A Bacteriological Study of Public Bus and Bus Stop Surfaces in Dhaka: Identifying GLASS Pathogens and Analyzing Their Antibiotic Resistance Pattern

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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

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing 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 which 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

The thesis titled "A Bacteriological Study of Public Bus and Bus Stop Surfaces in Dhaka: Identifying GLASS Pathogens and Analyzing Their Antibiotic Resistance Pattern" submitted by Britney Rahman (21126020), Isha Ieen (21326005) Mahmuda Anwar (21326016) and Mohosina Mokaddas (21326035) of Spring and Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 29 January, 2026.

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Ethics Statement

In this study no samples were collected from any human or animal subjects. Therefore, no ethical clearance was necessary.

Abstract

Due to overcrowding, humid environment and unsanitary conditions, public buses of Dhaka city can be a probable reservoir for pathogenic and antimicrobial resistant bacteria. This study focuses on identifying the Global Antimicrobial Resistance and Use Surveillance System (GLASS) guidelines' priority bacteria in common touch surfaces of buses and bus stops, and providing a proper insight on the antimicrobial resistance (AMR) pattern of these bacteria. Samples collected from seats, grab rails and bus stops were spread on different selective media. Based on colony morphology bacteria were presumptively selected, then polymerase chain reaction (PCR) was performed. Upon identification of target bacteria, the analysis of their AMR pattern was done using Kirby-Bauer disc diffusion method. Among the 30 samples, 60.0% were positive for *Klebsiella pneumoniae*, 36.7% for *Vibrio* species, 33.3% for *Acinetobacter baumannii*, 20% for *Staphylococcus epidermidis*, 16.7% for *Enterococcus* spp. and/or *P. aeruginosa*, and 10.0% for *Staphylococcus aureus* and/or *Salmonella* species. Samples taken from cloth surfaces had the highest bacterial count, while acrylic paint and steel surfaces had a relatively lower count. Among the nine bacteria, all bacteria except for *A. baumannii*, *Salmonella* species and *P. aeruginosa* showed an alarmingly high level of multidrug-resistance, with *K. pneumoniae* showing the highest resistance, 95%. The detection of multidrug-resistant bacteria in public buses, emphasise these transportation as being an important reservoir for clinically significant pathogens and the dissemination of antimicrobial resistance genes to the environmental microbiome. The findings of this study call for an urgent need for improving the overall conditions of buses and ensuring proper cleaning practices in public transports.

Keywords: Public bus; antimicrobial resistance; multidrug-resistance

Dedication

This work is dedicated to our loving family and friends who have been supportive of and patient towards us throughout our undergraduate life.

Acknowledgement

First of all, we would like to express our heartfelt gratitude to the Almighty Allah (SWT) for giving us the strength and determination to work on this project and completing it successfully. Secondly, we are deeply grateful to our lab mates and lab staff for always being patient and helpful to us. And lastly, we would like to articulate our heartfelt thanks to Amena Begum, Research Assistant, Department of Microbiology and School of Life Sciences, BRAC University, for helping, supporting and guiding us through every step of the project.

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List of Acronyms

A.baumannii	*Acinetobacter baumannii*
AMR	Antimicrobial Resistance
APC	Aerobic Plate Count
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
BRTC	Bangladesh Road Transport Corporation
CFU	Colony Forming Unit
CFU/cm²	Colony Forming Unit per Square Centimeter
cm	Centimeter
cm²	Square Centimeter
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
EtBr	Ethidium Bromide
FCC	Fecal Coliform Count
GLASS	Global Antimicrobial Resistance and Use Surveillance System
K. pneumoniae	*Klebsiella pneumoniae*
LB	Luria Bertani
MDR	Multidrug Resistance
mL	Milliliter
mFC	Membrane Fecal Coliform
MRSA	Methicillin-Resistant Staphylococcus aureus
MSA	Mannitol Salt Agar
MHA	Mueller Hinton Agar

NA	Nutrient Agar
PCR	Polymerase Chain Reaction
P. aeruginosa	*Pseudomonas aeruginosa*
rpm	Revolutions Per Minute
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
Salmonella spp.	*Salmonella* species
Shigella spp.	*Shigella* species
S. pneumoniae	*Streptococcus pneumoniae*
TCBS	Thiosulfate Citrate Bile Salts Sucrose
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
TNTC	Too Numerous To Count
UTI	Urinary Tract Infection
UV	Ultraviolet
V. cholerae	*Vibrio cholerae*
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate
µL	Microliter
µm	Micrometer

Chapter 1

Introduction

1.1 Background

One of the major public health concerns includes the rise of antimicrobial resistance amongst certain common bacteria lurking amongst us. With the misuse and overuse of antibiotics, drug-resistant pathogens are rising rapidly and are becoming a major threat for everyone across the globe. An estimate of 1.27 million deaths occurred in 2019 due to the bacterial Antimicrobial Resistance, AMR (World Health Organization: WHO, 2023). This is putting modern medicine at a huge risk as humanity continues to evolve backwards as the situation slowly moves toward the pre-antibiotic era.

Dhaka being one of the most populated cities in the world with a number of 36.6 million inhabitants (Dhaka Tribune, 2025) , the situation of public health continues to degrade. Millions of people have been migrating to the city as rapid unplanned urbanisation increases many health risks and poses a huge challenge for public health. A city so populated needs to have certain transportations for an easier commute system and public buses serve that quite well. Everyday, millions of people use the bus to reach their desired destinations, from schools to offices and even patients from hospitals and despite the number of commuters, the amount of buses available are quite low for which the majority of these buses are often overcrowded. Proper hygiene in these buses is an absolute myth which is very evident from the state of the bus' appearances which leads to many common pathogens retaining onto the surfaces of bus seats, handles and even the bus stops. Each of these bus takes on different routes throughout the city, passing by hospitals, schools offices etc and hence causing a rapid spread of disease

It is known that when met with a disease people rush their way to the pharmacy and the first thing they do in a country like Bangladesh is that they go for antibiotics. This unregulated usage of antibiotics where pharmacies sell them without any prescriptions and people not finishing a whole antibiotic course is the reason behind all the rise in AMR. Regardless of this alarming situation, much research has not been done here in Dhaka city so this paper will seek to address the situation of AMR of these 10 priority bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterococcus* spp., *Staphylococcus aureus*, and *Streptococcus pneumoniae.*

In order to tackle these and keep an eye on the situation of the AMR, World Health Organisation, WHO has launched the Global Antimicrobial Resistance and Use Surveillance System, GLASS, in the year 2015 which involved bacteria that commonly causes infections in humans. And according to that, this study has been laid out where samples from buses of different routes were collected to isolate and identify these 10 bacteria and their antimicrobial resistance.

1.2 Literature Review

Public transportation systems are increasingly recognized as important reservoirs for pathogenic and antimicrobial resistant bacteria due to continuous human contact, overcrowding, and inadequate cleaning practices. Frequently touched surfaces such as seats, grab rails, and bus stops surfaces facilitate the transfer of microorganisms between passengers and the environment, thereby posing significant public health risks. Numerous studies conducted globally have reported high bacterial loads and the presence of clinically important pathogens on public transport surfaces (Arowan et al., 2021).

In Bangladesh, research focusing specifically on the microbiological contamination of public transportation remains limited. However, several studies conducted on frequently touched objects in public places have consistently reported the presence of fecal coliforms and opportunistic pathogens, indicating poor hygiene and sanitation practices. Environmental surveillance studies conducted in Dhaka and Mymensingh have identified *E.coli*, *K. pneumoniae*, *Staphylococcus* spp., and *P. aeruginosa* on public contact surfaces, highlighting the role of urban infrastructure in pathogen transmission (Biswas et al., 2024; Arowan et al., 2021). Similar to the findings of the present study, *K. pneumoniae* was reported as one of the dominant organisms, reflecting its environmental persistence and increasing antimicrobial resistance reported in multiple environmental and clinical studies from Bangladesh (Islam et al., 2021).

A comprehensive study conducted in Sadar Upazila, Mymensingh, examined bacterial contamination on frequently touched surfaces of public buses, hospital wards, and public toilets. Among 90 swab samples collected, *E.coli* was detected in 76.67% of samples, *Klebsiella* spp. in 80%, and *Staphylococcus* spp. in 68%, indicating widespread environmental contamination with enteric and opportunistic pathogens. Molecular detection revealed virulence and resistance genes such as tetA, stx-1, and mecA in several isolates, while antibiotic susceptibility profiles showed high resistance to commonly used antibiotics like amoxicillin, azithromycin, and tetracycline and the presence of methicillin resistant *Staphylococcus aureus* (MRSA), emphasizing the public health risk posed by contact surfaces in communal spaces (Biswas et al., 2024).

Comparable evidence of bacterial contamination in public-contact environments was reported in a study involving street food vendors in Dhaka, where hands of vendors harbored high total viable counts, coliforms, and fecal coliforms. Enteric bacteria such as *E. coli*, *Klebsiella*

spp., and *Acinetobacter* spp. were isolated, many exhibiting antibiotic resistance to amoxicillin, while showing sensitivity to ciprofloxacin and azithromycin (Hassan et al., 2018). Although these studies focused on different environments, the pattern of bacterial prevalence particularly *E. coli* and *Klebsiella* spp. mirrors the dominant organisms identified on public bus surfaces in the present research, suggesting endemic contamination across varied public settings in Bangladesh.

Studies of microbial contamination on public contact surfaces beyond transportation also support this trend. For example, assessment of ready to eat food contact surfaces in local restaurants of Patuakhali demonstrated substantial total viable counts and fecal contamination, with *E. coli* and other coliforms present on hands of food handlers and utensils (Tareq et al., 2024). These findings reflect a broader issue of environmental hygiene and surface contamination in Bangladesh, where frequent human contact and inadequate sanitation contribute to microbial transfer and persistence on surfaces.

Comparable findings have been reported across South Asia. A study conducted in India examining microbial contamination in public buses reported elevated aerobic plate counts and frequent isolation of *E. coli*, *K. pneumoniae*, *Enterococcus* spp., and *P. aeruginosa* from seats and handrails, with a significant proportion of isolates exhibiting multidrug resistance (Sharawat et al., 2018).

Research from Southeast Asia further supports these findings. A Thai study analyzing microbial contamination on public bus surfaces reported significantly higher bacterial recovery from seats compared to grab rails, particularly on cloth-covered seats, indicating that porous materials favor bacterial persistence. Frequently isolated organisms included *K. pneumoniae*, *E. coli*, and *Acinetobacter* spp., which is consistent with the surface wise distribution observed in the present study (Boonman & Chutrtong, 2022).

Studies from East Asia have also demonstrated similar contamination patterns. A subway based investigation in South Korea reported seasonal variation in bacterial prevalence, with higher isolation rates of Gram-negative bacteria such as *Klebsiella* spp. and *Acinetobacter* spp. during warmer months, attributed to increased temperature, humidity, and passenger density (Lee et al., 2024). This seasonal trend closely mirrors the findings of the current study.

In Europe and North America, microbial surveillance of public transportation has revealed comparable public health concerns. A study conducted in Portugal found widespread contamination of bus and subway surfaces with fecal indicator bacteria and opportunistic pathogens, including *Enterococcus* spp. and *Staphylococcus epidermidis* (Smelikova et al., 2025).

Antimicrobial resistance among environmental isolates from public transport has been documented globally. Studies from China and Africa reported high resistance rates among *K. pneumoniae*, *E. coli*, and *A. baumannii*, particularly against beta-lactams and macrolides, while carbapenems and aminoglycosides retained higher efficacy (Simon et al., 2014). These resistance patterns are consistent with the present study, where *K. pneumoniae* showed high resistance to amoxicillin and erythromycin, while meropenem, imipenem, and amikacin remained effective.

Moreover, the detection of multidrug resistant organisms on public bus surfaces reinforces concerns that public transportation can act as a reservoir and transmission hub for antimicrobial resistance. The presence of MDR *K. pneumoniae*, *E. coli*, and *Vibrio* spp. aligns with global evidence indicating that environmental surfaces contribute to the dissemination of resistant bacteria outside clinical settings (World Health Organization [WHO], 2023).

Overall, the findings of this study are consistent with both regional and international literature, confirming that public bus surfaces harbor diverse bacterial populations, including GLASS priority pathogens with significant antimicrobial resistance. These similarities underscore the urgent need for improved sanitation practices and routine microbial surveillance in public transportation systems in Dhaka and comparable urban environments.

1.3 Objectives

1.3.1 General Objective

To investigate the microbiological contamination and antimicrobial resistance profile of frequently touched surfaces of public buses in Dhaka city by isolating, identifying, and characterizing GLASS priority bacterial pathogens using culture-based, molecular, and antibiotic susceptibility testing methods.

1.3.2 Specific Objectives

- To determine the aerobic bacterial load on selected public bus touch surfaces using Aerobic Plate Count (APC).
- To assess fecal contamination on bus surfaces by enumerating fecal coliforms using the membrane filtration technique on Membrane Fecal Coliform agar (mFC).
- To isolate presumptive GLASS-priority bacterial pathogens from bus seats, grab rails, and bus stops using selective and differential culture media.
- To perform presumptive identification of bacterial isolates based on colony morphology and biochemical reactions on selective media.
- To confirm the identity of selected bacterial isolates using species-specific PCR assays.

- To evaluate the antimicrobial susceptibility patterns of confirmed bacterial isolates using the Kirby Bauer disc diffusion method following CLSI guidelines.
- To identify the occurrence of MDR bacteria among the isolated pathogens from public bus environments.
- To compare bacterial diversity and distribution across different sampling sites, surface types, and seat or grab-rail materials.
- To analyze seasonal variation in bacterial contamination and pathogen prevalence between winter and summer sampling periods.

Chapter 2

Materials and Methods

2.1 Work Plan

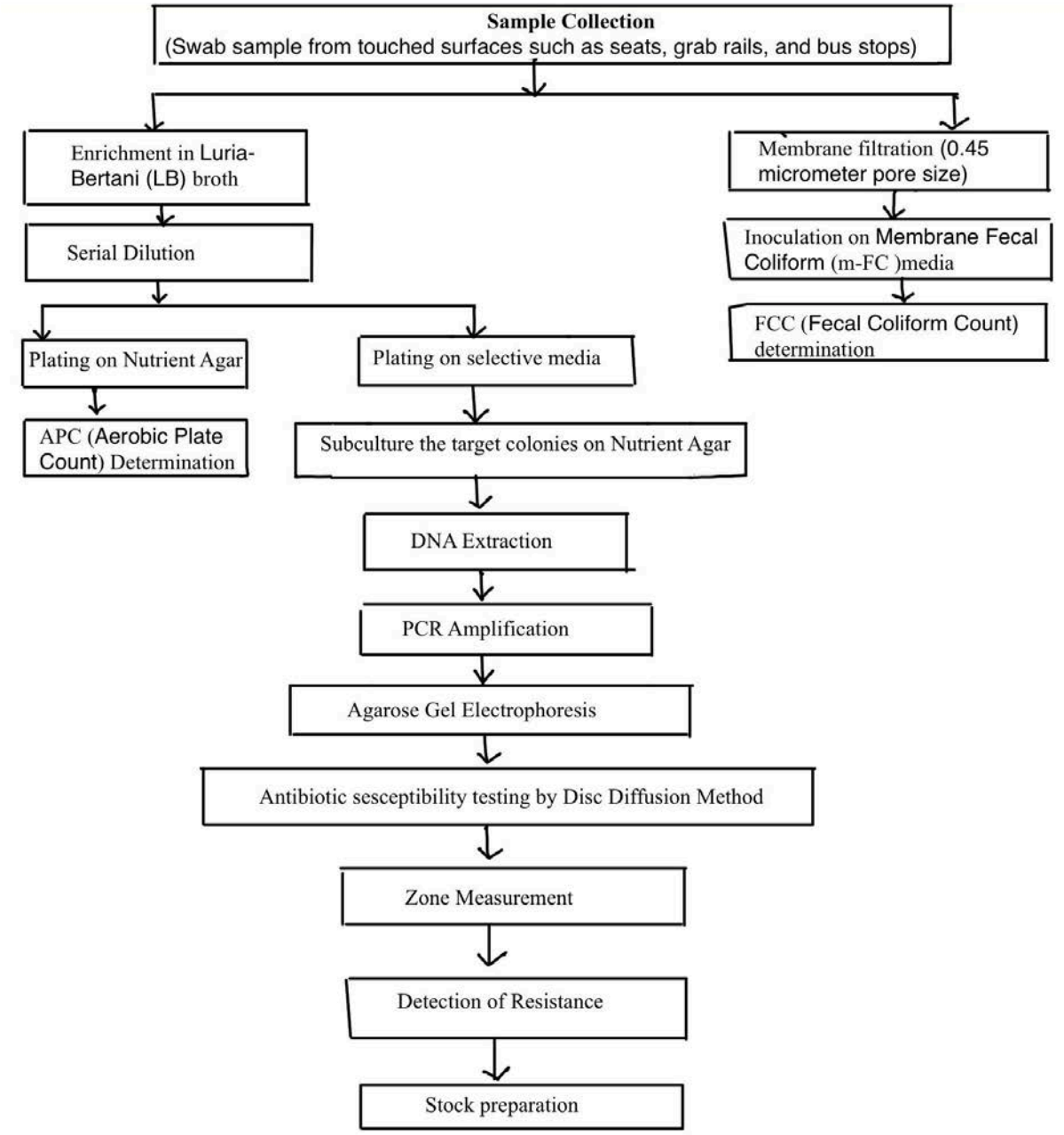

Figure 1: Work Plan of this study

2.2 Description of the study area and sample type

The study was conducted in Dhaka, Bangladesh, a densely populated megacity where public buses represent one of the most widely used modes of daily transportation. Given the high commuter turnover and limited sanitation practices on most bus routes, these vehicles serve as potential reservoirs for environmental and pathogenic microorganisms. The samples were taken from the buses that travel around different parts of Dhaka, including areas like Mirpur, Uttara, Badda, Gulshan, Motijheel, and Banani. They pass through busy markets, residential neighborhoods, hospitals, and important business districts.

Swab samples were collected from surfaces frequently touched by passengers, including metal grab rails, handrails and seat surfaces. These sites were chosen because repeated hand contact increases the likelihood of bacterial deposition and potential transmission of antimicrobial-resistant organisms. All laboratory procedures were conducted in the microbiology research facilities at BRAC University, where biosafety guidelines for handling environmental bacterial isolates were strictly followed. The controlled laboratory environment ensured accurate processing, incubation, and isolation of bacterial colonies obtained from the bus samples.

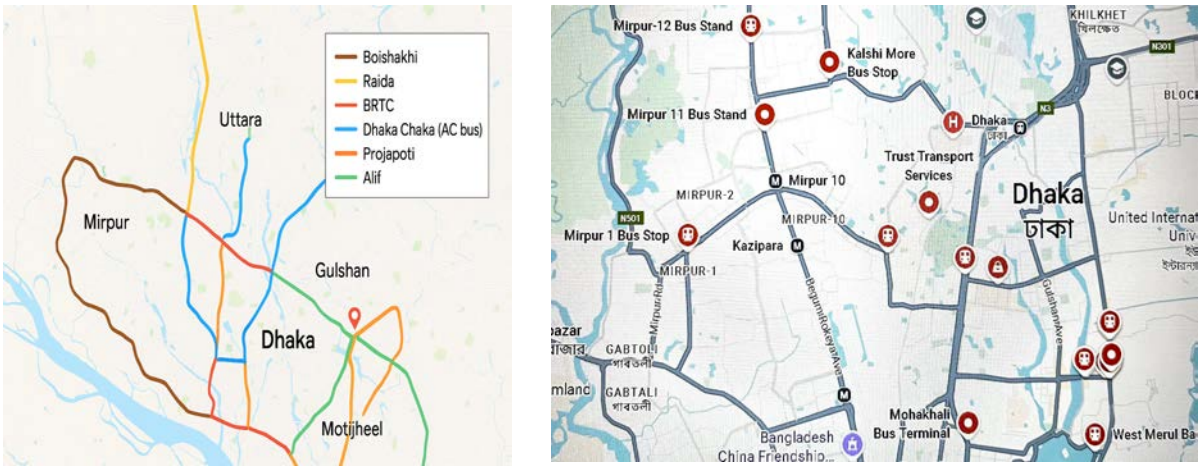

Figure 2: The bus routes of Dhaka city from where swab samples were collected

2.3 Sample Collection

Sample collection was followed by WHO recommended environmental swabbing procedures to ensure optimum recovery of microorganisms from dry and metallic surfaces (WHO, 2023). The Cotton swabs were autoclaved prior to use and transported in sterile containers. Each swab was moistened with sterile saline immediately before sampling to improve adherence of microorganisms onto the swab tip. Swabs were drawn across the selected surface areas (10 cm x 2 cm) using firm, consistent strokes, ensuring maximum coverage of regions with high passenger contact.

After sampling, swabs were inserted into two different types of sterile media: 10 mL of sterile saline for membrane filtration and 5 mL of Luria Bertani (LB) broth for enrichment. Tubes were immediately sealed and placed inside an insulated icebox to maintain the temperature 4°C during transportation. This prevented premature bacterial proliferation and maintained the viability of microorganisms until laboratory processing. All samples were transported to the laboratory within one hour of collection, adhering to recommended time constraints for environmental samples (APHA, 2017). Alongside each sample, metadata such as bus route,

surface type, time of sample collection, and environmental conditions were recorded to facilitate downstream analysis.

2.4 Sample Processing

Immediately after sample arrival at the laboratory, the swabs placed in sterile saline were vortexed to ensure homogenization. The entire 10 mL sample was subsequently subjected to membrane filtration through a 0.45 µm nitrocellulose membrane, which effectively retains most bacterial cells (APHA, 2017). The membrane was transferred onto mFC agar and incubated at 44°C for 22–24 hours to cultivate fecal coliforms. Blue colonies on mFC agar were recorded as fecal coliforms, while red or white colonies represented non-fecal coliforms.

Swabs stored in LB broth were incubated overnight at 37°C in a shaker incubator to encourage the proliferation of bacteria present in low numbers. Enrichment improved the recovery of fastidious organisms such as *V. cholerae*, *Salmonella*, *Shigella*, and *Enterococcus* spp., which may not grow well without prior enrichment. Enriched cultures were used for serial dilution and plating on selective media.

2.5 Serial Dilution and Culture on Selective Media

Serial dilution was performed using sterile saline to achieve bacterial separation and isolate distinct colonies. Dilutions ranging from 10^{-1} to 10^{-5} were prepared, and aliquots were plated onto selective and differential media. Media selection targeted specific pathogens: Thiosulfate Citrate Bile Salts Sucrose agar (TCBS) for *V. cholerae*, Xylose Lysine Deoxycholate (XLD) agar for *Salmonella* and *Shigella*; HiMedia Chromogenic Urinary Tract Infection Agar (HiCrome UTI) for *E. coli* and *K. pneumoniae*, Leeds medium for

Acinetobacter spp. ; cetrimide for *P. aeruginosa* and Mannitol Salt Agar (MSA) for *S. epidermidis* Plates were incubated at 35–37°C for 18–24 hours, depending on media requirements. Resulting colonies were evaluated based on characteristic reactions described in standard microbiological references (Cappuccino & Sherman, 2016).

2.6 Presumptive Identification of Bacteria

Presumptive identification of isolates relied on colony morphology and differential media reactions. Colony characteristics such as pigmentation on TCBS, H_2S production on XLD, chromogenic differentiation on UTI agar, green pigment on cetrimide and mannitol fermentation and salt tolerance on MSA were compared with published descriptions to predict organism identity (Forbes et al., 2018). These observations provided the first level of organism identification prior to molecular assays.

2.7 Colony Morphology on Selective Media

Table 1 summarizes the expected colony morphology of pathogens across the selective media used in this study. These morphological characteristics were used as preliminary indicators for identification of isolates.

Organism	Bacterial Type Based on Gram Staining	Media	Expected Colony Morphology
Escherichia coli	Gram-negative	UTI Agar	Pink–purple colonies
Klebsiella pneumoniae	Gram-negative	UTI Agar	Blue colonies
Enterococcus species	Gram-positive	Leeds Medium	Green colonies
Staphylococcus aureus	Gram-positive	MSA	Creamy white/yellow colonies
Staphylococcus epidermidis	Gram-positive	MSA agar	Red/pink
Pseudomonas aeruginosa	Gram-negative	Cetrimide Agar	yellow green, glows under UV ray
Salmonella species	Gram-negative	XLD Agar	Red colonies with black centers
Shigella species	Gram-negative	XLD Agar	Red or colorless colonies
Vibrio cholerae	Gram-negative	TCBS Agar	Yellow colonies
Acinetobacter species	Gram-negative	Leeds / NA	Pink on Leeds; opaque on NA
Fecal coliforms	Gram-negative	mFC Agar	Blue colonies
Non-fecal coliforms	Gram-negative	mFC Agar	Red or white colonies
Mixed flora	—	NA	White, cream, or colorless colonies

Table 1: Colony morphology of different bacteria on selective media

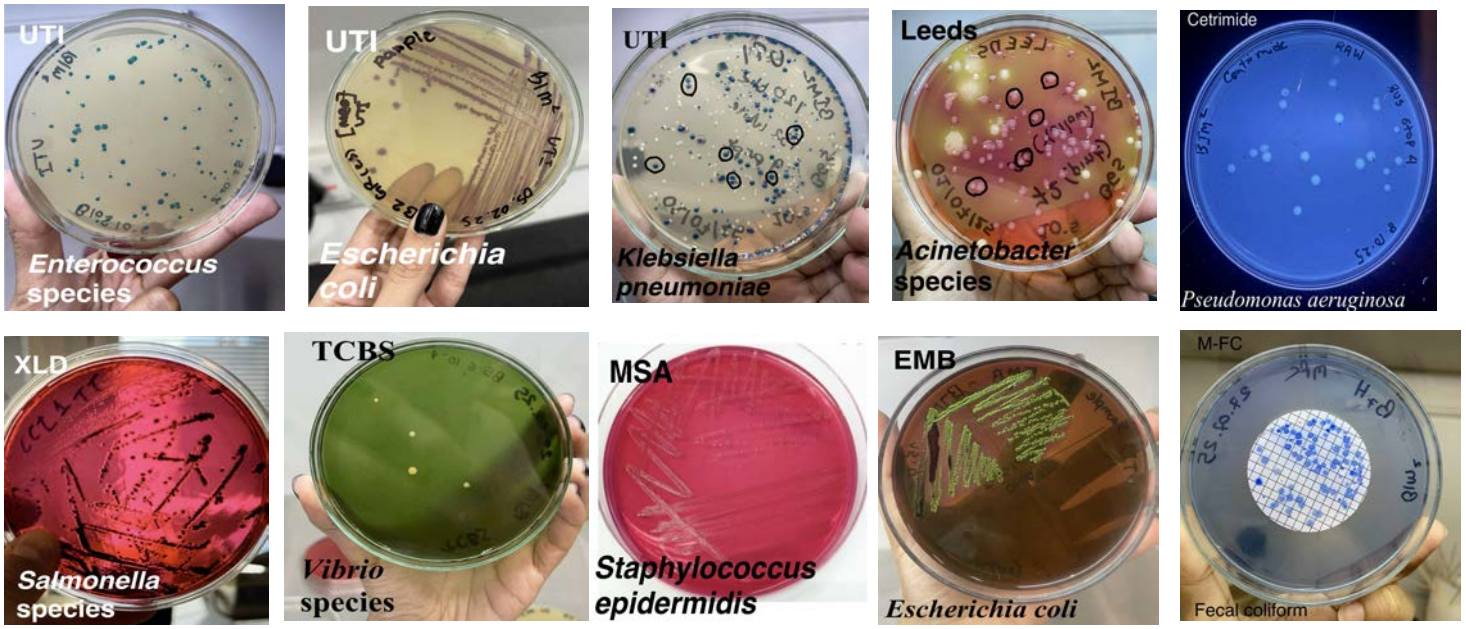

Figure 3: Different selective media used to isolate different organisms. The top left indicates the media used and the bottom left indicates the organism isolated

2.8 Further Screening of Suspected Colonies on Selective Media

Following the initial plating on selective media and enumeration, colonies obtained from TCBS, XLD, NA, Leeds, MSA and UTI agar were examined based on their color, morphology, and growth characteristics. Pathogens including *E. coli*, *K. pneumoniae*, *V. cholerae*, *Shigella* spp., *Salmonella* spp., *P. aeruginosa*, *Acinetobacter* spp., *Enterococcus* spp., *S. aureus*, *S. pneumoniae* and *S. epidermidis* were selected for further screening.

To obtain pure cultures, characteristic colonies were streaked onto their respective selective media and NA. Colonies from Leeds agar were re-streaked on fresh Leeds plates, isolates from TCBS were subcultured again on TCBS, suspected *Shigella* and *Salmonella* isolates from XLD were streaked on NA and selective confirmation media, isolates from MSA were further streaked on fresh MSA and nutrient agar and isolates from UTI agar were further

streaked on Cetrimide agar and EMB agar where appropriate. All plates were incubated at 35–37 °C for 24 hours to ensure isolation of pure colonies.

Distinct colony characteristics were used as preliminary indicators during this phase. For example, lactose fermenting organisms on EMB agar displayed characteristic coloration that aided in differentiating *E. coli* and *K. pneumoniae*, where *E. coli* typically forms dark colonies with a metallic sheen due to strong lactose fermentation, while *K. pneumoniae* forms mucoid pink-purple colonies. Similarly, isolates producing yellow colonies on TCBS were screened as potential *Vibrio* spp., whereas red colonies with black centers on XLD were evaluated as possible *Salmonella* spp. On MSA, *S. epidermidis* typically formed pink/red colonies indicating no mannitol fermentation, differentiating it from *S. aureus* which ferments mannitol producing yellow colonies.

These purified isolates were then transferred to fresh nutrient agar plates for preparation of DNA extraction and subsequent molecular confirmation. Species specific PCR assays and agarose gel electrophoresis were performed to verify the identity of each isolate based on the expected amplicon sizes.

2.9 DNA Extraction

Before further analysis, the DNA of the bacterial cells must be obtained, and hence their DNA must be extracted using the boiling method of DNA extraction. The process began with the preparation of 1X Tris-EDTA or TE buffer, which was the main solution for the DNA extraction process. Sterile Eppendorf tubes have been taken, and 400 µl of the 1X TE buffer was pipetted into the tubes. Then, using aseptic technique, a loopful of around 1µl colony has been taken from the growth media and mixed with the buffer. The suspension was then vortexed for 15 seconds for proper homogenisation.

Then the buffer mixture was subjected to centrifugation for 10 minutes at a speed of 13000 RPM. This caused the bacterial cells to separate into a pellet, which was obtained by gently discarding the supernatant from the tubes.

Another 400µl volume of the 1X TE buffer was added to the tubes containing the pellet, and they were mixed well by re-pipetting and then vortexing them well for 15 seconds.

The tubes were then heated up using heat blocks for 10 minutes at a temperature of 100 °C. After the heating, the tubes were allowed to cool down to room temperature for 5-10 minutes. After the samples were allowed to cool down, they were again subjected to a 10-minute spin in the centrifuge at 13000 RPM to separate the DNA from any other remaining impurities. The supernatant obtained from this has been transferred to a new Eppendorf tube for storage at -20°C in the refrigerator.

2.10 Polymerase Chain Reaction (PCR)

With DNA extraction, now Polymerase Chain Reaction was carried out to analyse and identify the obtained organisms. With a mixture of certain reagents along with the obtained DNA as the template, this molecular technique could yield an amplified amount of DNA as they were inserted into the thermal cycle machine. Each of the labeled PCR tubes contained 13µl of the sterile PCR mix, which included 6 µl PCR Master Mix, 3µl nuclease-free water, along with 1µl of each of the forward and reverse primers for the desired DNA template. Then each of the tubes had 11µl of the pcr mix added to them, followed by 2µl of the DNA according to their labels. A positive and negative control had been taken as well, where the positive control had DNA from an already confirmed sample and the negative control had no DNA added to it. The positive control ensured that the reaction mixture was able to produce reliable data, whereas the negative control ensured the absence of contamination in the PCR mix.

Once the mixture has been completed, it is subjected to a short spin that breaks down any form of bubbles. The tubes are then added to the thermal cycler, and the correct information is input into the machine, which causes the cycling to begin

Bacteria	Primer	Primer Sequence	Band size	PCR condition
Staphylococcus aureus	nuc	5'-GCGATTGATGGTG AT ACGGTT-3' -F 5'-AGCCAAGCCTTG ACG AACTAAA GC-3' -R	279bps	Initial denaturation: 94°C for 5 minutes, Denaturation: 94°C for 30 seconds, Annealing: 55°C for 30 seconds, Extension: 72°C for 1 minute. Cycles: 35 cycles. Final cycle: 72°C for 10 minutes.
Staphylococcus epidermidis	gseA	5'-GTAATATTACCTA ATAATAATAG-3'-F 5'-CCTTTACTTTCCC ACATTG-3'- R	503bps	Initial Denaturation: 94℃, 2 minutes Denaturation: 94℃, 10 seconds Annealing: 50℃, 10 seconds Elongation: 72℃, 60 seconds Final Elongation: 72℃, 4 minutes Cycle: 30
E. coli	ECO	5'-GAC CTC GGT TTA GTT CAC AGA-3' -F 5'-CAC ACG CTG ACG CTG ACC A3' -R	585bps	Initial Denaturation: 95℃, 3 minutes Denaturation: 94℃, 45 seconds Annealing: 58℃, 45 seconds Elongation: 72℃, 1 Min
Klebsiella pneumoniae	Kp-pf/pr	5'-ATTTGAAGAGGTT GCAAACGAT-3'-F 5'-TTCACTCTGAAGT TTTCTTGTGTTC-3'-R	130bps	Initial Denaturation: 94℃, 5 minutes Denaturation: 94℃, 60 seconds Annealing: 58℃, 1minute Elongation: 72℃, 1 minute Final Elongation: 72℃, 7 minutes Cycle: 35
Acinetobacter baumannii	BLA-OXA	5'-TTCAAGCCAAAG GCACGATAG-3' -F 5'-TCCGAGTTGACTG CCGGGTTG-3'-R	495bps	Initial denaturation: 94°C for 5 minutes, Denaturation: 94°C for 1 minute, Annealing: 55°C for 1 minute, and extension: 72°C for 1 minute. Cycles: 30 cycles. Final cycle: 72°C for 10 minutes.
***Enterococcus* spp.**	Tuf	5'-CCAATGCCACAAAC TCGT -3'-F 5'-CCTGAACCAACA GTACGT-3'-R	112bps	Initial denaturation: 95°C for 3 minutes, Denaturation: 95°C for 30 seconds,, Annealing: 55°C for 30 seconds, and extension: 72°C for 1 minute. Cycles: 35 cycles. Final cycle: 72°C for 7 minutes
Pseudomonas aeruginosa	PA-SS	5' -GGGGGATCTTCGGA C CTCA-3' -F 5'-TCCTTAGAGTGCCC A CCCG-3' -R	956 bps	Initial denaturation: 95°C for 2 minutes, denaturation: 94°C for 20 seconds, annealing: 58°C for 20 seconds, and extension: 72°C for 40 seconds. Cycles: 25 cycles. Final cycle: 72°C for 1 minute.
Salmonella	Salmonella	5'-GTATTGTTGATTAAT GA CA TCCG-3' -F 5'-ATATTACGCTACGGA AA C ACGTT-3'-R	403bps	Initial Denaturation: 94℃, 5 minutes Denaturation: 95℃, 30secs Annealing: 60℃, 30 seconds Elongation: 72℃, 1minute Final Elongation: 72℃, 8 minutes Cycle: 35
***Vibrio* spp.**	VG	5'-TGCAGATAATTCA CG CCCAG-3' -F 3'-ACCCGCTGGACGCC A T-5' -R	689bps	Initial Denaturation: 94°C, 5 minutes Denaturation: 94°C, 30 seconds Annealing: 60°C, 30 seconds Elongation: 72°C, 30 seconds Final Elongation: 72°C, 10 minutes Cycle: 25

Table 2: Primers and their respective PCR conditions

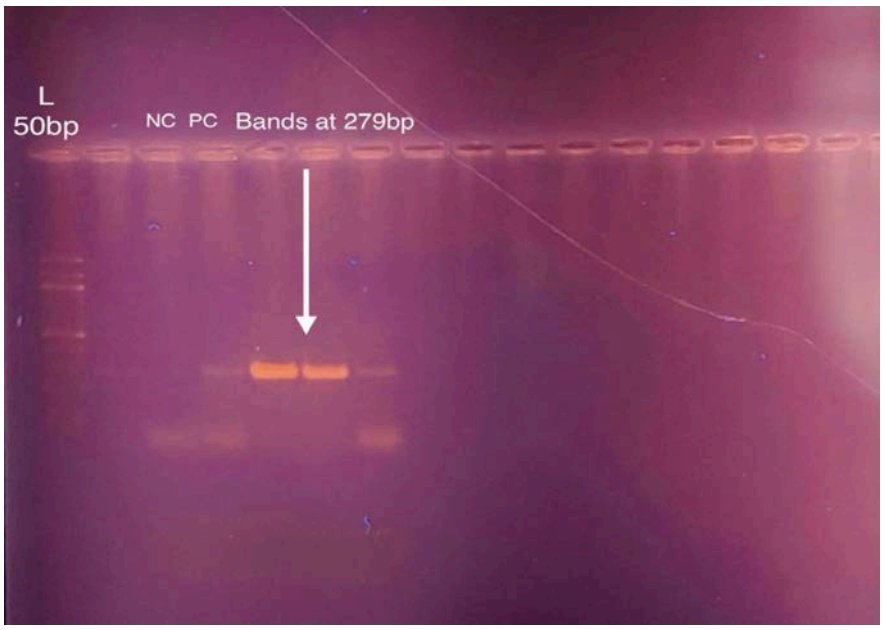

Figure 4: Gel Electrophoresis results of Staphylococcus aureus at 279bp

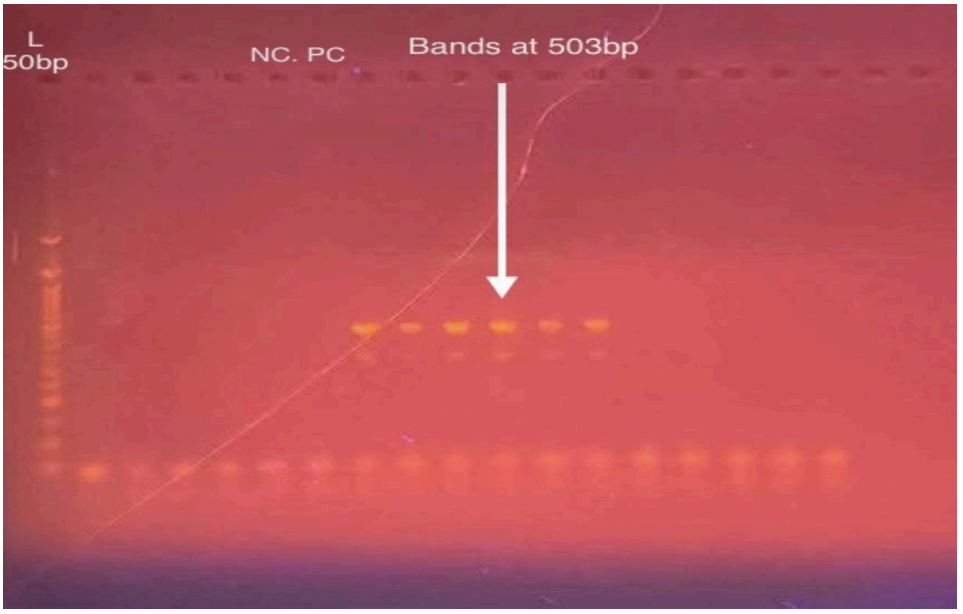

Figure 5: Gel Electrophoresis results of Staphylococcus epidermidis at 503bp

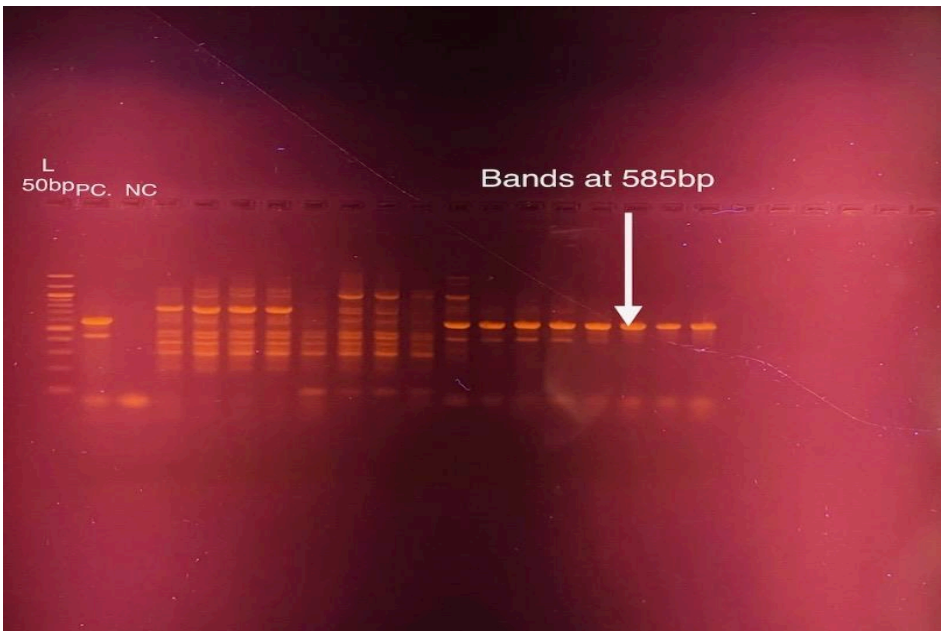

Figure 6: Gel Electrophoresis results of E.coli at 585bp

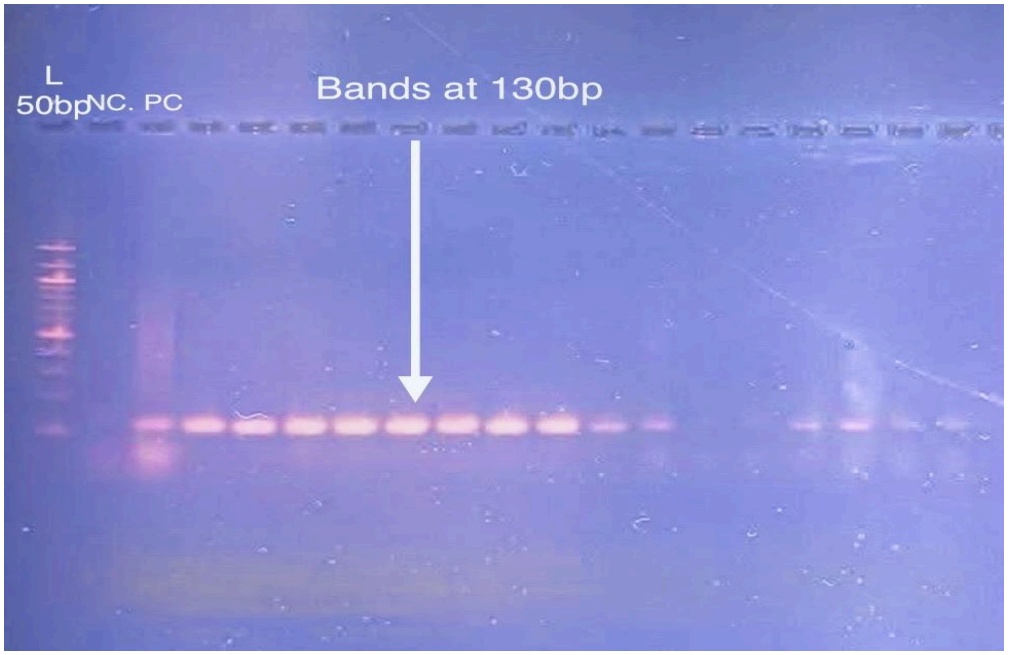

Figure 7: Gel Electrophoresis results of Klebsiella pneumoniae at 130bp

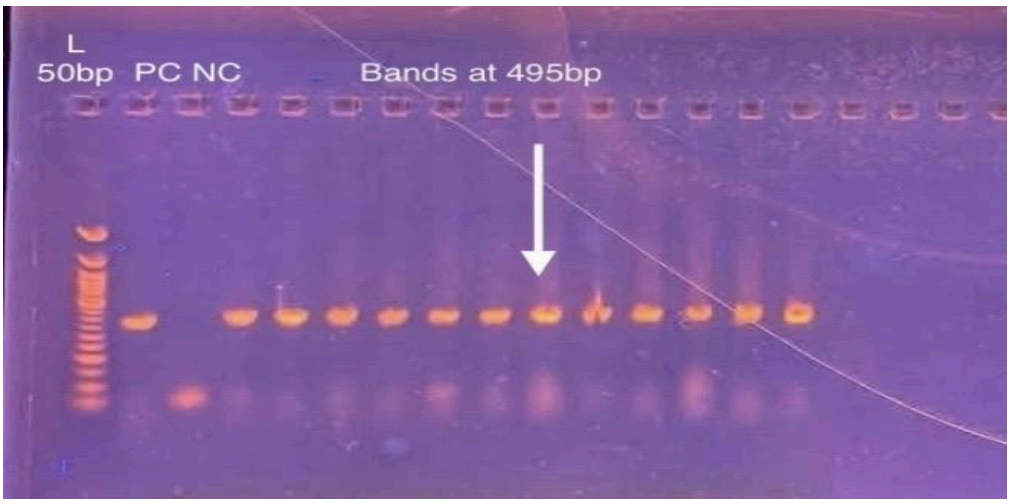

Figure 8: Gel Electrophoresis results of Acinetobacter baumannii at 495bp

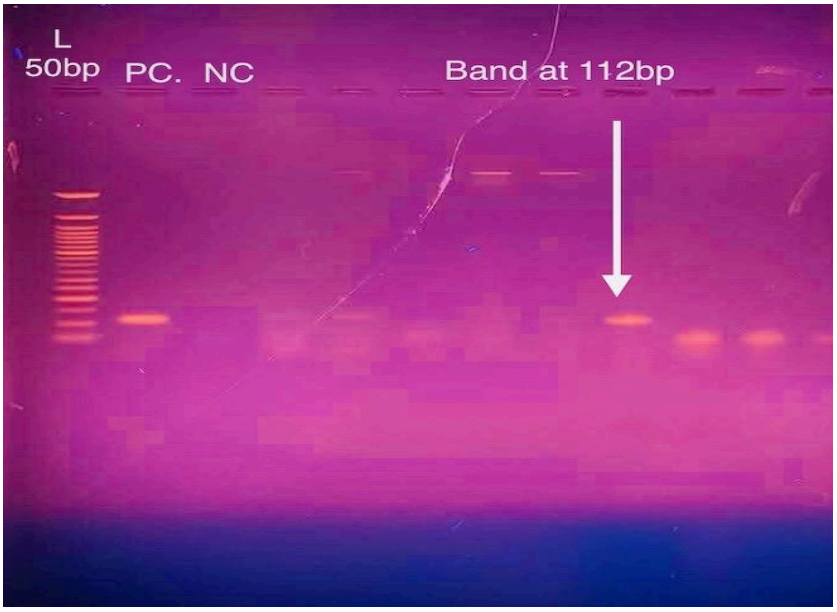

Figure 9: Gel Electrophoresis results of Enterococcus species. at 112bp.

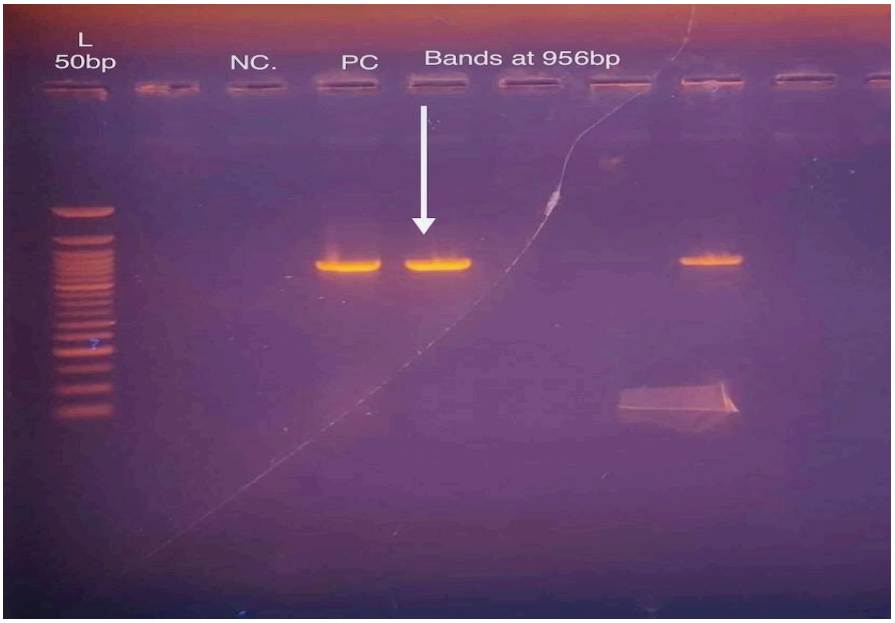

Figure 10: Gel Electrophoresis results of Pseudomonas aeruginosa at 956bp

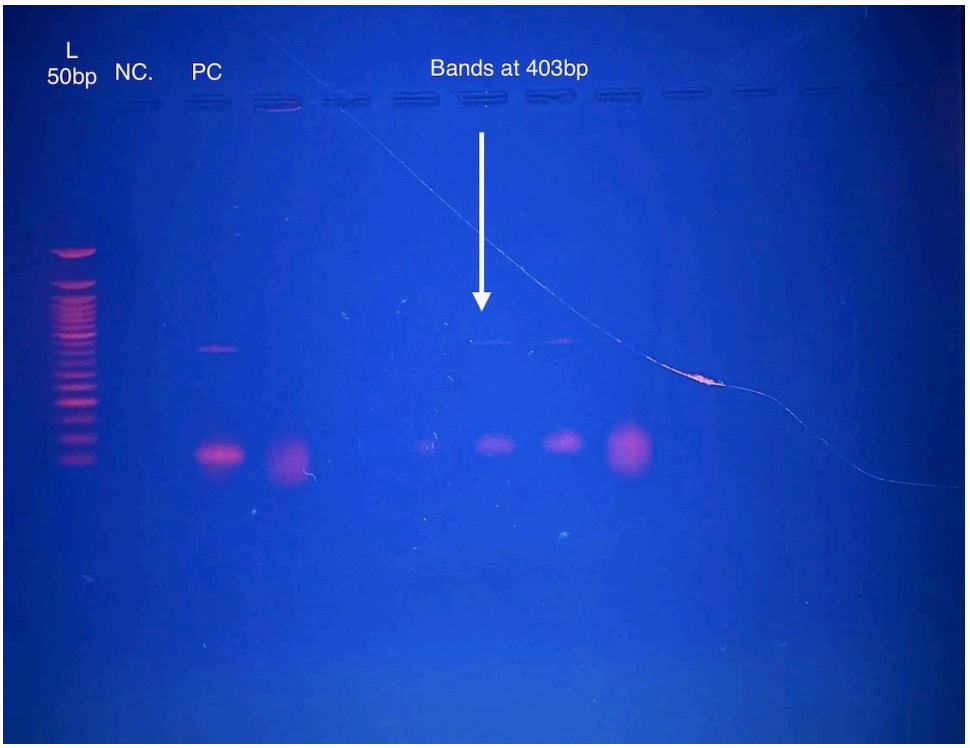

Figure 11: Gel Electrophoresis results of Salmonella species. at 403bp

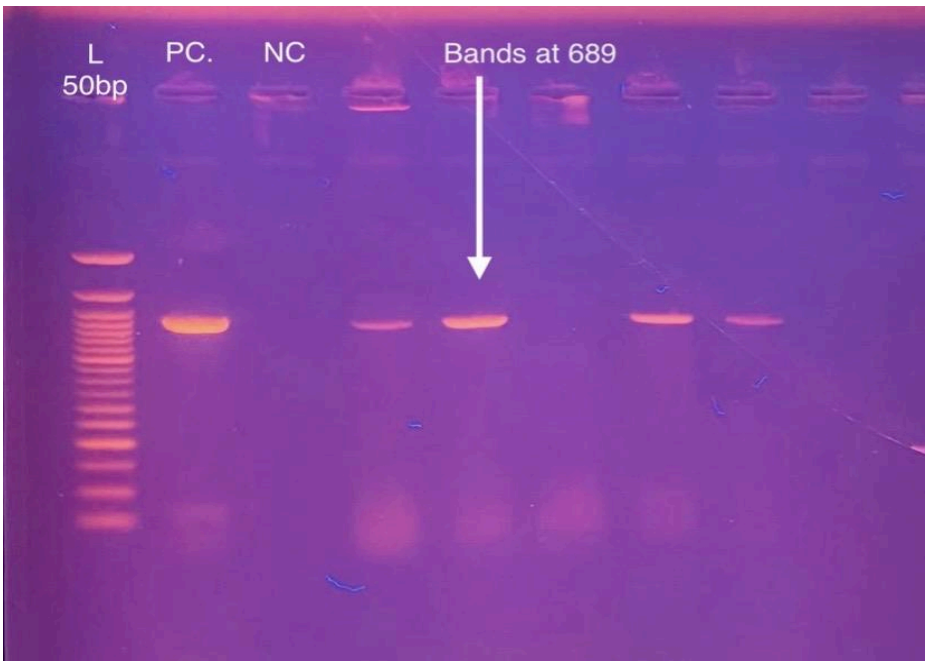

Figure 12: Gel Electrophoresis results of Vibrio species. at 689bp

2.11 Agarose Gel Electrophoresis

Now, with the obtained DNA amplicons from the PCR, there must be a way to visualise the data to know about the presence of a certain organism's DNA, and this is where the agarose gel electrophoresis comes into play.

The agarose gel was prepared with 1.5% of agarose powder, which was mixed with a water mixture of 98ml and 2ml of 1X Tris-EDTA (TBE) buffer. The mixture needs to be heated in the microwave until it started to boil. The mixture was then swirled to cool down, and once it was warm, 4µl EtBr or Ethidium Bromide, a DNA intercalating agent, was added to the solution until it dissolved properly. Then, the mixture was added to the casting tray with a comb already attached to it. The gel was then allowed to set, and the comb was lifted to create small wells where the amplicons were then added. The gel was then slowly placed inside the gel electrophoresis chamber, and 1M TBE running buffer was poured into the chamber. Once submerged, the wells were filled up one by one, starting with 6µl of the 50bp ladder, followed by 5µl of positive and negative control and then the DNA amplicons.

The apparatus was set to 100 volts for 40 minutes, and the DNA was allowed to move from the negative electrode to the positive electrode. As the DNA traveled through the porous agarose gel, the DNA fragments were separated based on their band sizes.

Once the run was complete, the gel was taken and placed inside a sealed UV illuminator, where the DNA could be visualised as the EtBr intercalated into the DNA amplicons. The ladder acted as a medium for the proper comparison and identification of the DNA bands.

2.12 Antibiotic Susceptibility Testing (AST)

The Kirby-Bauer or the disc diffusion method was carried out in this case, following the CLSI procedures. This method uses MHA media as the media forms a porous base which allows the antibiotics to diffuse through.

The media was prepared by autoclaving and then pouring onto sterile plates under the laminar. On the other hand, a small loop of the isolate was taken and mixed in a test tube of sterile saline of 10 ml, which was then vortexed to form a suspension. The suspension was then compared with a 0.5 McFarland solution. Then, swabbing was done where a sterile cotton swab was taken and submerged in the saline solution and lawned onto the MHA media plates. Then, one by one while maintaining a distance, the discs were placed onto the media and were incubated at 37 °C for 18-24 hours. The zones of inhibition were then measured and written down in an Excel sheet, where compared according to the CLSI guidelines and each of them was marked “sensitive”, “intermediate” and “resistant”.

Spectrum	Class of drug	Antibiotic	Content	Susceptible	Intermediate	Resistant
Broad Spectrum	Tetracycline	Tetracyclin	30	19	15-18	14
		Doxycyclin DO	30	16	13-15	12
	Nitrofurans	Nitrofurantoin	30	17	15-16	14
	Lincosamides	Clindamycin DA		21	15-20	14
	Fluoroquinolones	Levofloxacin lev	5	19	16-18	15
		Moxifloxacin MO	5	24	21-23	20
	cephalosporin	Cefoxitin FOX	30	22	–	21
	Macrolides	Azithromycin AZM	15	18	14-17	13
	Oxazolidinones	Linezolid LZD	30	26	23-25	22
	Penicillin	Penicillin G	10	29	–	28
	Phenicols	Chloramphenicol C	30	18	13-17	12

Table 3: Antibiotics used for Staphylococcus species

Spectrum	Class of drug	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad Spectrum	Tetracycline	Tetracyclin	30	15	12-14	11
	Cephems	Cefixime	5	19	15-18	14
		Cefepime	30	19	16-18	15
		Ceftriaxone CRO	30	23	20-22	19
	Fluoroquinolone	Ciprofloxacin	5	26	22-25	21
	Aminoglycosides	Amikacin AMK	30	20	17-19	16
		Kanamycin K	30	18	14-17	13
	Carbapenem	Imipenem IPM	10	23	20-22	19
		Meropenem MEM	10	23	20-22	19
	Phenicols	Chloramphenicol	30	18	13-17	12
	Penicillin	Amoxicillin AML	10	18	14-17	13
	Aminoglycosides	Streptomycin S	10	15	12-14	11
Narrow Spectrum	Macrolide	Erythromycin	15	19	15-18	14

Table 4: Antibiotics used for Escherichia coli

Spectrum	Class of drug	Antibiotic	Content	Susceptible	Intermediate	Resistant
Broad Spectrum	Aminoglycosides	Amikacin AK	30	20	17-19	16
		Kanamycin K	30	18	14-17	13
	Tetracycline	Tetracycline TE	30	15	12-14	11
	Cephems	Ceftriaxone CRO	30	23	20-22	19
		Cefixime CFM	5	19	16-18	15
		Cefepime FEP	30	25	19-24	18
	Fluoroquinolones	Ciprofloxacin CIP	5	26	22-25	21
	Carbapenem	Meropenem MEM	10	23	20-22	19
		Imipenem IPM	10	23	20-22	19
	Macrolides	Azithromycin AZM	15	16	-	15
	Penicillin	Amoxicillin AML	30	18	14-17	13
		Amoxyclav AMC	30	18	14-17	13

Table 5: Antibiotics used for Klebsiella pneumoniae

Spectrum	Class of drugs	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad spectrum	Carbapenem	Meropenem MEM	10	18	15-17	14
		Imipenem IPM	10	22	19-21	18
	Aminoglycosides	Amikacin AK	30	17	15-16	14
		Gentamicin CN	10	15	13-14	12
	Penicillin	Ampicillin AMP	10	17	—	16
	Fluoroquinolones	Ciprofloxacin CIP	5	21	16-20	15
		Levofloxacin LEV	5	17	14-16	13
	folate pathway antagonistic	Trimethoprim-Sulfamethoxazole SXT	1.25/23.75	16	11-15	10
	beta-lactam	Piperacillin PRL	100	21	18-20	17
	Tetracyclines	Doxycycline DO	30	13	10-12	9
	cephems	Cefepime FEP	30	18	15-17	14
		Ceftazidime CAZ	30	18	15-17	14
		Ceftriaxone CTR	30	21	14-20	13

Table 6: Antibiotics used for Acinetobacter species

Spectrum	Class of drug	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad Spectrum	Penicillin	Amipicilin AMP	10	17	—	16
	Aminoglycosides	Streptomycin S	25	17	15-16	14
	Aminoglycosides	Gentamicin CN	10	17	15-16	14
	Fluoroquinolones	Levofloxacin LE	5	17	14-16	13
		Ciprofloxacin CIP	5	21	16-20	15
	Tetracycline	Tetracycline TE	30	19	15-18	14
	Aminoglycosides	Streptomycin S	25	17	15-16	14
		Gentamicin CN	10	17	15-16	14
Narrow Spectrum	Oxazolidinones	Linezolid LZD	30	23	21-22	20
	Glycopeptides	Vancomycin VA	30	17	15-16	14

Table 7: Antibiotics used for Enterococcus species

Spectrum	Class of drug	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad Spectrum	Cephems	Ceftazidime CAZ	30	18	15-17	14
	Fluoroquinoles	Ciprofloxacin CIP	5	25	19-24	18
	penicillin	Piperacillin PIT	100	22	18-21	17
	Carbapenem	Imipenem IPM	10	19	16-18	15
		Meropenem MEM	10	19	16-18	15
	Fluoroquinolones	Levofloxacin LE	5	22	15-21	14
	Aminoglycosides	Amikacin AK	30	17	15-16	14
Narrow Spectrum	monobactams	Aztreonam ATM	30	22	16-21	15

Table 8: Antibiotics used for Pseudomonas aeruginosa

Spectrum	Class of Antibiotics	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad Spectrum	Tetracycline	Tetracycline TE	30	15	12-14	11
	Macrolides	Azithromycin AZM	15	13	—	12
	Folate pathway antagonistic	Trimethoprim-Sulfamethoxazole SXT	1.25/23.75	16	11-15	10
	Aminoglycosides	Amikacin AK	30	17	15-16	14
	Carbapenem	Imipenem IPM	10	19	16-18	15
		Meropenem MEM	10	23	20-22	19
	Fluoroquinolones	Levofloxacin LE	5	21	17-20	16
		Ciprofloxacin CIP	5	26	22-25	21
	Cephems	Ceftriaxone CTR	30	18	15-17	16

Table 9: Antibiotics used for Salmonella species

Spectrum	Class of drugs	Antibiotic	Content	Susceptible	Immediate	Resistant
Broad Spectrum	Macrolides	Erythromycin E	15	17	—	12
	Cephem	Cefepime CFM	30	25	19-24	18
	Carbapenem	Imipenem IPM	10	23	20-22	19
		Meropenem MRP	10	23	20-22	19
	Tetracycline	Tetracycline TE	30	15	12-14	11
	Aminoglycosides	Amikacin AK	30	17	15-16	14
	Penicillin	Amoxicillin AML	10	18	14-17	13
	Fluoroquinolones	Ciprofloxacin CIP	5	21	16-20	15

Table 10: Antibiotics used for Vibrio species

2.13 Stock Preparation

Stock preparation of the purified isolates was carried out to ensure long-term preservation of all confirmed bacterial strains recovered from public bus surfaces. After obtaining pure

colonies on nutrient agar, each isolate was freshly subcultured and incubated at 37°C for 18–24 hours to ensure active growth prior to storage. A single isolated colony was transferred aseptically into T1N1 semisolid nutrient medium using a sterile inoculating needle, and the culture was gently stabbed into the medium to facilitate deeper growth and prolonged viability. Following incubation at 37°C for 24–48 hours, the surface of the culture was carefully overlaid with approximately 200 µL of sterile liquid paraffin to create an anaerobic seal that prevents desiccation and oxidative stress, thereby maintaining the viability of both Gram-positive and Gram-negative isolates for extended periods. This storage method is widely used in microbiology laboratories for preserving non-fastidious environmental and clinical isolates due to its simplicity and reliability (Cappuccino & Sherman, 2016).

All stored isolates were labeled with organism code, sample ID, date of isolation, and storage batch number to ensure proper traceability throughout downstream molecular and antimicrobial susceptibility analyses. The stock cultures were maintained at room temperature under aseptic conditions, following standard laboratory guidelines for bacterial preservation. These stock preparations served as the primary source of isolates for subsequent DNA extraction, PCR confirmation, and antimicrobial resistance profiling performed in later stages of the project.

Chapter 3

Result

3.1 Aerobic Plate Count (APC)

The aerobic plate counts (APCs) were calculated using the following formula:

$$APC\ (CFU/cm^2) = Number\ of\ colonies\ on\ plate \times \frac{Initial\ volume\ of\ diluent}{Area\ sampled} \times Dilution\ Factor$$

Here "Initial volume of diluent" indicates the volume of the diluent in which the cotton swab was suspended in after sample collection.

The APC for 16 out of the 30 samples were Too Numerous To Count (TNTC) and the rest of the samples had an aerobic plate count ranging from 0.5 to $5x10^6$ CFU/cm^2.

Sample	CFU/cm^2	Sample	CFU/cm^2
Bus 1 Seat	1.5	**Bus 8 Grab Rail**	TNTC
Bus 1 Grab Rail	0	**Bus 9 Seat**	1175×10^3
Bus 2 Seat	0	**Bus 9 Grab Rail**	35×10^4
Bus 2 Grab Rail	0.5	**Bus 10 Seat**	325×10^3
Bus 3 Seat	TNTC	**Bus 10 Grab Rail**	TNTC
Bus 3 Grab Rail	2	**Bus 1.2 Seat**	TNTC
Bus 4 Seat	TNTC	**Bus 1.2 Grab Rail**	575×10^3
Bus 4 Grab Rail	TNTC	**Bus 2.2 Seat**	TNTC
Bus 5 Seat	TNTC	**Bus 2.2 Grab Rail**	TNTC
Bus 5 Grab Rail	TNTC	**Bus 3.2 Seat**	TNTC
Bus 6 Seat	$2x10^6$	**Bus 3.2 Grab Rail**	TNTC
Bus 6 Grab Rail	$5x10^6$	**Bus Stop 1**	TNTC
Bus 7 Seat	TNTC	**Bus Stop 2**	TNTC
Bus 7 Grab Rail	TNTC	**Bus Stop 3**	75×10^4
Bus 8 Seat	375×10^4	**Bus Stop 4**	2825×10^3

Table 11: Aerobic plate count of all samples

3.2 Fecal Coliform Count (FCC)

The fecal coliform counts (FCCs) were calculated using the following formula:

$$FCC\ (CFU/cm^2) = Number\ of\ blue\ colonies\ on\ plate \times \frac{Initial\ volume\ of\ diluent}{Area\ sampled} \times Dilution\ Factor$$

Here "Initial volume of diluent" indicates the volume of the diluent in which the cotton swab was suspended in after sample collection or the volume of the diluent used for membrane filtration, and the dilution factor is 10^0 or 1 since the raw diluent was used here

The FCC for 10 out of the 19 samples, positive for fecal coliform, were TNTC and the rest of the samples had a FCC ranging from 1 to 40 CFU/cm^2.

Sample	CFU/cm^2	Sample	CFU/cm^2
Bus 1 Seat	24.5	**Bus 8 Grab Rail**	0
Bus 1 Grab Rail	16.5	**Bus 9 Seat**	0
Bus 2 Seat	0	**Bus 9 Grab Rail**	0
Bus 2 Grab Rail	7	**Bus 10 Seat**	0
Bus 3 Seat	TNTC	**Bus 10 Grab Rail**	0
Bus 3 Grab Rail	TNTC	**Bus 1.2 Seat**	1.5
Bus 4 Seat	TNTC	**Bus 1.2 Grab Rail**	1
Bus 4 Grab Rail	TNTC	**Bus 2.2 Seat**	TNTC
Bus 5 Seat	TNTC	**Bus 2.2 Grab Rail**	TNTC
Bus 5 Grab Rail	TNTC	**Bus 3.2 Seat**	1
Bus 6 Seat	TNTC	**Bus 3.2 Grab Rail**	1
Bus 6 Grab Rail	TNTC	**Bus Stop 1**	0
Bus 7 Seat	10	**Bus Stop 2**	0
Bus 7 Grab Rail	40	**Bus Stop 3**	0
Bus 8 Seat	0	**Bus Stop 4**	0

Table 12: Fecal coliform count of all samples

3.3 Target Bacteria Detected in All Samples

Out of the 10 target organisms, no *Shigella* spp. or *S. pneumoniae* were detected in any sample. Moreover, no *V. cholerae* were identified in any of the samples, rather yellow colonies collected from TCBS media were PCR positive for *Vibrio* spp. Similarly, instead of *Acinetobacter* species, pink colonies picked from Leeds media were checked for *A. baumannii*, due to the unavailability of *Acinetobacter* species primer in the lab. Additionally, while running PCR for *S. aureus* a good number of samples were found to be positive for *S. epidermidis,* compared to *S. aureus*. Hence, *S. epidermidis* was also added to the list of detected organisms. So, a total of 9 different organisms were detected in the sample. All 30 samples collected in this study were positive for target bacterial growth, of which, 8 samples were positive for *E. coli*, 18 positive for *K. pneumoniae*, 5 positive for *Enterococcus* spp., 11 positive for *Vibrio* spp., 1 positive for *S. aureus*, 3 positive for *Salmonella* spp., 5 positive for *P. aeruginosa*, 10 positive for *A. baumannii* and 6 positive for *S. epidermidis*.

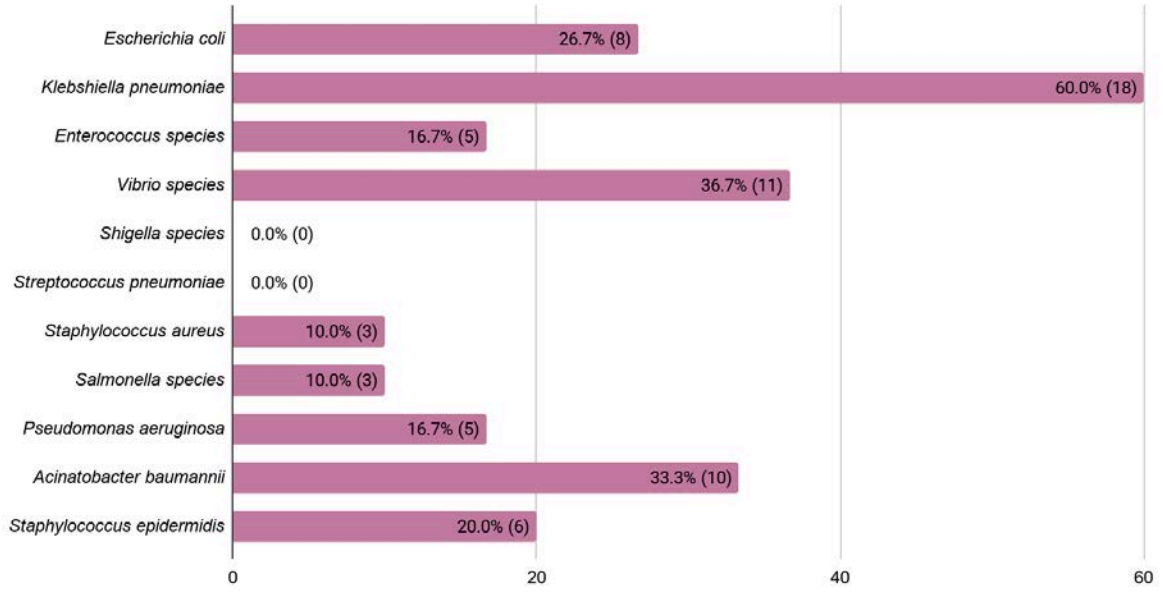

Figure 13: Percentage of samples positive for each organism. The number inside the parentheses indicates the number of isolates.

3.4 Bacterial Diversity in Samples Collected in Winter and Summer

In this study, the months of winter, from November to February, have been chosen due to the comparatively lower temperature and humidity seen in Dhaka during these months. And the months of summer, from March to October, have been chosen due to the higher temperature and humidity seen during these months in Dhaka, compared to the months of summer. Among the 30 samples collected, 10 samples from 5 buses were collected in winter and the rest were collected during summer. Except for *E. coli*, *K. pneumoniae* and *S. aureus*, no other target organisms were found in the samples collected in winter.

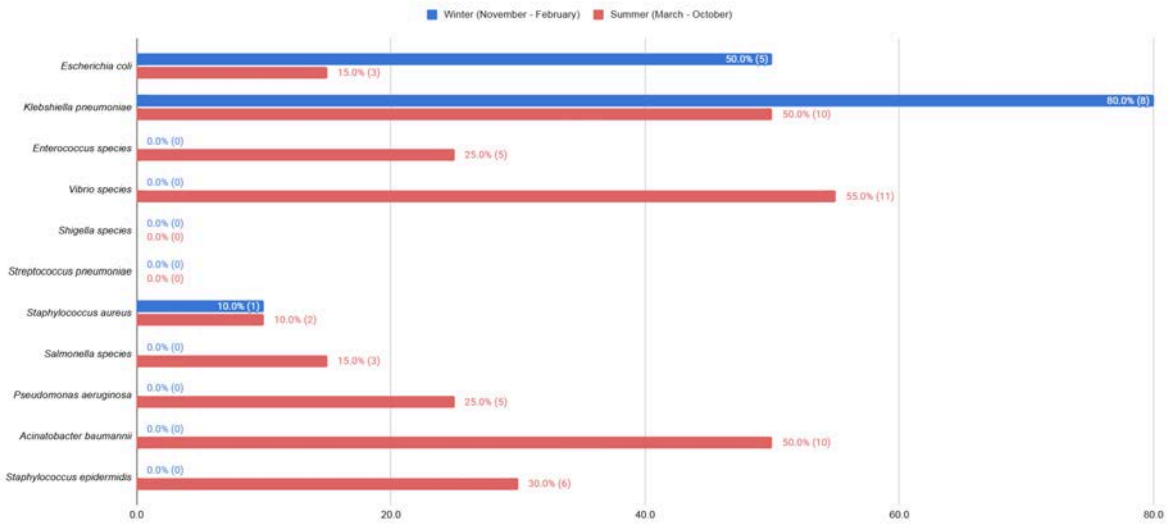

Figure 14: Percentage of samples, collected in winter and summer, positive for each organism. The number inside the parentheses indicates the number of isolates.

3.5 Bacterial Diversity in Samples Collected From Different Touch Surfaces

In this study, samples were collected from three different touch surfaces, seat and grab rail of public buses, and bus stops. Among the 30 samples, 13 were taken from seat head rests, 13 from grab rails and 4 from bus stops. All 9 organisms were detected in samples taken from seats and grab rails. Whereas in samples taken from bus stops, all organisms except for *S. aureus* and *Enterococcus* spp. were identified. Overall, a higher number of isolates were recorded in seats compared to grab rails and bus stops, which had almost the same number of isolates.

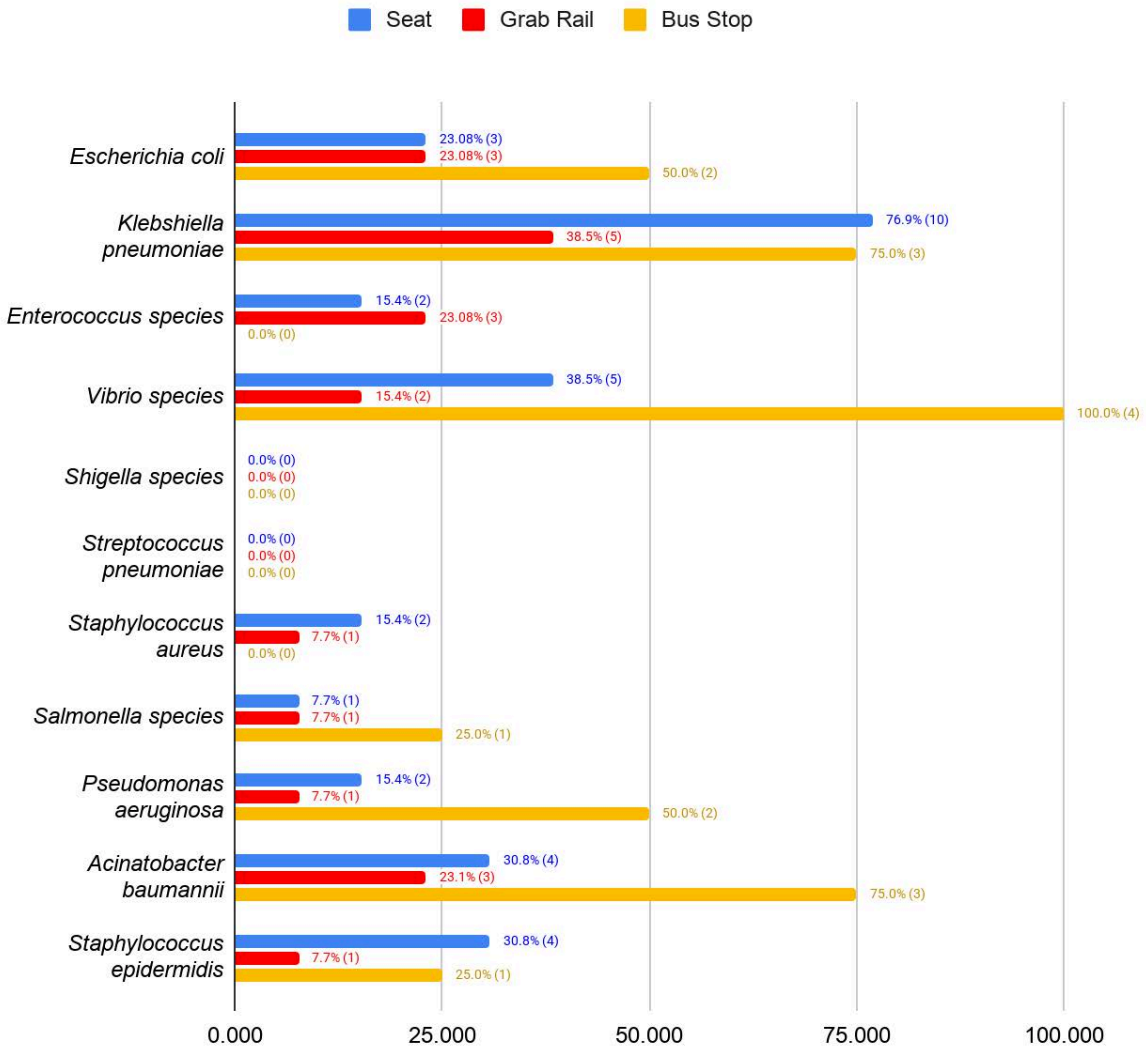

Figure 15: Percentage of positive samples, collected from seats, grab rails and bus stops, for each organism. The number inside the parentheses indicates the number of isolates.

3.6 Bacterial Diversity in Samples Collected From Seats Covered in Different Materials

A total of 13 seat samples were collected from 13 buses for this experiment, among which three types of seat cover materials, cloth, leather and plastic were seen. Although vinyl seat covers are seen in public buses of Bangladesh, out of all the buses boarded for sample collection, no buses had any seats with vinyl cover. Out of the 13 samples taken from seats, 10 seats were covered with cloth, 2 with plastic and 1 with leather. All 9 organisms except for

Salmonella spp. were detected in cloth seat samples. On the other hand, no other organisms except for *Salmonella* spp. were found in leather seat samples. And in plastic seat samples, only *K. pneumoniae, Enterococcus* spp., *Vibrio* spp., *P. aeruginosa*, *A. baumannii* and *S. epidermidis* were detected. No studies have been found so far which compare at least two out of the three materials mentioned in this research experiment.

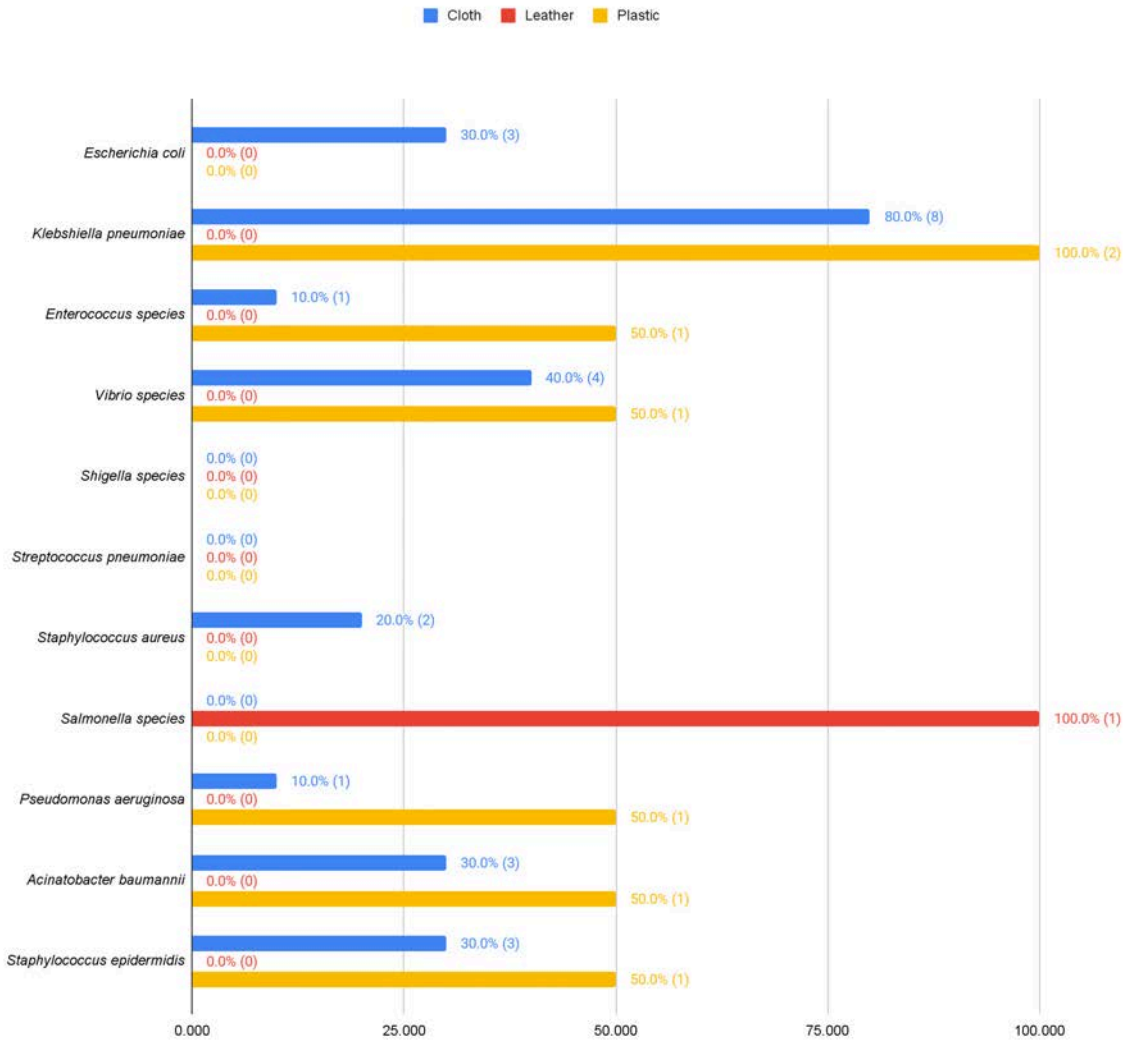

Figure 16: Percentage of positive samples collected from seats, covered in different materials, for each organism. The number inside the parentheses indicates the number of isolates.

3.7 Bacterial Diversity in Samples Collected From Grab Rails Covered in Different Materials

In public buses of Bangladesh, the grab rails on the inside of the bus are seen covered or made of different materials, like stainless steel, plastic and sometimes even cloth. Among the 13 grab rails from which samples were collected for this experiment, five different types of coating materials were seen. Among them 6 grab rails were made of stainless steel, 2 covered in stainless steel and cloth, 2 coated with rust, 2 made of plastic and 1 coated with acrylic paint. *E. coli*, *K. pneumoniae*, *Vibrio* spp., *P. aeruginosa* and *A. baumannii* were detected in swab samples collected from grab rails made of stainless steel and in samples taken from grab rails covered in both cloth and stainless steel, only *E. coli*, *K. pneumoniae*, *Enterococcus* spp. and *A. baumannii* were detected. Samples collected from rusty grab rails, came out positive for *K. pneumoniae*, *Vibrio* spp., *Enterococcus* spp., *A. baumannii* and *S. epidermidis*, while samples collected from plastic grab rails or handles tested positive for only *K. pneumoniae*, *Enterococcus* spp. and *Salmonella* spp. Among all organisms, the sample collected from the grab rail covered in acrylic paint was only positive for *E. coli*.

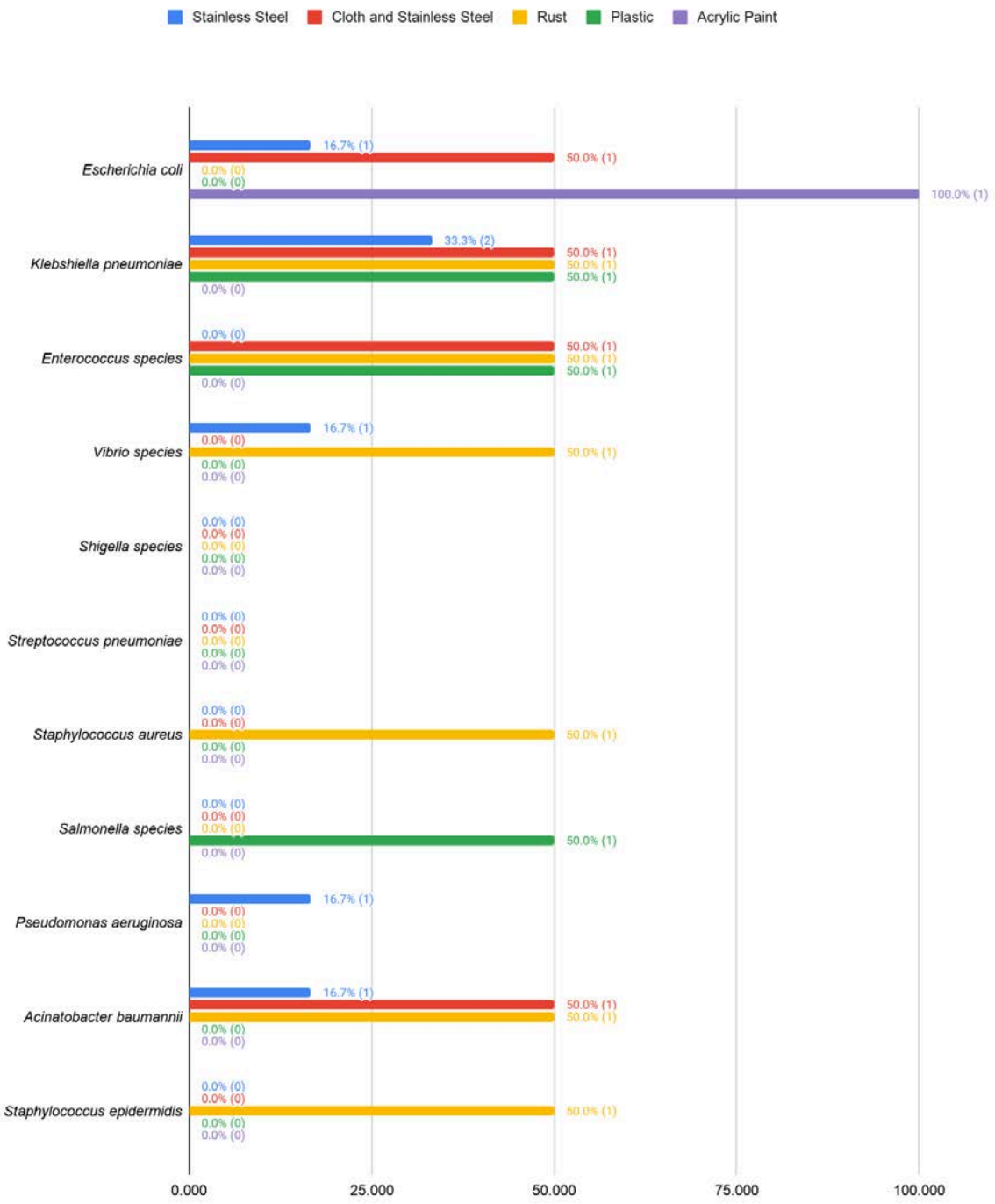

Figure 17: Percentage of positive samples collected from grab rail, covered in different materials, for each organism. The number inside the parentheses indicates the number of isolates.

3.8 Bacterial Diversity in Samples Collected From Bus Stops Covered in Different Materials

Swab samples from 4 different bus stops were collected for this experiment, among which 3 bus stops touch surfaces were covered in rust and 1 was painted with acrylic paint. *E. coli*, *K. pneumoniae*, *Vibrio* spp., *P. aeruginosa* and *A. baumannii* were detected in all bus stop samples. Whereas, *S. epidermidis* and *Salmonella* spp. were detected in rusty bus stop surfaces and the bus stop painted with acrylic paint, respectively.

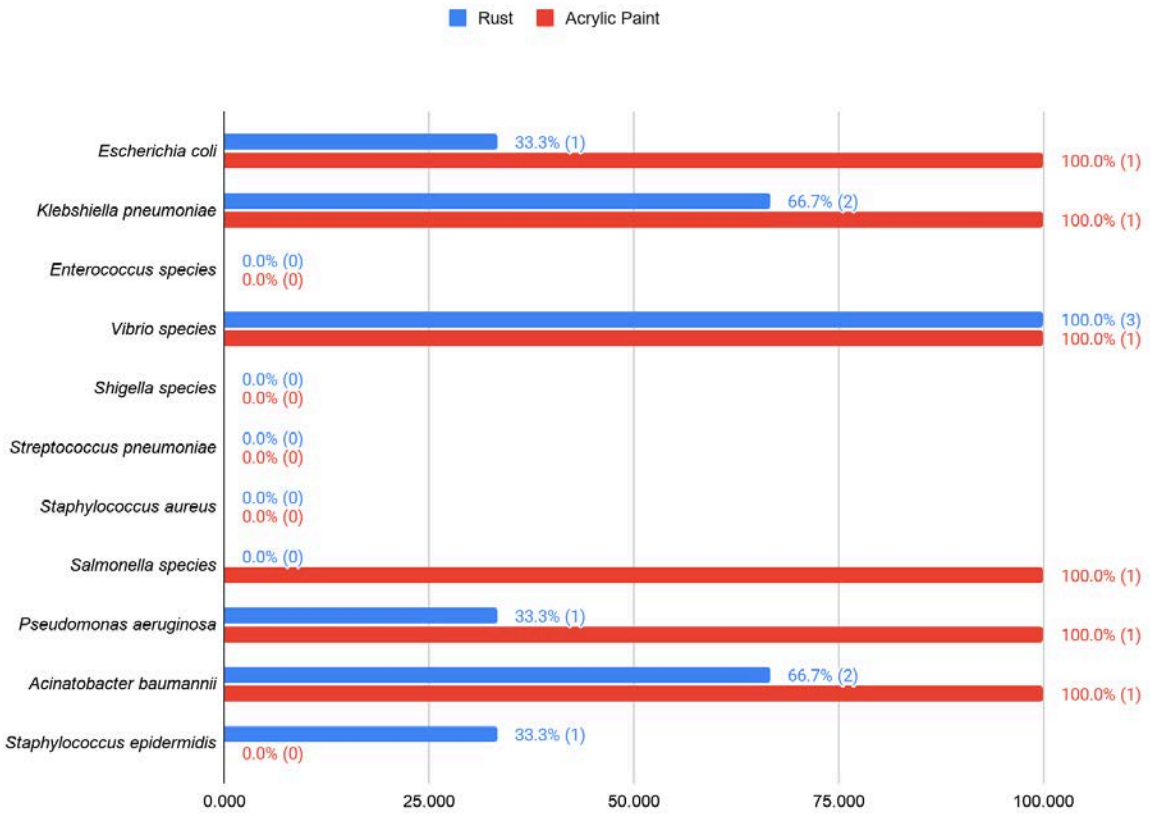

Figure 18: Percentage of positive samples collected from bus stops, covered in different materials, for each organism. The number inside the parentheses indicates the number of isolates.

3.9 Antibiotic Susceptibility Test

3.9.1 Antibiotic Susceptibility Test Result of *Klebsiella pneumoniae*

A total of 20 *K. pneumoniae* isolates were included for antimicrobial susceptibility testing. The resistant, intermediate, and susceptible isolate numbers in the table represent out of these 20 isolates. The graph shows that *K. pneumoniae* exhibits high resistance to most tested antibiotics like amoxicillin, erythromycin, and cefixime. Only a few antibiotics, such as amikacin and kanamycin showed better susceptibility. Cefixime, erythromycin, and amoxicillin demonstrated the highest resistance levels, with 100%, 95%, and 85% resistance. Azithromycin also displayed a high resistance rate of 60%.

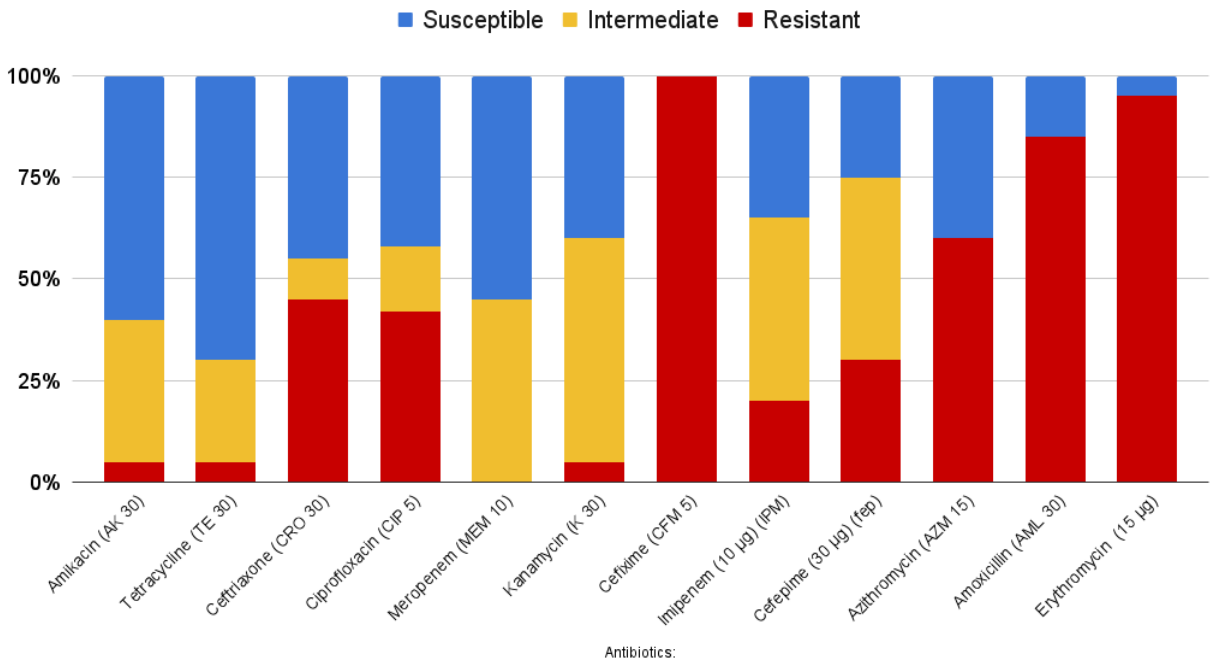

Figure 19: AST results of K. pneumoniae

3.9.2 Antibiotic Susceptibility Test Result of *Staphylococcus aureus*

A total of 7 *S. aureus* isolates were taken for the test. The antibiotic susceptibility results show that Nitrofurantoin and Chloramphenicol were the most effective, each with 100%

susceptibility and no resistance. Azithromycin and linezolid also demonstrated high effectiveness, with 85.7% susceptibility. Tetracycline, levofloxacin, doxycycline, and moxifloxacin each showed 71.4% susceptibility, indicating moderate activity. Clindamycin, cefoxitin, and penicillin had the highest resistance levels, each with 42.9% resistance.

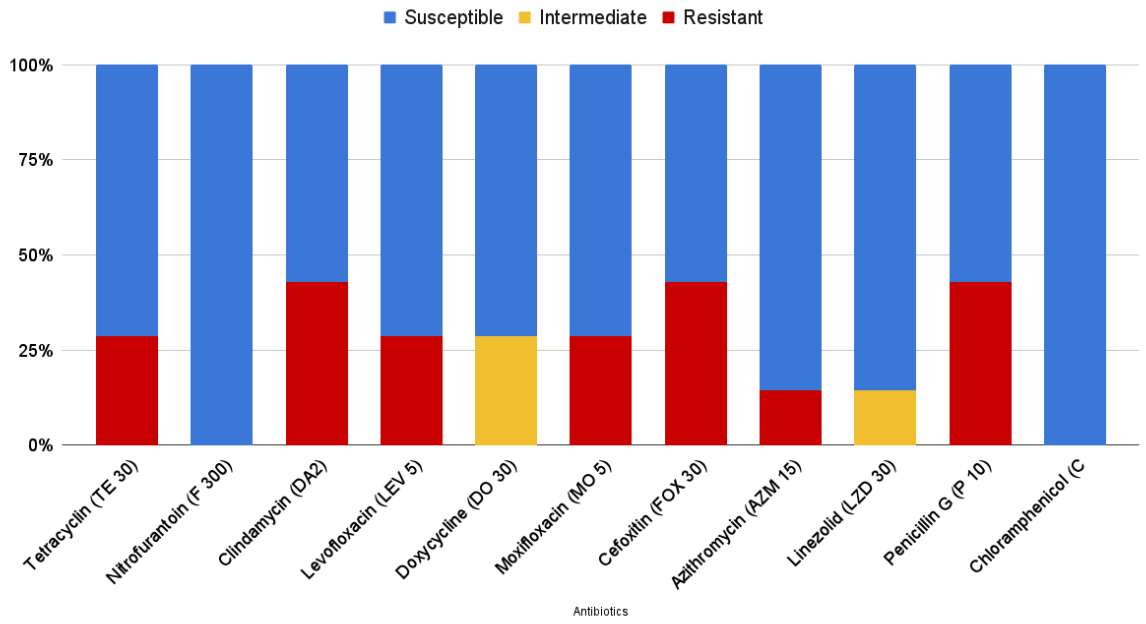

Figure 20: AST results of S. aureus

3.9.3 Antibiotic Susceptibility Test Result of *Staphylococcus epidermidis*

A total of 6 *S. epidermidis isolates were taken for this AST.* High levels of resistance were observed in penicillin and cefoxitin both showing 100% resistance. Azithromycin also showed a high resistance rate of 66.7%. Tetracycline and chloramphenicol have 83.3% and 66.7% susceptibility. 100% of isolates were susceptible to moxifloxacin, gentamicin, doxycycline, levofloxacin, and trimethoprim–sulfamethoxazole.

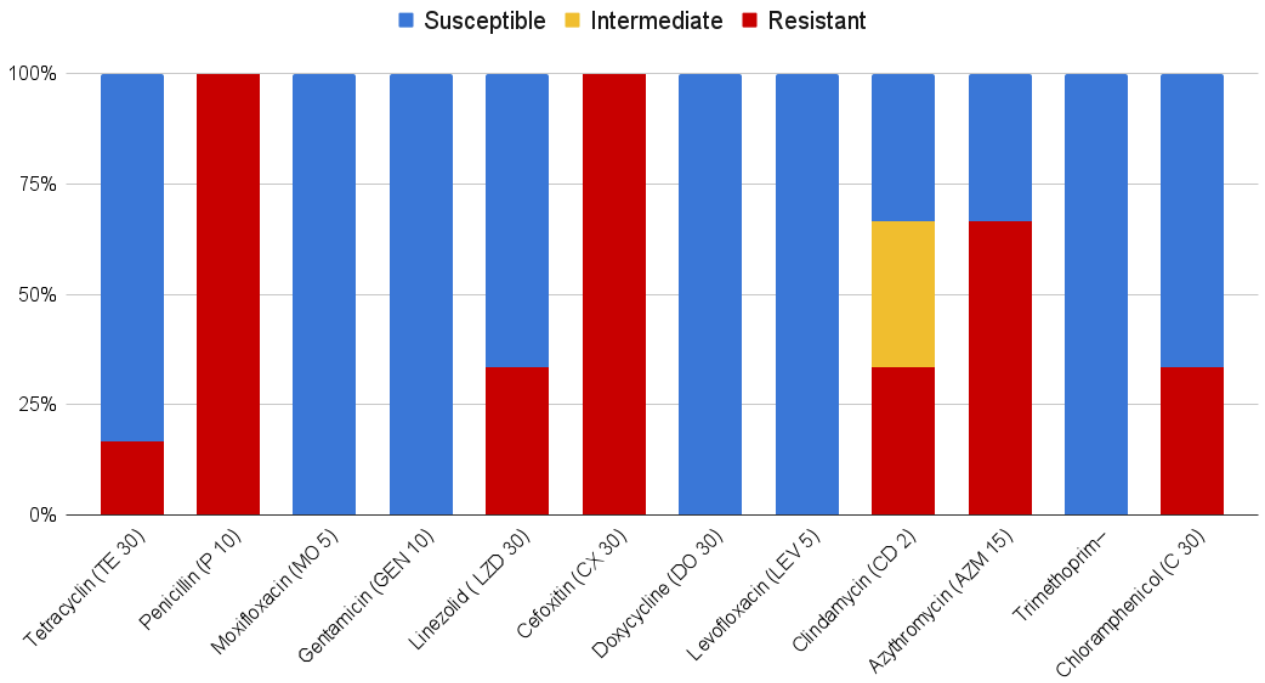

Figure 21: AST results of Staphylococcus epidermidis

3.9.4 Antibiotic Susceptibility Test Result of *Enterococcus* species

Total 4 *Enterococcus* spp. isolates from 4 different samples were taken for this test. The antibiotic susceptibility results show that levofloxacin, ciprofloxacin, tetracycline, streptomycin, and gentamicin exhibited 100% sensitivity. Linezolid showed mixed results with 50% of isolates being susceptible and 50% resistant. Penicillin showed limited effectiveness, with 75% resistance and only 25% sensitivity. Vancomycin had the same result with 50% resistance and 50% intermediate response. Ampicillin was the least effective antibiotic which was showing 100% resistance to all the isolates.

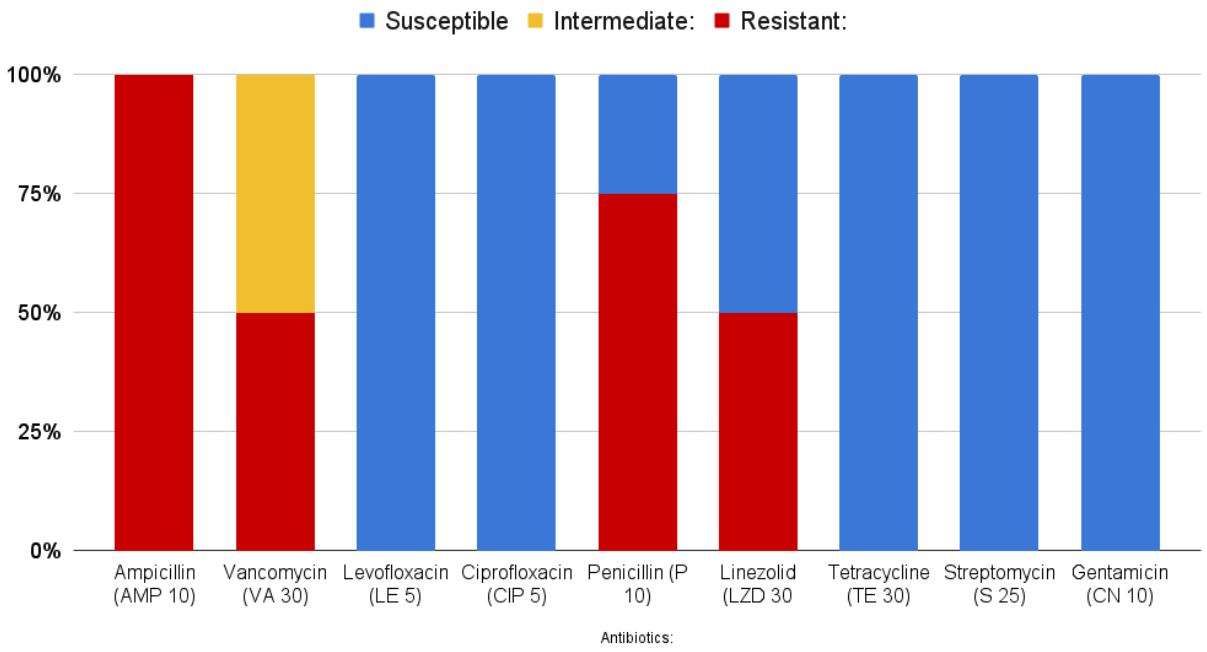

Figure 22: AST results of Enterobacter species

3.9.5 Antibiotic Susceptibility Test Result of *Acinetobacter baumannii*

A total of 13 isolates of *A. baumannii* from different samples were taken for this test. The antibiotic susceptibility profile of the isolates showed that ampicillin and ceftriaxone had the highest resistance, with 100% of isolates resistant. Imipenem, meropenem, doxycycline, gentamicin, and trimethoprim-sulfamethoxazole were fully effective, with 100% susceptibility. Ciprofloxacin showed 23.1% resistance and 76.9% susceptibility, while Cefepime had 30.8% resistance and 69.2% susceptibility. Levofloxacin exhibited 15.4% intermediate and 84.6% susceptibility, Piperacillin had 7.7% intermediate and 92.3% susceptibility, and Amikacin had 7.7% intermediate with 92.3% susceptibility. Ceftazidime showed 46.2% intermediate and 53.8% susceptibility

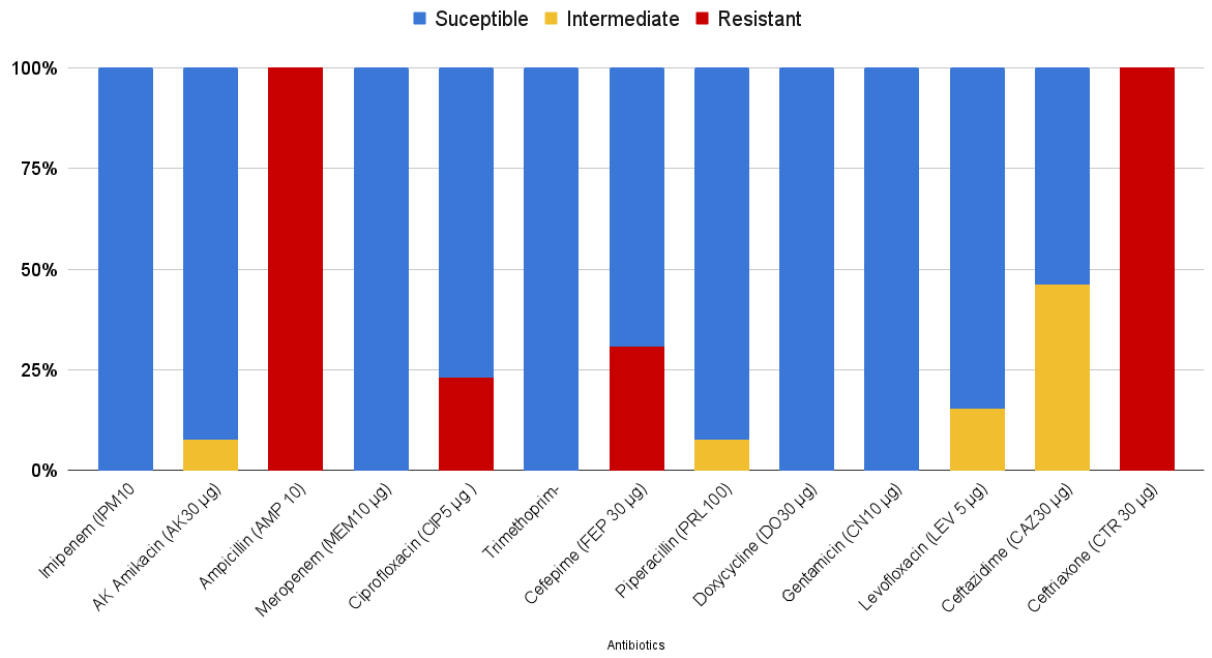

Figure 23: AST results of Acinetobacter baumannii

3.9.6 Antibiotic Susceptibility Test Result of *Vibrio Species*

Total 12 samples of *Vibrio* spp. were taken for this test. Erythromycin and amoxicillin showed high resistance, with 91.7% and 75% of isolates resistant, respectively. Cefepime and meropenem had moderate resistance at 58.3% and 66.7%. Tetracycline and imipenem were largely effective, showing 91.7% and 91.7% susceptibility, while ciprofloxacin and amikacin were fully effective with 100% susceptibility. Overall, carbapenems like imipenem, meropenem and amikacin were mostly effective, whereas erythromycin and amoxicillin showed poor activity against the isolates.

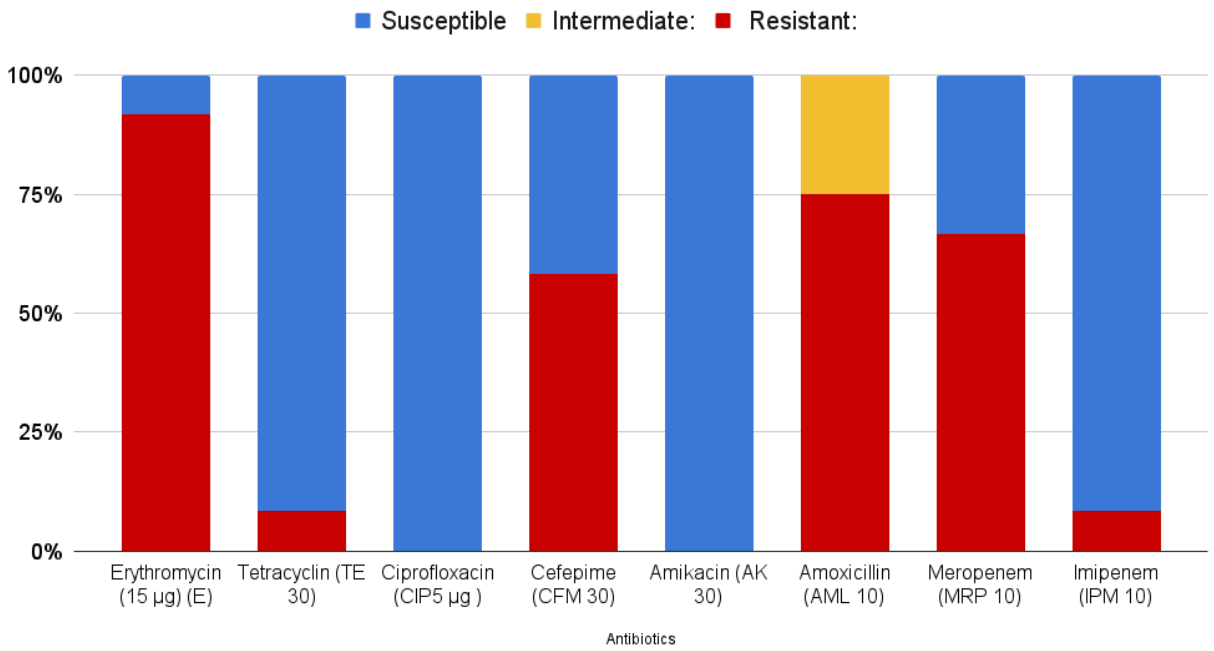

Figure 24: AST results of Vibrio species

3.9.7 Antibiotic Susceptibility Test Result of *Salmonella* species

Three isolates of *Salmonella* spp. were selected. Most isolates were fully sensitive to tetracycline, trimethoprim-sulfamethoxazole, imipenem, and meropenem (100% susceptibility). Azithromycin showed partial resistance (33.3%) with 66.7% sensitivity. Amikacin had a high intermediate response (66.7%). Levofloxacin, ceftriaxone, and ciprofloxacin showed complete intermediate response (100%), indicating reduced effectiveness in these isolates.

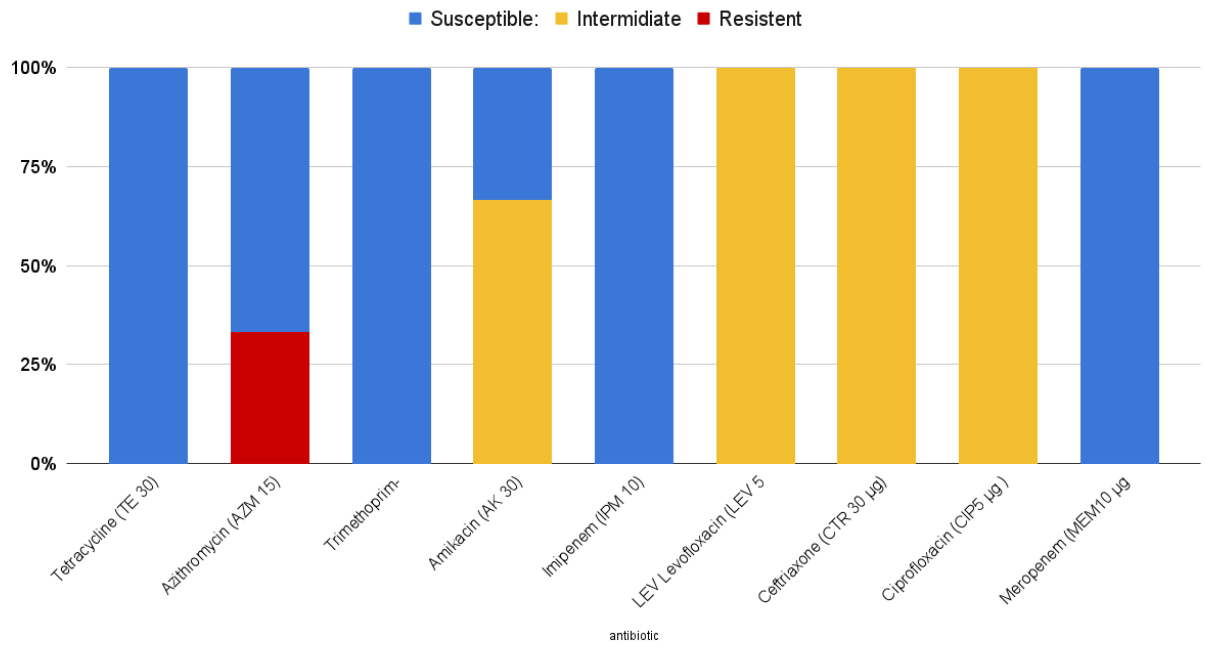

Figure 25: AST results of Salmonella species

3.9.8 Antibiotic Susceptibility Test Result of *Pseudomonas aeruginosa*

Total 7 *P. aeruginosa* isolates were taken. Ceftazidime showed complete resistance (100%), while aztreonam had 71.4% resistance. Ciprofloxacin, piperacillin, and levofloxacin had 28.6% intermediate response and 71.4% susceptibility. Imipenem, meropenem, and amikacin were fully effective, showing 100% susceptibility among the isolates.

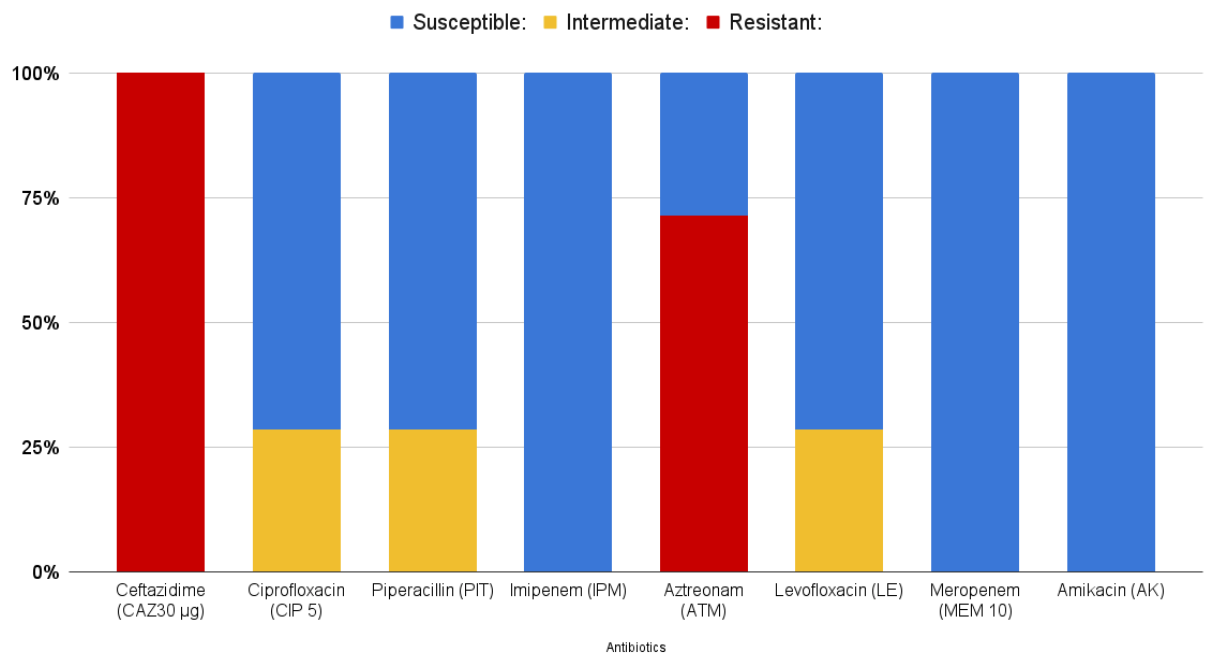

Figure 26: AST results of Pseudomonas aeruginosa

3.9.9 Antibiotic Susceptibility Test Result of *Escherichia coli*

A total of 12 *E. coli* isolates were taken. Erythromycin showed complete resistance (100%), followed by tetracycline (91.7%) and cefixime (83.3%). Ciprofloxacin had moderate resistance (66.7%) with 25% susceptibility. Imipenem and cefepime displayed mixed resistance and intermediate responses, while chloramphenicol had 75% susceptibility. Meropenem was fully effective, showing 100% susceptibility among the isolates.

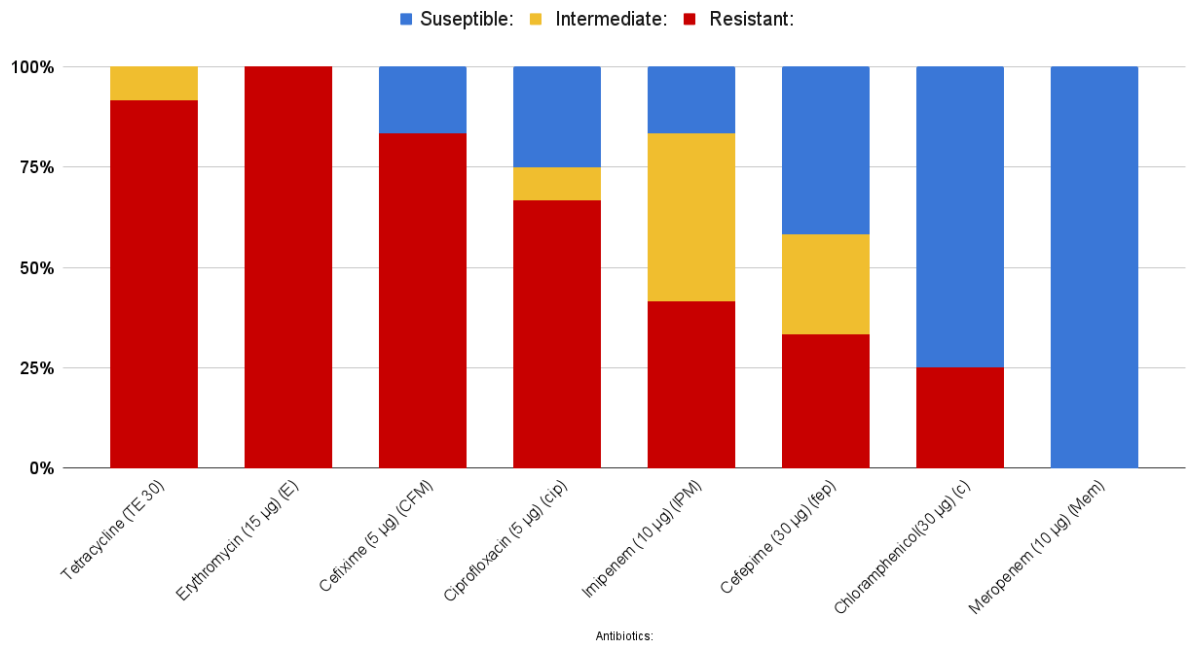

Figure 27: AST results of E. coli.

3.9.10 Multidrug Resistance Patterns

The antimicrobial susceptibility analysis revealed varying levels of resistance among the isolated bacterial species. *K. pneumoniae* exhibited the highest proportion of multidrug resistant isolates (95%). *E. coli* and *V. cholerae* demonstrated high multidrug resistance rates (75% each). Moderate resistance was observed in *S. epidermidis* (83.33%) and *Enterococcus* spp. (50%), while *S. aureus* showed comparatively lower resistance (42.86%). *A. baumannii, Salmonella* spp., and *P. aeruginosa* showed no multidrug resistant isolates (0%).

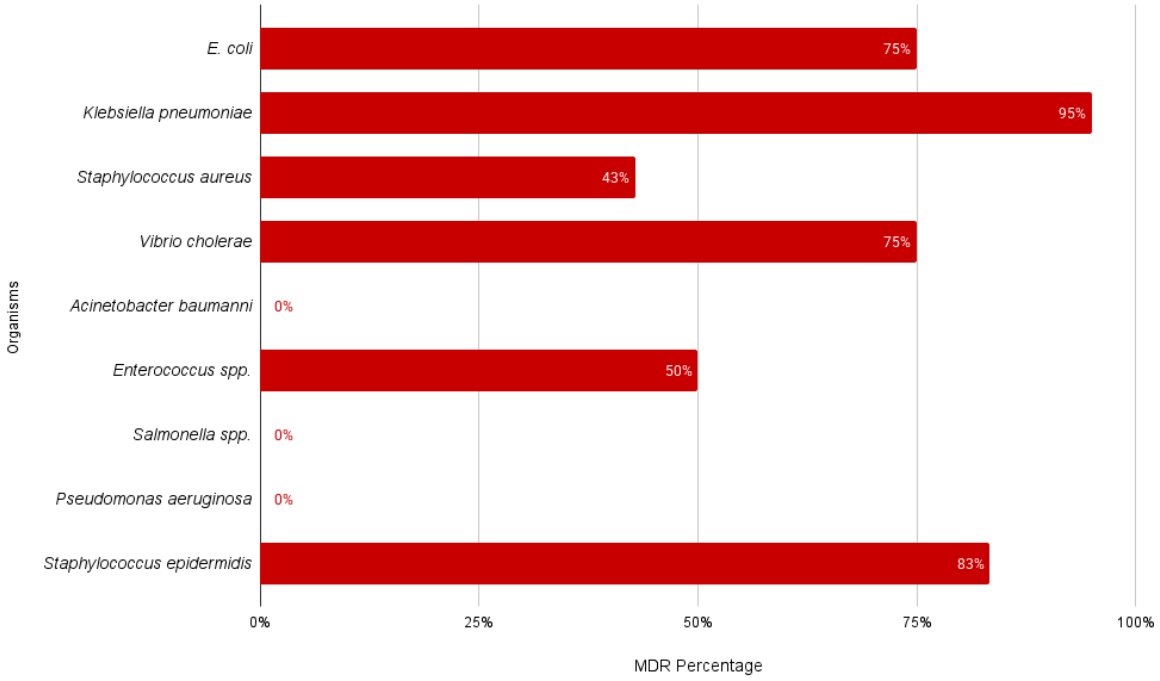

Figure 28: MDR patterns of the isolates.

Chapter 4

Discussion

There are currently no national or international standards for the limit of APC and FCC allowed on touch surfaces of public transports or public spaces. But different studies concerning the microbiological profile of public transport touch surfaces have mentioned the microbial load in the samples they have analyzed. A bacterial load ranging from 0.12×10^6 to 86×10^6 CFU/ml was recorded in samples collected from vehicles in the Tamale metropolis of Ghana (Abdulai et al, 2020). And in a study conducted by Kalb (2022), a microbial burden ranging from 0.1 to 77.1 CFU/cm^2 in vehicle touch surfaces was found.

In comparison to a study conducted by Chowdhury et al. (2016) in Chittagong, Bangladesh, the rate of positive samples for *E. coli, Salmonella* spp. and *S. aureus* is much less in this study. Whereas, the rate of *Vibrio* spp. found in another study conducted on public buses of Dhaka city is much less than the rate seen in this study (Arowan et al., 2021). In this study the rate of *K. pneumoniae* and *P. aeruginosa* is almost twice as much as, and the rate of *S. epidermidis* is lesser than, the rate of these organisms recorded in a study conducted in Sagamu, Ogun state, Nigeria (Famojuro et al., 2024). The percentage of *Enterococcus* spp. detected in this study is almost half as much as the rate of the said organism detected by Tan and Erdoğdu (2017) in their study, conducted in Istanbul, Turkey. Although, no studies have been found regarding finding *A. baumannii* in public buses or bus stops, a study conducted on touch surfaces of subways or mass transit railways (MTR) of Hong Kong shows *A. baumannii* as one of the the most abundant species found in the samples, which is consistent with the findings in this study (Kang et al., 2018).

Analysis of bacterial diversity in environmental samples collected in different seasons is necessary since, a pattern is usually noted between occurrence of and infection caused by

different organisms, and different seasons. According to Kaplan (2005), an increase in MRSA infections is seen during summer. In case of the seasonal variation it is seen that only *E. coli*, *K. pneumoniae* and *S. aureus* were present in the winter samples, while all the nine other organisms were detected in samples collected during summer. In this study the rate of positive samples for *E. coli* and *K. pneumoniae* is less in summer than in winter, which is contradictory to the findings of Yadav et. al (2019). This is unusual for *K. pneumoniae* and *E. coli* as higher humidity and temperature favour the growth of both the organisms, making summer season in a tropical country like Bangladesh a more favourable season than the dry winter season, for the growth of these organisms. A reason behind the number of positive samples for *E. coli* being comparatively less in summer could be the extreme solar insolation that Bangladesh experiences during the months of summer (Miskat et al.,2022). Some studies have shown that although *E. coli* has a higher growth rate in warmer temperatures, the growth rate exponentially declines if the solar insolation is high in an environment (Petersen and Hubbart, 2020). In case of *K. pneumoniae* no studies have been found that explain the reason behind this unusual finding, but a probable reason could be that, the winter season in a tropical country like Bangladesh is not very cold and has lesser rainfall compared to summer season. The temperate weather of tropical winter lets *K. pneumoniae* proliferate with ease, while less rainfall during winter could reduce washing out or dilution of *K. pneumoniae* populations and increased rainfall during summer could dilute *K. pneumoniae* populations in environmental reservoirs. Just like the findings in this study, *S. aureus* was also detected in samples collected from subway compartments of different cities of South Korea during winter and summer seasons (Lee et al., 2024). Finding *S. aureus* during winter and summer simultaneously is not unusual as *S. aureus* is tolerant to low and high temperatures of tropical weather, and desiccation (Bianchi et. al., 2022; Maudsdotter et. al., 2015). Although the same study showed *P. aeruginosa* being detected in the samples collected from the subway

compartments in winter, no *P. aeruginosa* was detected in the samples collected during winter for this study (Lee et al., 2024). Just like *S, aureus, P. aeruginosa* can also survive in the low and high temperatures of tropical weather, but it is more abundant in the tropical environment during the summer because about 37°C temperature and high humidity favours its growth (LaBauve & Wargo, 2012; Ramos et al., 2013). Unlike the findings in this study, a study conducted by Solaiman et al. (2022) found a homogeneous distribution of *Enterococcus* spp. in samples collected in different seasons. Although no study has been found as to why *Enterococcus* spp. is found in the tropical environment during summer and not during winter, as according to Byappanahalli et al. (2012) and Ramsey et al. (2014) they can grow in temperatures ranging from 10°C to 45°C and are highly tolerant to dessication, a reason behind this could be the lack of nutrient they might have faced during the winter. According to Di et al. (2017) there is no specific season that favours the growth of all *Vibrio* spp., as different species of *Vibrio* are found in the environment in different seasons, like *V. cholerae and Vibrio vulnificus* are found in the environment during summer, or throughout the year like Vibrio alginolyticus and Vibrio parahaemolyticus. Findings of Abdalla et al. (2022) showed that the optimum temperature for growth for most *Vibrio* spp. is around 37°C, which is a strong reason for finding *Vibrio* spp. during summer when the temperature is usually 30°C - 40°C. Parallel to the findings of this study, the findings of Monter et al. (2021) show that *Salmonella* species are seasonally specific to summer. *Salmonella* spp. have an optimal growth temperature of about 35°C - 37°C, its shedding by animal hosts increases during warmer weathers and its spread in the environment increases with an increase in rainfall, which makes tropical summer more favourable not only for the growth of *Salmonella* spp. but also their distribution in the environment (Alharbi et al., 2025; Haley et al., 2009; Labib, 2020). A study conducted in the Hebei Province of China found an increased detection of *A. baumannii* in samples collected during summer compared to samples collected in winter,

which is consistent with the results of this experiment (Liu et al., 2024). Doughari et al. (2011) reported in their finding that the optimum growth temperature of *A. baumannii* is 42°C - 45°C and its growth starts to decline under 28°C according to Pulami et al. (2020). High temperatures starting from 37°C also promote biofilm formation and nutrient intake in *A. baumannii* which helps in their spread, adhesion and rapid proliferation (Eze et al., 2018). Since, high temperatures from 37°C - 42°C can only be experienced during the tropical summer of Bangladesh, this is the only season here when *A. baumannii* can be found in the environment. Lastly, a study conducted by Afrif et al. (2019) showed an increase in *S. epidermidis* during winter, which is contradictory to the results of this experiment. Most studies have shown that occurrence of *S. epidermidis* has no relationship with any seasons, as it is found in the environment steadily regardless of low or high temperature and humidity. Although, a reason behind *S. epidermidis* being more abundant in samples during summer could be the increased exposure of skin, people wearing short sleeves, during summer compared to winter. *S. epidermidis* can float on moisture and is disseminated faster when it does so, thus sweat forming on skin during warmer days can aid in the quick dissemination of *S. epidermidis*, which could be another reason why *S. epidermidis* is more abundant during the summer time (Katsarou et al., 2023).

When analyzing the surfaces from which the samples were taken, it is noted that, a higher number of isolates were recorded in seat samples compared to grab rail and bus stop samples, which had almost the same number of isolates. These findings align with the finds of Boonman and Chutrtong (2022) in Thailand, who found *S. aureus* in 17 grab rail samples and 30 seat samples, among the total 60 samples they collected. A study conducted in Portland, USA, shows a higher number of bacterial colonies in swabs taken from bus seats compared to swabs taken from grab rails and bus stops, which also aligns with the findings of this experiment (Yeh et al., 2011).

The previously mentioned study of Portland, USA also showed a higher concentration of bacteria in seats covered in cloth, compared to seats covered in other materials, which is similar to the finds of this study (Yeh et al., 2011). According to the findings of Ketema and Worku (2023), both gram positive and negative bacteria are able to grow effortlessly on cloths and at the same rate, which is also seen in this study in case of samples collected from cloth seats. Surface roughness, charge and hydrophobicity/hydrophilicity of fabrics aid both gram positive and negative bacteria in adhesion to cloth, while skin shedding, sweat or moisture and nutrients from surrounding environments and naturally occurring particles in fabrics aid in the proliferation of bacteria (Bruinsma et al., 2001; Dixit et al., 2023 Szostak-Kotowa, 2003 Zheng et al., 2021;). But in case of grab rails covered in cloth and steel, more samples were positive for gram negative bacterial growth than gram positive bacterial growth, which is contradictory to this finding. No studies have been found which has explored the reason behind only *Salmonella* spp. being identified on leather seat swabs, but a reason behind *Salmonella* spp. being able to survive on properly tanned leather, which has a very little water activity, could be its ability to survive in a desiccated state for an extended period of time and the fact that bacteria, if present on tanned leather surface, can grow on leather under relatively high humidity (Finn et al., 2013; Orlita, 2004). A study on microbial growth and survival period on inanimate objects showed that *A. baumannii* among other gram negative bacteria had a higher survival rate on stainless steel and plastic than *S. aureus* and other gram positive bacteria, which is congruent with the results of this experiment (Katzenberger et al., 2021). Higher hydrophobicity and a lower intensity of negative charge on the surface of gram negative bacteria compared to gram positive bacteria, make adherence of gram negative bacteria on stainless steel stronger than gram positive bacteria (Harimawan et al., 2011). Similarly, due to gram negative bacteria's higher hydrophobicity, they are able to adhere more strongly to hydrophobic plastics than gram

positive bacteria are (Abram et al., 2021). Although no studies have been found which showed any specific relation between growth of gram negative and positive bacteria on rusted surfaces, Li and Logan (2004) have explained in their study the reason behind finding a good load of bacteria on rusted surfaces. According to their findings, due to metal oxides' positive charge and hydrophobicity, and surface roughness of rusted material, adherence of bacteria on rusted surfaces is high. Although the findings of Shazadi et al. (2024) regarding acrylic paint lessening microbial growth aligns with the results from samples taken from grab rails in this experiment, it does not support the findings in case of bus stops. Due to having lower surface roughness and no electron accepting capability at all, bacterial growth on surfaces painted with acrylic paint is always limited (Sousa et al., 2009). It is to be noted that the acrylic painted surface of the bus stop was peeling at some places including the area from where the swab was collected from. Disruption in the acrylic coating thus creation of roughness could be the reason why bacteria were able to grow on that acrylic paint coated bus stop touch surface.

In this study *K. pneumoniae* shows high resistance in many commonly used antibiotics. For example: cefixime (100%), erythromycin (95%), amoxicillin (85%). Good susceptibility was observed in meropenem, amikacin, kanamycin and tetracycline. A study done in China, where the samples were collected from public transport shows high resistance to amikacin (81.82%), tetracycline (81.82%), ciprofloxacin (81.82%), meropenem and cefepime (81.82%) (Cao et al., 2020). When compared to this study, it was observed to have good susceptibility in most of these antibiotics with exceptions being ciprofloxacin (42.1%) and cefepime (30.0%). However, both studies show high resistance to β-lactam class antibiotics. Another study which was done in Mymensingh, Bangladesh on the public surface of buses and bus stations shows 100% resistance to amoxicillin. (Biswas et al., 2024).This was also observed in this study as high resistance was observed in amoxicillin (85%).

E. Coli has shown high resistance to erythromycin (100%), tetracycline (91.67%), cefixime (83.33%). In a similar study done in Mymensingh, 100% resistance was shown in tetracycline. (Biswas et al., 2024). Complete resistance to macrolides is also observed. A study done in Mekella city, Tigray, Ethiopia on the bus surface of that city showed high resistance to tetracycline (75%) (Kahsay, et al., 2019) which is similar to this study. However complete susceptibility was observed for erythromycin. Chloramphenicol was found to be highly resistant to about 75%. (Kahsay et al., 2019). However in this study it was significantly low to 25%. Ciprofloxacin was found to be 37.5% resistance (Kahsay et al., 2019) compared to this study which was 66.7%.

In this study *Vibrio* spp. Has shown complete resistance to erythromycin, amoxicillin and meropenem (100%). For cefepime it has shown 75% resistance. No published study was found on antibiotic susceptibility test results of *Vibrio* spp. for public bus or public surface. However when comparing the results to public waterbodies studies in Nigeria, 86-100% resistance in erythromycin is observed. 50% resistance in amoxicillin was found. (Adesiyan, et al., 2021).

No data were available for *A. baumannii* specifically from public bus surfaces or public transports. Even though one specific study shows the presence of this organism in various public places in urban areas like shopping carts, ATMs, playground slides and various other areas where it's frequently touched, (Ababneh et al., (2022) no antibiotic susceptibility testing was reported.

In this study, *Enterococcus* spp. from public bus surface, it was found 100% resistance to ampicillin and penicillin. 66.7% resistance to vancomycin and linezolid was observed. In another study done in Bangladeshi restaurants (Shadique et al., 2025), it shows 100% resistance to penicillin. It also showed moderate resistance to tetracycline (44.4%),

ciprofloxacin (33.3%) and erythromycin (33.3%). In a study done in Nigeria on door handles being a possible reservoir of drug resistance *Enterococci* (Otokunefor et al., 2020), high levels of resistance can be seen in many β-lactam antibiotics. Resistance to aminoglycosides like gentamicin and fluoroquinolones was found low in both studies. This comparison shows that *Enterococcus* spp. Resistance towards β-lactam antibiotics are extremely concerning.

In this study, *Salmonella* spp. isolated from public bus surfaces showed 0% resistance to ciprofloxacin, ceftriaxone, tetracycline, trimethoprim–sulfamethoxazole, imipenem, and meropenem, while resistance to azithromycin was observed in one isolate. In contrast, a study conducted on public bus surfaces in Mekelle city, Ethiopia reported *Salmonella* spp. showing 100% resistance to ciprofloxacin, cefepime, and aztreonam (Kahsay et al., 2019). The complete susceptibility of *Salmonella* spp. to ciprofloxacin and carbapenems observed in the present study differs from the Ethiopian bus surface study. *Salmonella* species showed resistance to chloramphenicol at 28.7% (6), 61.9% to ceftriaxone. (Sultan et al., 2025).

A study done in Dhaka, where samples were collected from different sources, analyzing the susceptibility of *P. aeruginosa* (Bhuiya et al., 2018). indicate that *P. aeruginosa* isolates from environmental sources showed complete resistance (100 %) to penicillin, ampicillin, and cefixime, while 90 % were resistant to cefpodoxime. Many isolates displayed intermediate resistance to other cephalosporins: 90 % to cefotaxime, 70 % to ceftriaxone, 20 % to aztreonam, and 10 % to cefpodoxime. All isolates were fully sensitive to many antibiotics, including levofloxacin, tobramycin, gentamicin, imipenem, piperacillin, amikacin, ciprofloxacin, meropenem, and ceftazidime. Partial sensitivity was observed for aztreonam (80 %), ceftriaxone (30 %), and cefotaxime (10 %). Comparing this with the data in this study, environmental *P. aeruginosa* isolates were 100 % resistant to ceftazidime and aztreonam, while remaining fully susceptible to ciprofloxacin, levofloxacin, piperacillin, imipenem, meropenem, and amikacin. This pattern is consistent with the data from

environmental samples in Dhaka, as both studies show high resistance to some β-lactams and strong susceptibility to fluoroquinolones, aminoglycosides, and carbapenems.

In this study, *S. aureus* isolates showed resistance to many commonly used antibiotics. Resistance to tetracycline, levofloxacin, and moxifloxacin was 28.6%, while clindamycin, cefoxitin, and penicillin G exhibited higher resistance levels of 42.9%. Azithromycin resistance was comparatively lower at 14.3%, and resistance to linezolid was 0%. Nitrofurantoin and chloramphenicol showed 0% resistance. Similarly, the study on bacteria isolated from door handles of the University of Benin Teaching Hospital, Benin City, Nigeria, (Otokunefor et al., 2020) reported very low resistance to gentamicin (0%) and streptomycin (2%). Moderate resistance was observed for fluoroquinolones such as ciprofloxacin (24%) and ofloxacin (22%). Higher resistance rates were recorded for beta-lactam antibiotics, including ampicillin (34%) and cephalexin (46%), with the highest resistance observed against nalidixic acid (89%). Overall, both studies demonstrate a similar resistance trend in which commonly used antibiotics, particularly beta-lactams, show increased resistance, whereas antibiotics with more restricted use showed more susceptibility.

In this study, *S. epidermidis* resistance was highest to penicillin (100%) and cefoxitin (100%), azithromycin (66.7%) and linezolid (33.3%). Antibiotics such as moxifloxacin, gentamicin, doxycycline, levofloxacin, and trimethoprim–sulfamethoxazole showed 0% resistance. In comparison, a study of *Staphylococcus* isolates from public transportation surfaces like buses and trains in Portland, Oregon (Yeh et al., 2011) found that among environmental *Staphylococcus* spp., about 45% of isolates were resistant to penicillin or ampicillin, while all isolates were sensitive (0%) to trimethoprim–sulfamethoxazole, vancomycin, and tetracycline, indicating that clinically important resistance to β-lactams was common in both settings, whereas susceptibility to drugs like trimethoprim–sulfamethoxazole and tetracycline remained high.

4.1 Limitations

Only two samples from acrylic paint covered surfaces were collected which is very less to be able to draw a good conclusion regarding the microbial growth on acrylic paint cover surfaces.

4.2 Future Prospects

In the future, the study will be focusing on detection of virulent genes and serum resistance in the target organism found in the samples. Moreover, the number of samples will also be increased, with samples being collected from every major bus route within Dhaka city. Samples will be taken from air conditioner (AC) buses in order to study the comparison between samples taken from AC and non-AC buses.

Chapter 5

Conclusion:

This study focused on the bacteriological quality and antimicrobial resistance patterns of bacteria isolated from public bus seats, grab rails, and bus stop surfaces. Standard culture techniques and antibiotic susceptibility testing were used to identify important bacteria which can cause public health concerns. Several multidrug resistant organisms were detected, including *E. coli*, *K. pneumoniae*, *Staphylococcus* spp., and others, indicating that frequently touched public transport surfaces can act as reservoirs of resistant bacteria. The resistance patterns observed in this study highlight a potential risk of environmental transmission of antimicrobial resistant bacteria among daily commuters traveling across the city. Similar to findings reported in other environmental studies, higher resistance was observed against commonly used antibiotics on the other hand better sensitivity was seen with some advanced antibiotics. The presence of resistant bacteria on public transport surfaces emphasizes the importance of regular cleaning, use of proper material to inhibit the growth of microbes, public hygiene awareness, and proper antimicrobial use policies. Overall, this study highlights very important local data which supports the need for improved surveillance of antimicrobial resistance organisms in public spaces in Bangladesh.

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