety precautions. All the experiments needed for this project were performed at Brac University Life Sciences Laboratory. It should also be noted that no animal or human models were used in this study.

Abstract

Pathogenic bacteria pose a significant threat to public health, particularly impacting vulnerable populations with compromised immune systems. Identifying the sources of pathogenic bacteria is important to prevent infections. This study aimed to isolate five pathogenic bacteria—*Klebsiella pneumoniae, Escherichia coli, Salmonella* spp., *Shigella* spp., and *Vibrio cholerae* from the surface of tables in local restaurants in Dhaka city. These pathogens cause illnesses such as pneumonia, bloodstream infections, urinary tract infections, salmonellosis, shigellosis, and cholera. The antibiotic susceptibility pattern of these pathogenic bacteria revealed varying resistance patterns. All isolated species of *Salmonella* (n=5), *E. coli* (n=13), *Shigella* (n=9) and *K. pneumoniae* (n=5), showed resistance to Vancomycin. Additionally, 100% isolates of *Salmonella* spp., *E. coli* and *Shigella* spp. exhibited resistance to Erythromycin and 100% isolates *E. coli* and *Shigella* spp. demonstrated resistance to Clindamycin. Moreover, *V. cholerae* (n=5) exhibited variable degree of resistance to Azithromycin (60%), Clindamycin (40%), Erythromycin (60%), Vancomycin (60%), Cefepime (40%) and Imipenem (40%). Understanding these resistance profiles is crucial for effective treatment and mitigation strategies against these pathogenic bacteria.

Keywords: Pathogenic bacteria, *Salmonella* spp.; *Shigella* spp.; *Escherichia coli*, *Vibrio cholerae*, *Klebsiella pneumoniae*, table surface, antibiotic-resistant bacteria, antibiotic susceptibility pattern.

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 Professor **A M F Yusuf Haider**, Associate Professor Dr. **Nadia Sultana Deen**, for always appreciating and encouraging us to complete our undergraduate thesis.

We would like to acknowledge our respected Supervisor Dr. **Nadia Sultana Deen**, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, Brac University 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.

Table of Contents

List of Figures

List of Tables

List of Acronyms

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

Chapter 1

1 Introduction

1.1 Background

Food contact surfaces refer to any surface that comes into contact with food, such as knives, spoons, trays, tableware, chopping boards, cups, glasses, and highchairs (Tenna *et al*., 2023). It is important to note that areas where food may spill, leak, or drain, like the apron of food handlers, desiccators, the interior of refrigerators, or microwave ovens, surface of tables can become significant sources of different pathogenic organisms. In modern cities, many people rely on restaurant foods for their daily meals. People may become contaminated with dangerous pathogens from surface of tables of the restaurants and contract food-borne illnesses.

In recent years, researchers reported microbial contamination antimicrobial-resistant patterns of contaminating bacteria from different sources (Bello *et al*., 2023, Mohamedin *et al*., 2015, Tenna *et al*., 2023). Bello *et al.* reported that on the eating tables, *Pseudomonas, Enterobacter*, and *Citrobacter* spp. had an occurrence of 11.76%, both *Listeria,* and *Staphylococcus* spp. had around 17.64%, and *E. coli*around29.41%(Bello *et al*., 2023). Moreover, Bukhari, M. A., *et al.* also identified *Klebsiella* spp. (18.7%), *Escherichia coli* (17,7%), *Staphylococcus aureus* (4.4%), *Pseudomonas* spp. (1.7%), *Proteus* spp. (0.7%), *Bacillus cereus* (0.7%), and *Candida sp.* (0.3%) in the food contact surfaces of Makkah cities restaurants (M *et al*., 2021). Furthermore, Mohamedin *et al.* discussed the significant amounts of *S. aureus* and *E. coli* found in numerous restaurants and catering services as well as on food processing surfaces and utensils (Mohamedin *et al*., 2015). These pathogenic bacteria may cause severe health issues *e.g.,* severe urinary tract infection, abdominal, pelvic infection, pneumonia, bacteremia and meningitis even death.

A different study by Tenna *et al.* discussed that the rate of contamination on cleaned and ready-to-use utensils was quite high for aerobic plate count, total coliform, fecal coliform, *E. coli*, and *Staphylococcus aureus* and also fecal coliform and *E. coli* were identified in 14.37% and 3.12% in the surface of utensils (Tenna *et al*., 2023). Alsallaiy *et al.,* have found that restaurant menus are a potential source of bacterial contamination with *Staphylococcus* spp. And *E. coli*. Moreover, the study encountered that bacteria from menus can transfer to the hands of consumers, as confirmed by the presence of *E.coli* on both menus and hands (Alsallaiy *et al*., 2016). The transfer rate of *E. coli* to menus was 11.17% and *E. coli* JM109

was identified on plastic-laminated menus in both studies confirming its ability to adhere to plastic (Torres *et al*., 2005).

Beyi *et al.,* conducted a study in the Central Ethiopia and found that *E. coli* O157 was identified in 4.5% of beef carcass swabs and 3.6% of cutting board swabs at butcher shops (Beyi *et al*., 2017). Another study claimed that non-food contact surfaces were also a major source of bacteria that cause foodborne illnesses as they found bacterial growth on tables, sinks, chairs, counters, and walls of a restaurant.

1.2 Epidemiology and Impact of Foodborne Illnesses

Foodborne pathogens are responsible for numerous illnesses that have substantial impacts on both human well-being and the economy. These pathogenic bacteria, *e.g. Bacillus. cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Cronobactersakazakii, E. coli, Listeria monocytogenes, Salmonella*spp., *Shigella*spp., *Staphylococcus aureus, V. cholerae, and Yersinia enterocolitica*, exhibit distinct characteristics and contribute significantly to foodborne diseases (Bintsis T. *et al*., 2017). *E. coli*, commonly found in the large intestines of various animals, including humans and cattle, is generally harmless. However, certain strains of *E. coli* can pose significant health risks when consumed by humans. One such strain, *E. coli* 0157:H7 typically resides in the intestines of cattle and is often contracted through the consumption of undercooked ground beef (Beyi *et al*., 2017). Additionally, *E. coli* 0157:H7 can be transmitted through unpasteurized milk, fruit juice, contaminated water, raw fruits, and vegetables, as well as person-to-person contact.

Salmonellosis is an illness caused by *Salmonella*spp. bacteria, which are frequently present in the gastrointestinal tracts of mammals, reptiles, and birds. Typically, humans contract *Salmonella*spp. infections by consuming animal-derived foods such as eggs, meat, and milk. *Shigella* spp. is a type of bacteria responsible for causing shigellosis, also referred to as bacillary dysentery. These bacteria are highly contagious, and foodborne outbreaks frequently occur due to infected food handlers. Notably, unlike many other prevalent foodborne pathogens, *Shigella* spp. primarily infects humans and does not have other natural hosts (Boslaugh, S. E. *et al*., 2023).

V. cholerae produces *cholerae* toxin and is a noninvasive pathogen that colonizes mainly the small intestine and results in severe diarrhea (Weil *et al*., 2019).
Individuals working with food can serve as a source of highly pathogenic strains of foodborne illnesses that can lead to various symptoms, including diarrhea, abdominal cramps, nausea, and vomiting. The most severe symptoms tend to occur in infants and the elderly, although these diseases can affect individuals of all age groups (Bintsis T. *et al*., 2017).

While many foodborne infections go unnoticed and unreported, the Public Health Agency of Canada estimated that approximately 1 in 8 Canadians may fall ill each year due to foodborne pathogens. Among these cases, more than 11,500 individuals require hospitalization, each year, tens of millions of individuals worldwide experience diarrheal diseases. To illustrate, in 1988, China witnessed an outbreak of hepatitis A, due to the consumption of contaminated clams, which affected over 300,000 individuals.

Similarly, in 1994, the United States faced a salmonellosis outbreak linked to tainted ice cream, affecting 224,000 people(Boslaugh, S. E.*et al*., 2023). A huge portion of the global population faces the threat of consuming food that is not safe for consumption. Hence, it is crucial to maintain cleanliness in food preparation, transportation, and consumption areas, and for those involved in food handling to practice rigorous personal hygiene (Uçar, A., Yilmaz, M. V., & Çakiroglu, F. P. *et al*., 2016).

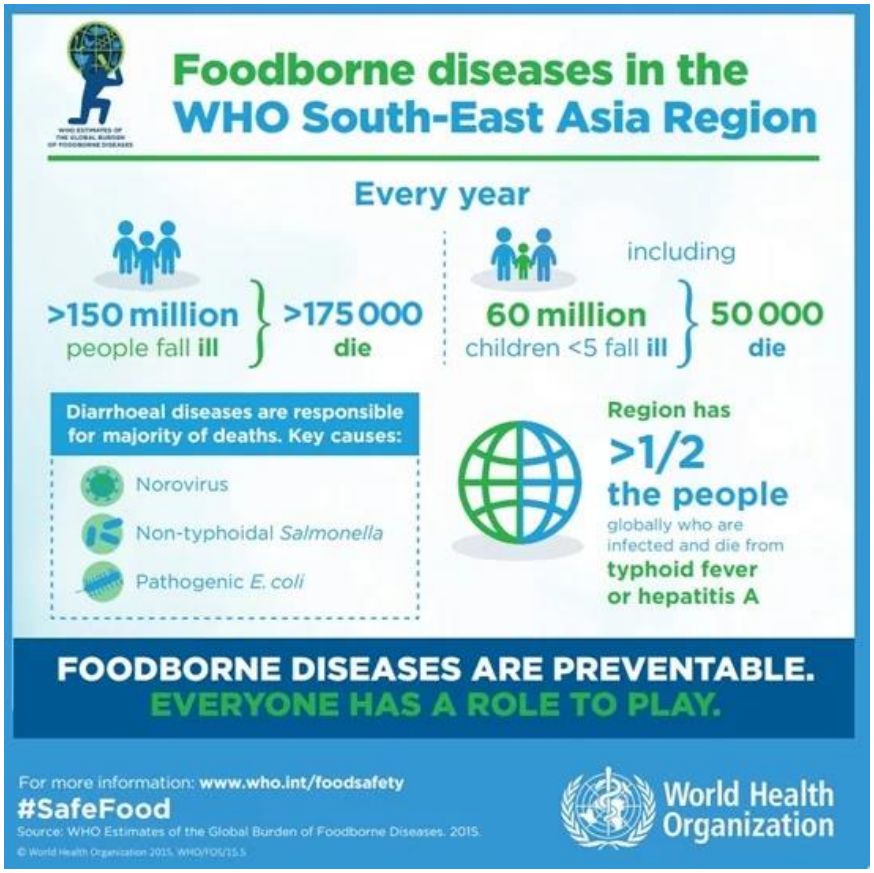

Figure 1.1: Foodborne diseases in the Asia Region (WHO, 2023). The World Health Organization (WHO) estimates that eating unhealthy food results in the loss of 33 million healthy lives per year (WHO, 2023).

Every year, millions of people suffer from foodborne illnesses because of unsafe food, causing 600 million cases of foodborne illnesses and 420,000 deaths globally. Shockingly, 30% of these deaths are reported to occur among children under the age of 5. According to the World Health Organization (WHO), about 33 million healthy lives are lost due to the consumption of unsafe food yearly (WHO, 2023). In addition, antibacterial susceptibility test (AST) of the bacteria causing food-borne illnesses is important to know the sensitivity and resistance of an antibiotic against a bacterium. Besides, it also helps us to find out which antibiotic will be most effective in treating a specific bacterial infection. It also determines the effectiveness of antibiotics against bacteria.

Table 1: Studies of Restaurant Isolates with Their Antimicrobial Susceptibility Characteristics

Route of Transmission	**Bacteria**	**Types of Disease**	**Test Performed**	**Antibiotics to Which Bacteria Are Resistant**	**Reference**
Kitchen sink, stove knob, cutting board, refrigerator handle, phone handle, etc.	*E. coli*	Food-borne illness	BT, AST, PCR	Cefpodoxime, Nitrofurantoin	(Kunhiraman *et al*., 2023)
Cooked and sold food samples	*K.pneumoniae*	Bacterial infection	BT, AST	Amoxicillin Clavulanic Acid, Ticarcillin, Clavulanic Acid, Ceftriaxone, Cefotaxime, and Ceftazidime.	(Kunhiraman *et al*., 2023)
Carcass and cutting board of butcher shops	*E. coli O157*	Severe diarrhea	Slide agglutination test, AST	Amoxicillin, streptomycin and Chloramphenicol	(Beyi *et al*., 2017)
Local restaurant tables, fast food restaurants, hospital canteens, and academic institutions tabletops	*E. coli, K. pneumoniae, Staphylococcus aureus, Salmonella*sp.	Foodborne illness	BT, AST	Most of the isolates showed a greater degree of susceptibility towards cotrimoxazole and tetracycline and were resistant to ceftriaxone	(Paul *et al*., 2020)
Tables of the dining hall	*S. aureus, E. coli, K. pneumonia* and *Shigella* spp.	Food poisoning	BT, AST, gram staining,	Gentamycin and resistant to most other antibiotics.	(Kelvin *et al*., 2020)
Restaurant menus	*E. coli, Staphylococcus aureus*	Foodborne illnesses	BT	N/A	(Alsallaiy *et al*., 2015)
Food contact surfaces of hotels and restaurants	*E. coli, S. aureus*	Foodborne illnesses	BT	N/A	(Tenna*et al*., 2023)

Route of Transmission	**Bacteria**	**Types of Disease**	**Test Performed**	**Antibiotics to Which Bacteria Are Resistant**	**Reference**
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* sp.	Food-borne illnesses	Vitek 2 compact test	N/A	(Bukhari *et al*., 2021)
Raw food, Cooked food, Preparation area, tools, hands, fridge, and cashier in restaurants	*E. coli, K. aerogenes Salmonella*sp p., *Shigella* spp., *S. aureus*, and *S. epidermidis*	foodborne infections	BT, gram staining	N/A	(Melebari*et al*., 2023)
Meat and Swab Samples of Various Contact Surfaces	*E. coli* and *E. coli*O157:H7	foodborne disease	BT, ASTtest by Kirby-Bauer disc diffusion method	Higher resistance against erythromycin and ampicillin with the prevalence of (97.1%) and (92.6%) and (91.2%) and (88.9%), respectively. The y were susceptible to cefotaxime, ceftriaxone, ceftazidime, ciprof loxacin, kanamycin, genta mycin, and streptomycin.	(Sebsibe& Asfaw, 2020)
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	(Bradley *et al*., 2011)
Tables and sink surface	*S. aureus, E. coli, Proteus, K. pneumoniae*	Food poisoning	BT, AST	N/A	(Obi *et al*., 2021)

Route of Transmission	Bacteria	Types of Disease	Test Performed	Antibiotics to Which Bacteria Are Resistant	Reference
Restaurant plates, spoons, chopping boards, and table	*E. coli*, *Salmonella*sp., and *S. aureus*	Causing early-onset Sepsis in very-low-birth-weight infants	BT, AST	Ciprofloxacin, amoxicillin (46.2 %) and sparfloxacin(53.8 %) were resistant.	(Obi *et al*., 2021)

BT: Biochemical test; AST: Antimicrobial Susceptibility Test; PCR: Polymerase Chain Reaction.

1.3 Aims of Study

This study aims to isolate five targeted pathogenic bacteria e.g., *E. coli*, *Salmonella*spp., *Shigella* spp., *V. cholerae*, and *K. pneumoniae* from the table surface of restaurants. Moreover, the prevalence of the antimicrobial resistance of the bacterial isolates to a wide range of antibiotics will also be evaluated.

1.4 Hypothesis and Objectives

This study focused on the gram-negative bacteria (*E. coli*, *Salmonella* spp., *Shigella* spp., *V. cholerae* and *K. pneumoniae*) since most of the food-borne pathogens are gram-negative. In addition, microbes can survive not only on inanimate objects but also can be transmitted through direct contact with hands. When pathogens come into contact with a person's hands, they can be easily transmitted to the mouth, allowing them to enter the body. Since the restaurants are used by the general people, there is a huge possibility that the tables might be a reservoir of food-borne pathogens that lead to food-borne illnesses.

Moreover, antibiotics are a type of medicine that is used to treat bacterial infections such as pneumonia, urinary tract infections, and skin infections. They can be life-saving when used appropriately. As a result, it is necessary to select a proper antibiotic against a specific bacterial infection and that can be done by conducting AST.

Chapter 2

2. Methods and Materials

2.1. Workflow

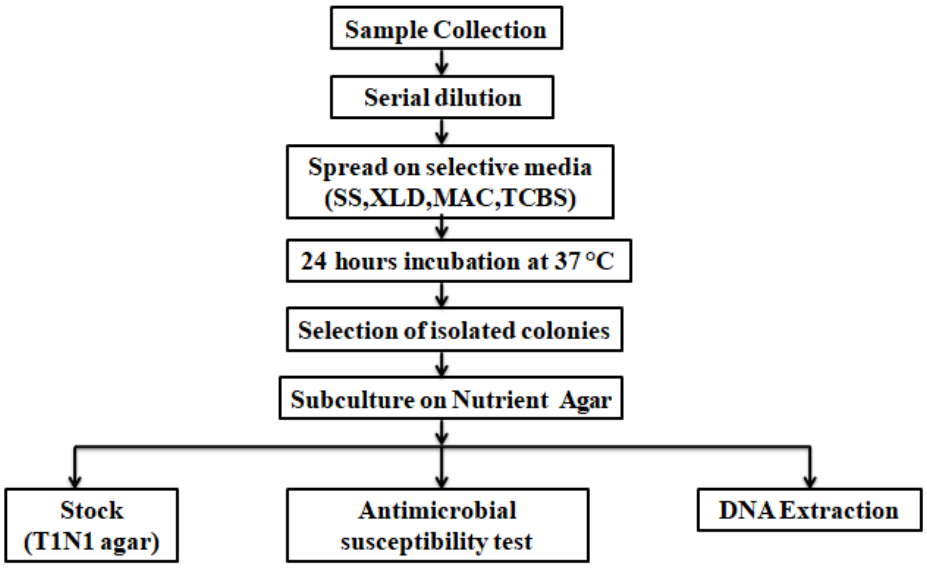

Figure 2.1: Workflow diagram of Isolated Organisms from Restaurant Table Surfaces. To perform this research study, this flow chart was followed step by step which includes serial dilution, spreading on selective media, AST and DNA extraction. Antibiotic Susceptibility Test: AST.

2.2. Media, Solutions, and Reagents

Nutrient agar (NA), MacConkey agar (MAC), *SalmonellaShigella* agar (SS), Thiosulfate-Citrate- Bile-Salts-Sucrose agar (TCBS), Xylose Lysine Deoxycholate agar (XLD), Sodium Chloride (NaCl) were purchased from Mark Chemicals and HI Media Laboratories Pvt. Ltd, India. Glycerol ($C_3H_8O_3$) for stocking was obtained from FUJIFILM Wako Pure Chemical Corporation, Japan.

2.3. Sample Collection Site

For this project, five different local restaurants within a 1 km area of BRAC University, MerulBadda, Dhaka, were selected. During the period of January 2024 to February 2024, samples were collected from the table surfaces of these restaurants.

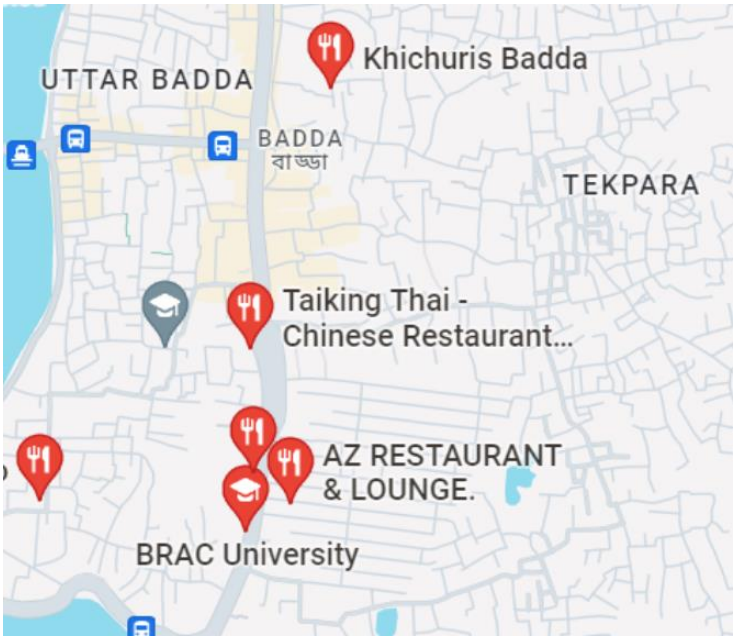

Figure 2.2: Sample collection sites of our study. The samples were collected from table surfaces of restaurants around BRAC University, MerulBadda.

2.4. Sample Collection

In brief, a cotton swab was dipped in a 10 ml sodium chloride (NaCl) solution. After that, the swab was used to wipe a 1*1 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. All tests were conducted in triplicates.

2.5. Isolation and Identification of Bacterial Strains

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. 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 developed to isolate microorganisms, mainly bacteria from the specimen or the sample cultures (Sanders, 2012). To perform this technique, at first agar

growth 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 onto the selective media by using a sterile glass rod. After that, the plates were incubated in an inverted position for 24-48 hours at 37^0C. Finally, the plates were observed to see the bacterial growth and the number of total bacteria was calculated. Besides, isolated colonies from the medium were selected for further investigation using the streak plate technique (Sanders, 2012).

Table 2: Colony Morphology of Specific Bacteria on Selective Media

Organism	**Gram Positive/Negative**	**Media**	**Expected colony morphology**
E. coli	Gram-negative	MAC agar	Pink to dark pink, dry and donut-shaped
E. coli	Gram-negative	XLD agar	Large, Flat, Yellow Colonies
Shigella spp.	Gram-negative	XLD agar	Red/pink colonies
Shigella spp.	Gram-negative	SS agar	Colorless colony, transparent
*Salmonella*spp.	Gram-negative	XLD agar	Colorless colonies with a black center
*Salmonella*spp.	Gram-negative	SS agar	Colorless, transparent, with a black center if H2S is produced
V. cholerae	Gram-negative	TCBS agar	Green/Yellow colonies
K. pneumoniae	Gram-negative	SS agar	Pink to red colony. Larger than *E. coli*, mucoid, pale, opaque cream to pink.
K. pneumoniae	Gram-negative	MAC agar	Pink colonies, mucoid

SS: *SalmonellaShigella* Agar, TCBS: Thiosulfate-Citrate- Bile-Salts-Sucrose agar, XLD: Xylose Lysine Deoxycholate agar.

2.7. Streaking of the Selected Isolated Colonies on the NA Media

The streak plate technique is a method used to isolate and purify a bacterial colony. Single isolated colonies were chosen from selective media and streaked onto NA within a laminar

airflow cabinet. Following this, the culture plates undergo a 24-hour aerobic incubation at 37°C. After the incubation, the colonies that were formed on the agar surface were observed. An individual and properly isolated colony was selected for the next analysis, *e.g.* DNA extraction and stocking (Sanders, 2012).

2.8. Antibiotic Susceptibility Testing (AST)

The Kirby-Bauer disk diffusion test is the most commonly used method to determine antibiotic resistance or susceptibility in various bacteria. 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. 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 (CLSI, 2023). In this study, 14 antibiotics from 11 different groups were utilized for microbiological sensitivity testing.

These subcultures were then incubated under aerobic conditions at 37°C for 24 hours. After obtaining pure cultures, a small amount of each bacterium was taken from a colony and placed in a tube containing 10 ml of 0.9% NaCl. The turbidity was adjusted to match the McFarland 0.5, thereby standardizing the size of the inoculums. Then, an autoclaved cotton swab was dipped, and excess liquid from the swab was removed by gently rotating the swab against the tube's surface. This swab was used to evenly distribute the bacteria across the entire surface of MHA. Following this, antibiotic discs were kept to the surface of MHA media by 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.

The bacterial isolates that show resistance to more than one antibiotic are classified as multidrug-resistant strains (MDR) (Basak *et al*., 2016). Furthermore, a Multiple Antibiotic Resistance Index of more than 0.2 indicated a high-risk source of cross-contamination in the sample where antibiotics were commonly used (Ritchell & Paul, *et al*.2016).

Table 3: The antibiotic discs, their concentrations, and the sizes of the zones of inhibition (mm) for Enterobacteriaceae

S. No.	Antibiotic	Antibiotic group	Disc code	Disc potency (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
1	Azithromycin	Macrolide	AZM	15	≥18	14-17	≤13
2	Clindamycin	Lincosamides	CLN	2	21	15-20	14
3	Ampicillin	β-lactam	AMP	10	17	14-16	13
4	Vancomycin	Glycopeptide	VAN	30	18	15-17	≤17
5	Tetracycline	Tetracycline	TET	30	15	12-14	11
6	Levofloxacin	Quinolones	LEV	5	17	14–16	13
7	Cefepime	Cephalosporin	CPM	30	25	19–24	18
8	Amoxicillin	Beta-lactamase	AML	30	18	14-17	13
9	Gentamicin	Aminoglycoside	GEN	10	17	14-16	14
10	Kanamycin	Aminoglycoside	KAN	30	18	14–17	13
11	Erythromycin	Macrolide	ERY	15	23	14-22	13
12	Meropenem	Carbapenem	MEM	10	23	20–22	19

TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin

2.9. DNA Extraction

To obtain deoxyribonucleic acid (DNA) from cells, DNA extraction was performed, which entails breaking the cell wall as well as the nuclear membrane. Initially, the cell gets ruptured so that it can release the nucleus, and subsequently, the nucleus is opened to release the DNA. Tris-EDTA(TE) buffer was utilized for DNA extraction, as it maintains pH of the solution and solubilizes DNA while protecting the nucleic acids from enzymatic lysis.

The DNA extraction by boiling method is a simple and cost-effective technique commonly used in molecular biology laboratories. To perform the test, 150 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. To lyse or release the DNA, resuspended cells were boiled for 10 minutes at 95°C. After that, it was centrifuged at 10,000 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 (Ahmed & Dablool *et al*., 2017).

Chapter 3

3. Results

3.1. Total bacterial count from the samples

Nutrient agar (NA) was used in the experiments for the total count of bacteria isolated from five different samples. Colony forming unit (CFU) were counted from the NA spread plates of the samples (Figure 3.1). Bacterial count on NA ranged between $7.67x10^5$ CFU/sample to $9.67x10^5$ CFU/sample. The CFU count of all samples such as R1, R2, R3, R4 and R5 was$8.33x10^5$, $7.67x10^5$, $9.0x10^5$, $9.67x10^5$ and $9.33x10^5$ CFU/sample respectively.

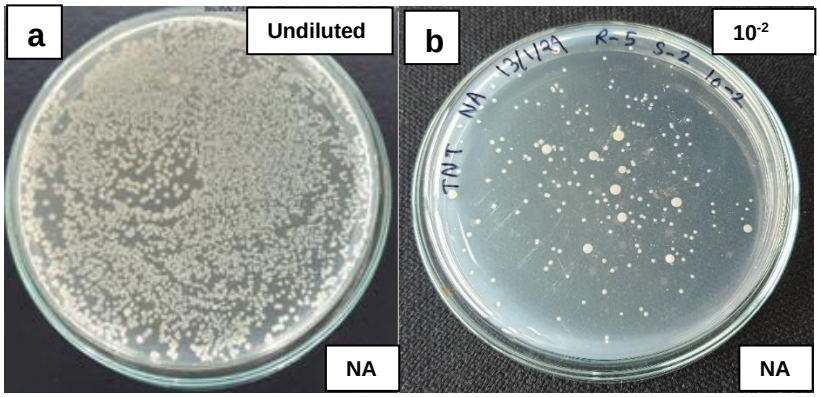

Figure 3.1: Total colony counts on NA. After diluting and inoculating on NA by spread plate techniques, samples were incubated for 24 hours and observed for individual colonies by counting CFU per sample. a) Spread plating of the original sample and b) Spread plating of the 1 in 100 dilution from the original sample.

3.2. Presumptive Bacterial Identification on Selective Media

Five samples were collected from local restaurants near BRAC University, MerulBadda, Dhaka. We aimed to isolate *E. coli*, *Salmonella* spp., *Shigella* spp., *K. pneumoniae*, and *V. cholerae*. The spread plate technique was performed on different selective media including MAC, TCBS, XLD, and SS to detect the targeted bacteria. After 24 hours of incubation at 37°C, colony morphology was observed.

Table 4: Average number of CFU per Sample

Sample ID No.	Replicate ID No.	CFU per replicate	Average CFU per sample
R1	R11	$9x10^5$	$8.33x10^5$
	R12	$1.2x10^6$	
	R13	$4x10^5$	
R2	R21	$8x10^5$	$7.67x10^5$
	R22	$1.1x10^6$	
	R23	$4x10^5$	
R3	R31	$1x10^6$	$9.0x10^5$
	R32	$1x10^6$	
	R33	$7x10^5$	
R4	R41	$1x10^6$	$9.67x10^5$
	R42	$1.4x10^6$	
	R43	$5x10^5$	
R5	R51	$1x10^6$	$9.33x10^5$
	R52	$1.5x10^6$	
	R53	$3x10^5$	

R1: Restaurant 1; R2: Restaurant 2; R3: Restaurant 3; R4: Restaurant 4; R5: Restaurant 5.

3.2.1. 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 pink colonies and appeared flat yellow colonies on the XLD agar due to lactose fermentation.

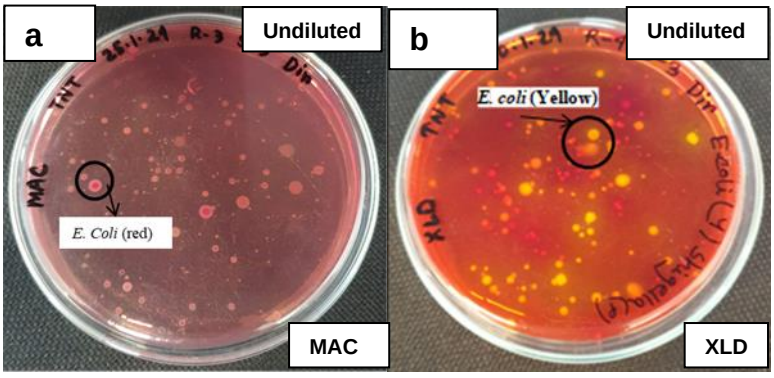

Figure 3.2: *E. coli* observed on MAC and XLD agar plates. Undiluted suspension prepared from the samples were inoculated on the selective media by the spread plate technique and incubated for 24 hours. Growth characteristics were observed on a) MAC agar, b) XLD agar.

3.2.2. Presumptive identification of *K. pneumoniae*

K. pneumoniae appeared as a mucoid pink due to lactose fermentation on MAC agar and mucoid yellow colonies on XLD agar. The differential and selective properties of MAC and XLD agar enable the isolation and presumptive identification of *K. pneumoniae.*

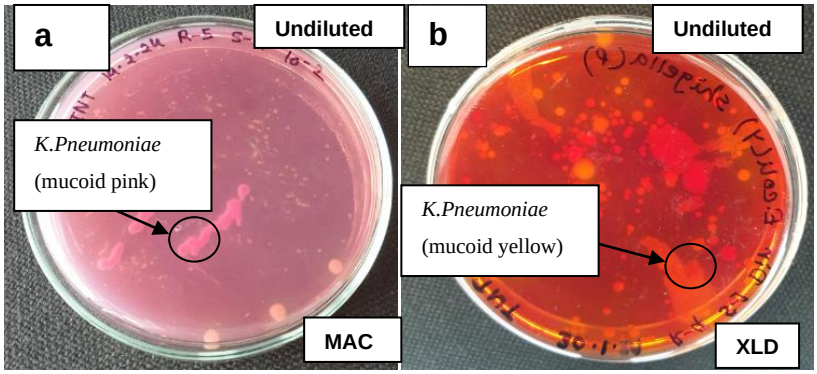

Figure 3.3: *K. pneumoniae* observed on MAC and XLD agar plates. A spread plate technique was performed to inoculate the undiluted suspension prepared from sample. After incubation for 24 hours, growth characteristics were observed on a). MAC agar, b). XLD agar.

3.2.3. Presumptive identification of *V. cholerae*:

In TCBS agar, *V. cholerae* formed yellow colonies due to the sucrose fermentation. TCBS agar contains sucrose, which is digested by *V. cholerae* to produce acidic byproducts. This acid alters the pH indicator in TCBS agar, causing *V. cholerae* colonies to turn yellow. This distinctive color change helps to distinguish *V. cholera* from other bacteria present in the sample, making it easier to identify.

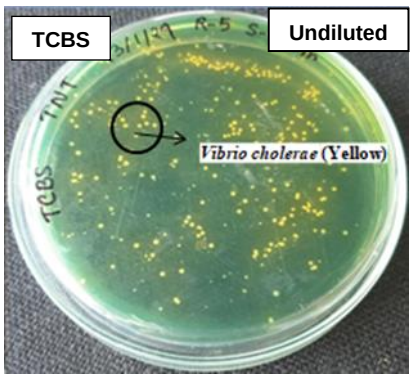

Figure 3.4: *V. cholerae* observed on TCBS agar plate. After incubating the inoculated plate for 24 hours, growth characteristics were observed.

3.2.4. Presumptive identification of *Salmonella* spp.:

Salmonella spp. formed colorless or transparent colonies on MAC agar due to their non-lactose-fermenting feature and showed colorless black center colonies on SS agar due to H_2S production.

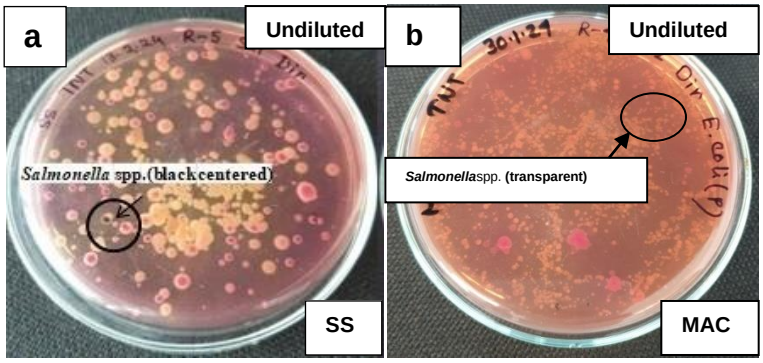

Figure 3.5: *Salmonella* spp. observed on SS and MAC agar plates. After incubation for 24 hours, growth characteristics were observed on a) SS agar, and b) MAC agar.

3.2.5. Presumptive identification of *Shigella* spp.:

In XLD, *Shigella* spp. formed pink colonies and it does not ferment xylose. 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 and pink or red with black centers on XLD agar due to hydrogen sulfide production.

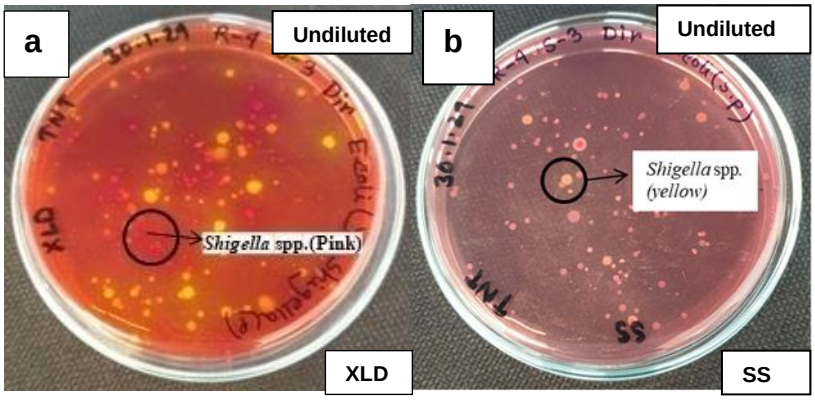

Figure 3.6: *Shigella* spp. observed on SS and XLD agar plates. Following an incubation period of 24 hours, growth characteristics were observed on both a) XLD agar and b) SS agar**.**

3.3. Presence of targeted bacteria in the samples

We aimed to detect five types of pathogenic bacteria such as *E. coli*, *Shigella* spp., *Salmonella* spp., *K. pneumoniae*, and *V. cholerae* from the samples from five different local

restaurants, namely R1, R2, R3, R4, and R5. Based on the presumptive identification, both *E. coli* and *Shigella* spp. were found to be present in R1, R2, R3, R4, and R5. Besides, *Salmonella* spp. was present in R2, R3, R4, R5 and absent in R1. *V. cholerae* was present in R1, R3, R4, R5 and absent in R2. *K. pneumoniae* was present only in R1, R2 and absent in rest of the samples.

Table 5: Presence of targeted bacteria in restaurant sample

Types of bacteria	Sample number				
	R1	**R2**	**R3**	**R4**	**R5**
E. coli	+	+	+	+	+
Shigella spp.	+	+	+	+	+
*Salmonella*spp.	-	+	+	+	+
V. cholerae	+	-	+	+	+
K. pneumoniae	+	+	-	-	-

3.4. Antibiotic Susceptibility Test

In this experiment, 37 bacterial isolates were selected for an antibiotic susceptibility test. A total of 12 different antibiotics were used in this test. The Kirby-Bauer disc diffusion method was used to perform this test on Mueller-Hinton Agar (MHA) media. After 18-24 hours of incubation, the zone diameter was measured to evaluate the antibiotic sensitivity patterns of these isolates by following the CLSI guideline (Table 3**).**

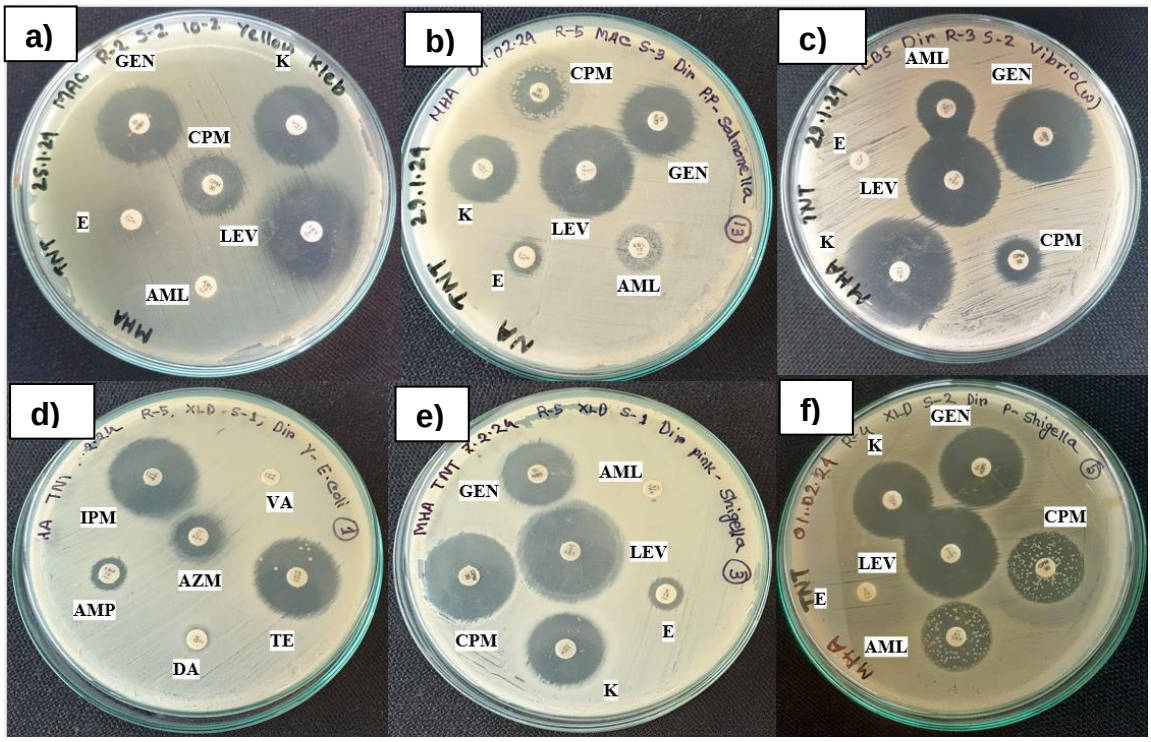

Figure 3.7: MHA plates after incubation for AST. Following the incubation, zones of inhibition surrounding antibiotic disks were observed on the MHA plates a, b, c, d, e and f, indicating bacterial sensitivity to the tested antibiotics.

Most of the isolates were resistant to Clindamycin (CLN), Erythromycin (ERY), and Vancomycin (VAN). The antibiotics Gentamicin (GEN) and Levofloxacin (LEV) were found to be the most effective against all bacterial isolates. Besides, satellite colonies were visible in the area of some zones. Some of the isolates were multidrug-resistant.

3.5. Antibiotic resistance profile of *E. coli*:

At first, 100% isolates of *E. coli* (n=13) showed resistant against Clindamycin (CLN) and Vancomycin (VAN); Followed by Erythromycin (ERY) (75%), Amoxicillin (AML) (66%), Ampicillin (AMP) (41%), Imipenem (IPM) (25%) and Kanamycin (KAN) (8.33%). In contrast, 100% isolates of *E. coli* showed susceptibility to the antibiotics Gentamycin (GEN), Tetracycline (TET), Levofloxacin (LEV), and Cefepime (CPM) was effective against the bacteria (Figure 3.5).

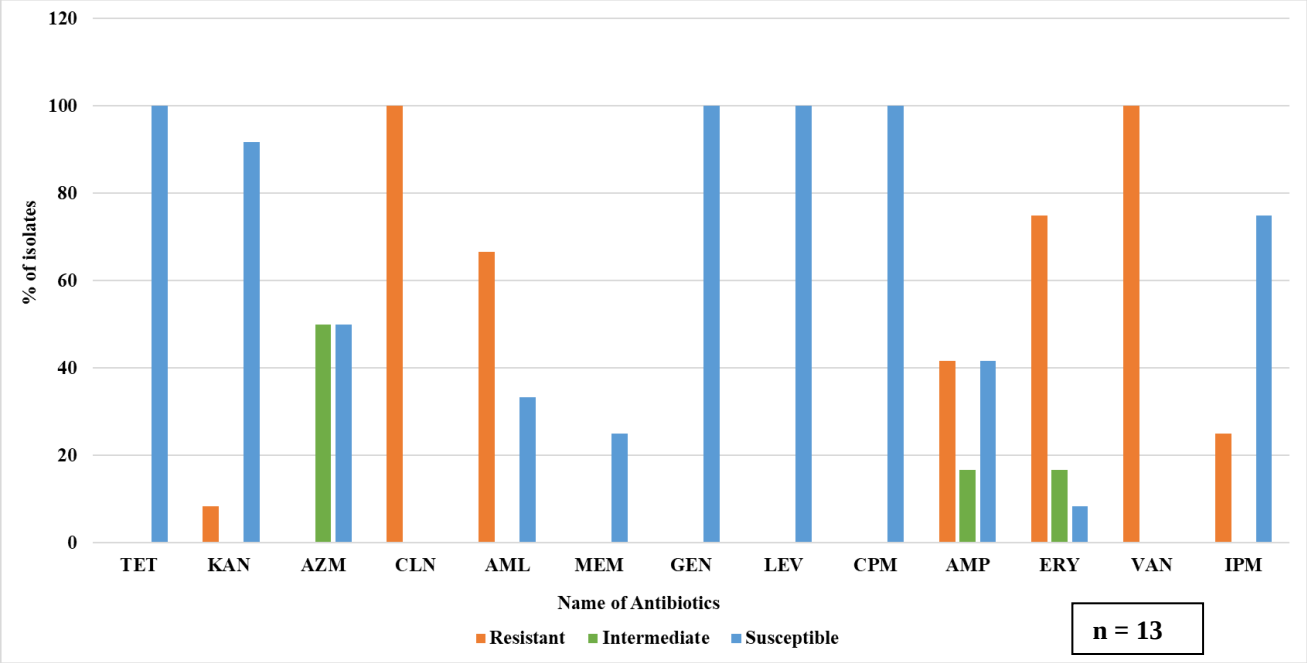

Figure 3.8: Antibiotic resistance pattern observed in *E. coli.* 100% isolates of *E. coli* showed resistant against CLN and VAN and were 100% susceptible to GEN, TET, LEV, and CPM.TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin

3.6. Antibiotic resistance profile of *Salmonella* spp.:

In the same way, 100% isolates of *Salmonella* spp. (n=5) showed resistant against ERY and VAN. Besides, other resistant antibiotics were AML (80%), AMP (80%), IPM (40%), AZM (40%), TET (20%), CPM (80%) and KAN (20%). On the other hand, *Salmonella* spp.has a susceptibility rate of 100% against GEN and LEV which were the most effective antibiotics against these bacteria (Figure 3.6).

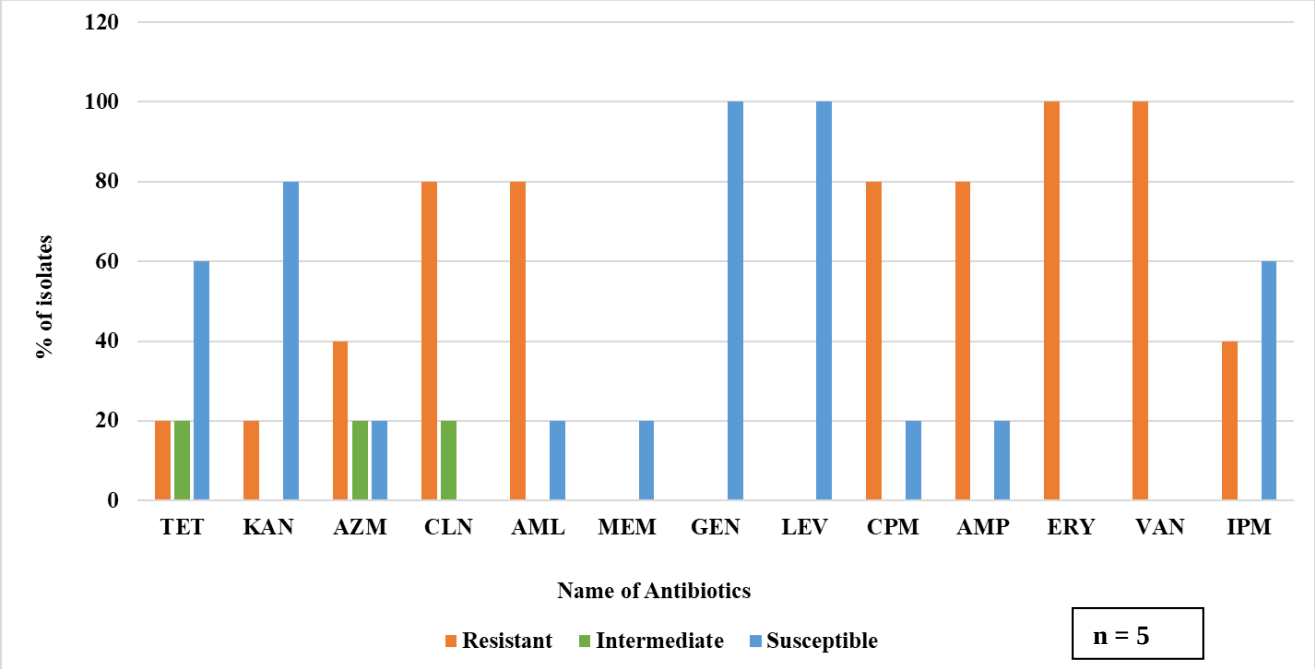

Figure 3.9: Antibiotic resistance pattern observed in *Salmonella* spp. 100% isolates of *Salmonella*spp. showed resistant against ERY and VAN and susceptibility against GEN and LEV.TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin.

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

Besides, 100% isolates of *Shigella* spp. (n=9) demonstrated resistant against CLN, ERY, and VAN. Followed by other resistant antibiotics were AZM (50%), AML (87%), CPM (12%), AMP (75%), and IPM (37%). In contrast, 100% isolates of *Shigella* spp. showed susceptibility against the antibiotics GEN, LEV, and KAN. According to the study, these antibiotics are most effective against the bacteria. (Figure 3.7).

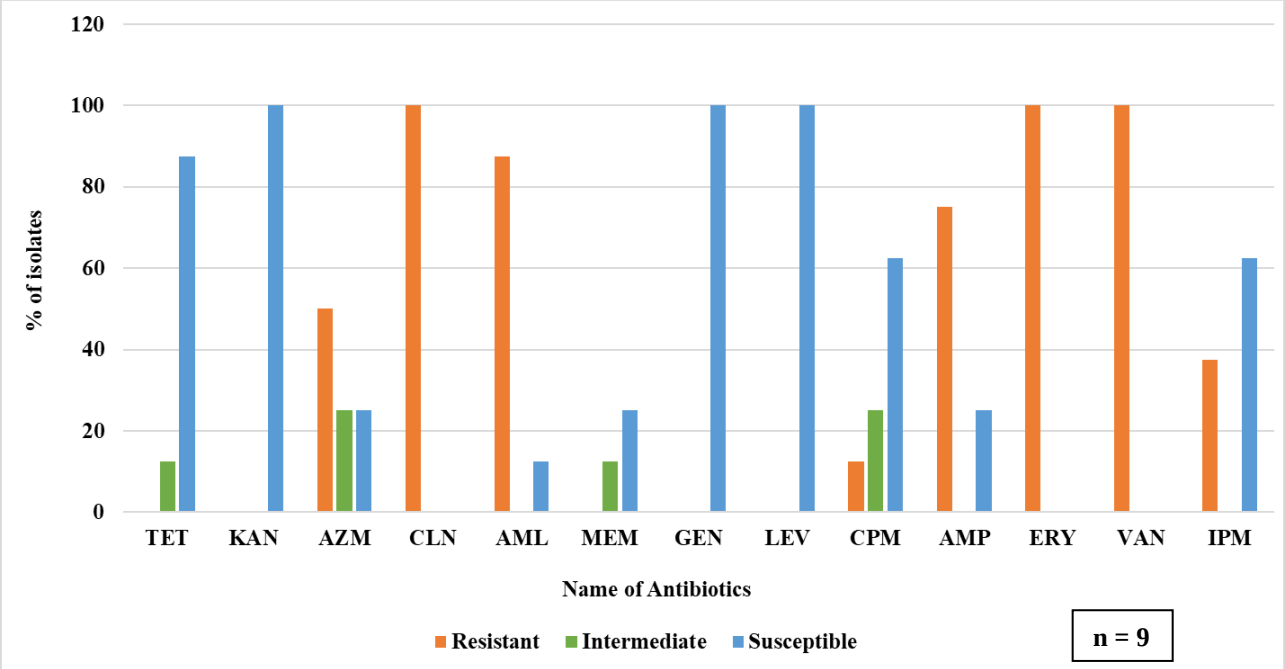

Figure 3.10: Antibiotic resistance pattern observed in *Shigella* spp. 100% isolates of *Shigella* spp. were resistant to CLN, ERY, and VAN and susceptible to the antibiotics GEN, LEV, and KAN. TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin.

3.8. Antibiotic resistance profile of *V. cholerae:*

V. cholerae (n=5) showed resistant against TET (20%), KAN (20%), AZM (60%), CLN (40%), ERY (60%), VAN(60%), CPM(40%) and IPM(40%). On the other hand, 100% isolates of *V. cholerae* showed susceptibility rate against GEN and LEV (Figure 3.8).

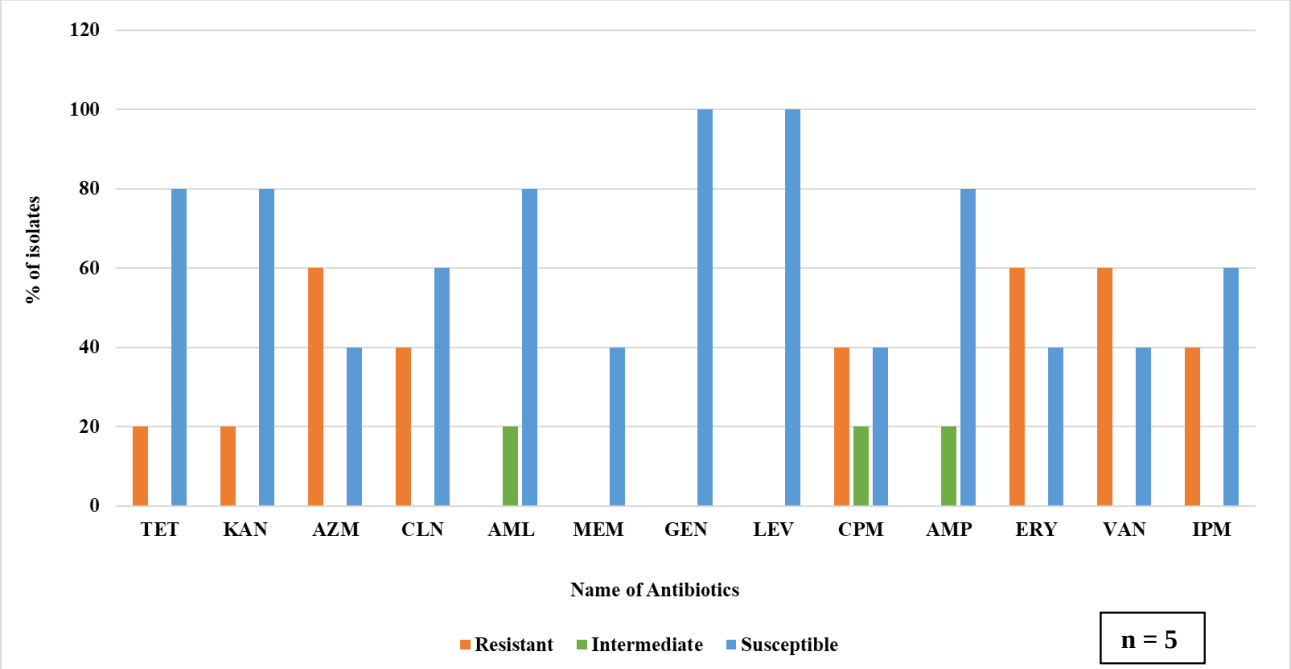

Figure 3.11: Antibiotic resistance pattern observed in *V. cholerae.* *V. cholerae* was resistant to AZM (60%), CLN (40%), ERY (60%), VAN (60%), CPM (40%) and IPM (40%) and100% isolates of *V. cholerae* were susceptible to GEN and LEV.TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin.

3.9. Antibiotic resistance profile of *K. pneumoniae*

In addition, 100% isolates of *K. Pneumoniae* (n=5) demonstrated resistant to VAN and other resistant antibiotics were KAN (25%), CLN (75%), AML (75%), AMP (75%), ERY (75%), and IPM (25%). In contrast, 100% isolates of *K. pneumoniae* were susceptible against TET, AZM, GEN, and LEV. According to the study, these antibiotics were more effective against the bacteria (Figure 3.9).

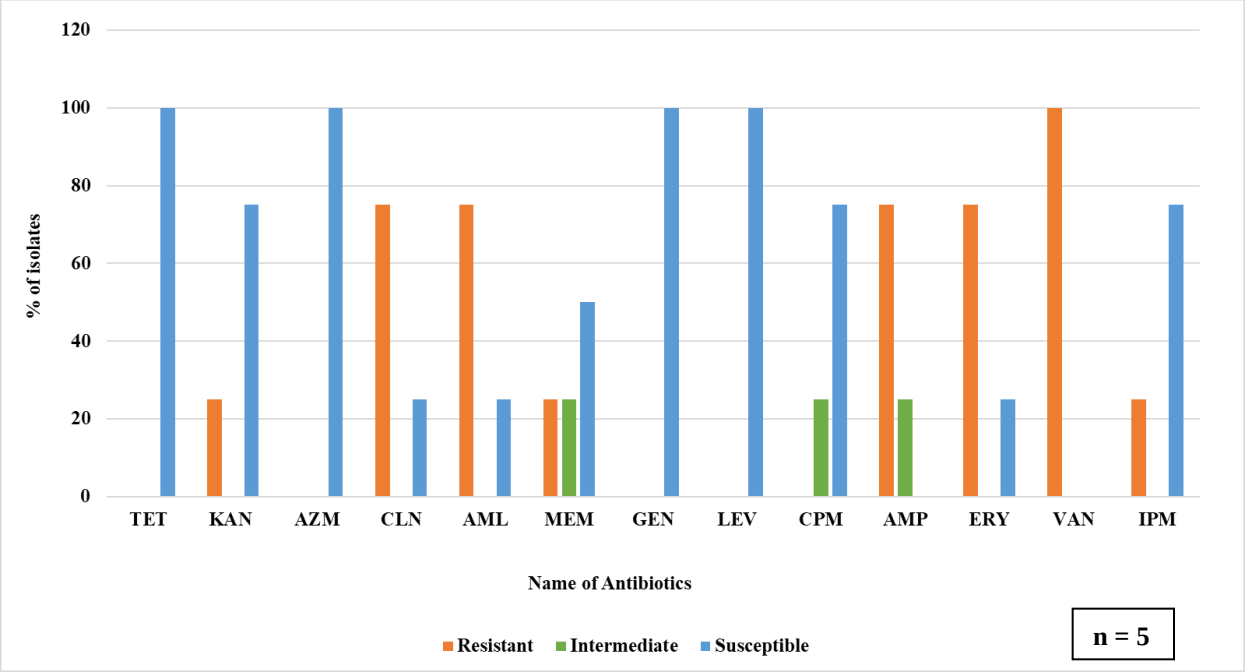

Figure 3.12: Antibiotic resistance pattern observed in *K. pneumoniae*. 100% isolates of *K. pneumoniae*, showed resistant only to VAN and was susceptible to TET, AZM, GEN, and LEV.TET: Tetracycline, CLN: Clindamycin, VAN: Vancomycin, ERY: Erythromycin, AML: Amoxicillin, AMP: Ampicillin, IPM: Imipenem, KAN: Kanamycin, GEN: Gentamycin, LEV: Levofloxacin, KAN: Kanamycin, AZM: Azithromycin.

3.10. DNA Extraction

The boiling method was used for extracting DNA. DNA samples were stored at -20°C for future use in molecular identification and detection of antibacterial resistance genes of the isolated bacteria.

Chapter 4

4. Discussion and Conclusion

4.1 Discussion

This research aimed to isolate five targeted pathogenic bacteria *Salmonella* spp., *Shigella* spp., *E. coli*, *V. cholerae*, and *K. pneumoniae* from restaurant table surfaces, significant sources of foodborne pathogens. Unsafe food consumption contributes to 600 million cases of foodborne diseases annually, resulting in 56 million deaths (Ritchie and Roser, 2018; WHO, 2015). Five table surface samples were collected from different local restaurants. *Salmonella* spp. growth was observed on MAC and SS agar, displaying colorless or transparent colonies on MAC due to non-fermenting lactose and colorless black center colonies on SS agar due to H_2S production. *Salmonella* spp. infection remains a major cause of acute diarrheal disease despite preventive measures. Antibiotic susceptibility testing of 100% isolates of *Salmonella* spp. exhibited resistance to ERY and VAN, with varying resistance levels to other antibiotics. Besides, 100% isolates of *Salmonella* spp. showed susceptibility against GEN and LEV.

On the other hand, *E. coli* was found in both MAC and XLD agar. It formed red colonies on MAC and flat yellow colonies on XLD agar due to lactose fermentation. A total of 12 antibiotics were used for the antibiotic susceptibility test. 100% isolates of *E. coli* demonstrated resistance against CLN and VAN. Followed by ERY (75%), AML (66.66%), AMP (41.1%), IPM (25%) and KAN (8.33%). In contrast, 100% isolates of *E. coli* showed susceptibility to GEN, TET, LEV, and CPM. According to a study, *E. coli* found on meat and swab samples of various contact surfaces had higher resistance against ERY and AMP (Sebsibe & M. A; Asfaw *et al*., 2020).

Shigella spp. was identified on both XLD and SS agar. In XLD agar, *Shigella* spp.formed pink colonies due to their inability to ferment xylose. In SS agar, it showed yellow colonies and did not ferment lactose. In addition, *Shigella* spp. bacteria cause an infection called shigellosis, leading to diarrhea. In this study, 100% isolates of *Shigella* spp. showed resistance against CLN, ERY, and VAN. Followed by AZM (50%), AML (87.5%), CPM (12.5%), AMP (75%), and IPM (37.5%). In opposition 100% isolates of *Shigella* spp. exhibited susceptibility to GEN, LEV, and KAN. In a study, *Shigella* spp. was found on tables in the dining hall that were resistant to GEN and most other antibiotics (Kelvin *et al*., 2020).

In addition, *K. pneumoniae* is a gram-negative bacterium found in the intestine and it is pathogenic to the human body. Besides, they cause various types of human disease including pneumonia, bronchitis, and UTI. The growth of *K. pneumoniae* was found on both XLD and MAC agar plates. In XLD agar, the colony color of *K. pneumoniae* was yellow whereas on MAC agar formed a transparent colony. However, 100% isolates of, *K. pneumoniae* showed resistance only to VAN. In contrast, 100% isolates of, *K. pneumonia* demonstrated susceptibility to TET, AZM, GEN, and LEV and these were the most effective against these bacteria.

In our study, we observed the growth of *V. cholerae* only on TCBS media with yellow colonies. *V. cholerae* produces *cholerae* toxin, which is a noninvasive pathogen that colonizes mainly the small intestine and results in severe secretory diarrhea (Weil *et al*., 2019). Besides, *V. cholerae* showed resistance to TET (20%), KAN (20%), AZM (60%), CLN (40%), ERY (60%), VAN (60%), CPM (40%) and IPM (40%). On the other hand, 100% isolates of *V. cholerae* demonstrated susceptibility against GEN and LEV.

All the targeted isolated bacteria are responsible for the food-borne or water-borne disease as they transfer from hand to table to food and are present in the intestine. Comprehensive antibiotic susceptibility testing revealed a concerning prevalence of multi-drug resistant isolates.

4.2 Conclusion

The study highlights the presence of pathogenic bacteria on the table surface of the restaurant. The targeted five organisms were found in the restaurant near MerulBadda. The isolates were presumptively identified by their morphology on the selective agar plate. Comprehensive antibiotic susceptibility testing revealed a concerning prevalence of multi-resistant strains among the isolates. Notably, Levofloxacin (LEV) and Gentamicin (GEN) exhibited notable efficacy against the majority of tested isolates. Statistical analysis underscored a significant degree of bacterial contamination on restaurant table surfaces, indicative of suboptimal cleanliness practices. Consequently, the presence of enteric bacteria underscores inadequate hygiene standards within the restaurant premises.

4.3 Limitations of our study

Our research focused on five restaurants in MerulBadda, Dhaka, limiting the clarity of our understanding of table surface organisms. To enhance the comprehensiveness of our study, a broader sampling from more restaurants would be beneficial. PCR and gel electrophoresis, which could confirm the presence of target organisms, were not conducted due to the unavailability of specific primers in our laboratory. Additionally, the lack of a biochemical test kit hindered further identification of bacteria based on attributes such as lactose fermentation, motility, and urea production. Integration of these techniques would bolster the accuracy and depth of our analysis.

4.4. Recommendations for future work

Expanding the sample size beyond the current five samples would enhance our ability to identify target organisms across various locations in Dhaka city. A comprehensive study incorporating numerous biochemical tests, PCR, gel electrophoresis, among other techniques, would offer a more detailed understanding. Additionally, examining the surface areas of kitchen utensils, cloths, and preparation tables in restaurant kitchens, in addition to dining tables, would increase the likelihood of detecting target organisms.

Chapter 5

References:

Alsallaiy, I., Dawson, P., Han, I., & Martinez-Dawson, R. (2016). Recovery, Survival, and Transfer of Bacteria on Restaurant Menus. *Journal of Food Safety, 36*(1), 52-61. https://doi.org/https://doi.org/10.1111/jfs.12212

Alsallaiy, I., Dawson, P., Han, I., & Martinez-Dawson, R. (2015, 08/01). Recovery, Survival, and Transfer of Bacteria on Restaurant Menus. *Journal of Food Safety, 36*. https://doi.org/10.1111/jfs.12212

Aryal, S. (2022, January 7). *SalmonellaShigella* (SS) Agar- Composition, Principle, Preparation, Results, Uses. Microbe Notes. https://microbenotes.com/*Salmonella-shigella*-ss-agar/

Aryal, S. (2023, January 13). TCBS AgAr- Composition, Principle, Preparation, Results, Uses. Microbe Notes. https://microbenotes.com/thiosulfate-citrate-bile-salts-sucrose-tcbs-agar/

Bello, A. Y., Jumare, F. I., Hussein, R. A., Haruna, Z. A., Sanusi, A., & Nafi'U, A. (2023, 08/09). ASSESSMENT OF BACTERIAL LOAD OF SOME SELECTED EATING TABLES OF UDUSOK MINI MART. *BIMA JOURNAL OF SCIENCE AND TECHNOLOGY (2536-6041), 7*(02. 1), 90-96. https://doi.org/10.56892/bima.v7i02. 1.460

Beyi, A. F., Fite, A. T., Tora, E., Tafese, A., Genu, T., Kaba, T., Beyene, T. J., Beyene, T., Korsa, M. G., Tadesse, F., De Zutter, L., Goddeeris, B. M., & Cox, E. (2017, 2017/03/03). Prevalence and antimicrobial susceptibility of Escherichia coli O157 in beef at butcher shops and restaurants in central Ethiopia. *BMC Microbiology*, *17*(1), 49. https://doi.org/10.1186/s12866-017-0964-z

Bukhari, M. A., Banasser, T. M., El-Bali, M., Bulkhi, R. A., Qamash, R. A., Trenganno, A., Khayyat, M., Kurdi, M. A., Majrashi, A. A., & Bahewareth, F. (2021). Assessment of microbiological quality of food preparation process in some restaurants of Makkah city. *Saudi Journal of Biological Sciences*, *28*(10), 5993–5997. https://doi.org/10.1016/j.sjbs.2021.06.050

Bradley, K., Williams, J., Burnsed, L., Lytle, M. B., McDermott, M. D., Mody, R. K., Bhattarai, A., Mallonee, S., Piercefield, E., McDonald-Hamm, C., & Smithee, L. (2011). Epidemiology of a large restaurant-associated outbreak of Shiga toxin-producingEscherichia coliO111:NM. *Epidemiology and Infection*, *140*(9), 1644–1654. https://doi.org/10.1017/s0950268811002329

Bayot, M. L., & Bragg, B. N. (2022, October 10). Antimicrobial susceptibility testing. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK539714/

Kunhiraman, S., Sukumaran, D., Antony, A., Silvester, R., & Abdulla, M. H. (2023, 04/30). Antibiotic Resistance and Pathogenic Potential of Escherichia coli Isolated from Food Contact Surfaces of a Commercial Kitchen. *60*, 143-147.

M, A. B., T, M. B., El-Bali, M., R, A. B., R, A. Q., Trenganno, A., Khayyat, M., M, A. K., Al Majrashi, A., & Bahewareth, F. (2021, Oct). Assessment of microbiological quality of food preparation process in some restaurants of Makkah city. *Saudi J Biol Sci*, *28*(10), 5993-5997. https://doi.org/10.1016/j.sjbs.2021.06.050

Melebari, M. (2023). Incidence of potential pathogenic bacteria at restaurants in Al-Mandaq City, Saudi Arabia: first study. *Journal of Pure and Applied Microbiology*, *17*(3), 1916–1925. https://doi.org/10.22207/jpam.17.3.57

Mohamedin, A., Michel, M., & Tolba, M. (2015, 09/25). Assessment of the Microbiological Quality of Public Restaurants and Street Vended Ready-To-Eat "Koshari" Meals. *International Journal of Applied Sciences and Biotechnology*, *3*(3), 452-458. https://doi.org/10.3126/ijasbt.v3i3.12933

Mohammed, S. S. D., Ayansina, A. D. V., Mohammed, S. R., & Shaba, O. A. O. A. M. (Feb 18, 2019). Evaluation of food contact surfaces in selected restaurants of Kaduna State University for the presence of Escherichia coli and Staphylococcus aureus.

Obi, C., Amakor, O., Oshim, I., Monique, O., & Ochiabuto, O. (2021, 10/12). Quantitative Measurement of Enteric Bacteria Load From Public Surfaces in Restaurants in Nnewi-Town. *International Journal of TROPICAL DISEASE & Health*, 32-40. https://doi.org/10.9734/ijtdh/2021/v42i1430516

Ogochukwu Ochiabuto, B., Ugochinyere Okeke, M., Onyema Oshim, I., Patrick Amakor, O., & Maureen Obi, C. (2021). Quantitative Measurement of Enteric Bacteria Load From Public Surfaces in Restaurants in Nnewi-Town. *International Journal of TROPICAL DISEASE & Health*, 32-40. https://doi.org/10.9734/ijtdh/2021/v42i1430516

Paul, A., Mahmud, M., & Ahmed, T. (2020, 08/07). Identification of pathogenic bacteria from food handling surfaces (tabletops) from different areas with demonstration of their drug resistance properties ARTICLE INFO ABSTRACT. *Journal of Food Safety and Hygiene*, *5*. https://doi.org/10.18502/jfsh.v5i3.5693

Rojas-Lopez, M., Monterio, R., Pizza, M., Desvaux, M., & Rosini, R. (2018, 2018-March-20). Intestinal Pathogenic Escherichia coli: Insights for Vaccine Development [Review]. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.00440

Sanders, E. R. (2012, May 11). Aseptic laboratory techniques: plating methods. *J Vis Exp*(63), e3064. https://doi.org/10.3791/3064

Sebsibe, M. A., & Asfaw, E. T. (2020). Occurrence of Multi-Drug Resistant Escherichia Coli and Escherichia Coli O157:H7 in Meat and Swab Samples of Various Contact Surfaces at Abattoir and Butcher Shops in Jimma Town, Southwest District of Ethiopia *Infection and Drug Resistance*, *Volume 13*, 3853–3862. https://doi.org/10.2147/idr.s277890

Sanders, E. R. (2012). Aseptic Laboratory Techniques: Plating Methods. *Journal of Visualized Experiments: JoVE*, (63). https://doi.org/10.3791/3064

Torres, A. G., Jeter, C., Langley, W., & Matthysse, A. G. (2005). Differential Binding of Escherichia coli O157:H7 to Alfalfa, Human Epithelial Cells, and Plastic Is Mediated by a Variety of Surface Structures. *Applied and Environmental Microbiology, 71*(12), 8008-8015. https://doi.org/doi:10.1128/AEM.71.12.8008-8015.2005

Tenna, A., Amare, K., Tekola, H., Kidan, Y. W., Melese, D., & Medhin, G. (2023). Assessment of the microbial quality of food Contact Surfaces (Utensils) of hotels and restaurants in Addis Ababa. Research Square (Research Square). https://doi.org/10.21203/rs.3.rs-2596388/v1

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
CrystalViolate	0.001
Neutral red	0.03
Agar	15.00

Mueller Hinton Agar

Component	Amount(g/L)
Beef	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
ShakingIncubator,Model:WIS-20R	Daihan Scientific Companies,Korea
Spectrophotometer,UVmini–1240	Shimadzu Corporation,Australia
NanoDrop2000Spectrophotometer	Thermo Scientific, USA
Microscope	A. Krüssoptronic, Germany
UVTransilluminator, Model:MD-20	WealtecCorp, USA
-20°CFreezer	Siemens, Germany
magnetic stirrer,Model:JSHS-180	JSR, Korea
Vortex Machine	VWR International
MicrowaveOven,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