Assessment of Airborne Bacterial Pathogens and Antimicrobial Resistance in Selected Urban Areas of Dhaka City

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfilment of the requirements for the degree of
Bachelor's in Microbiology

Department of Mathematics and Natural Sciences, Microbiology Program
BRAC University
December' 2025

Declaration

It is hereby declared that,

1. The thesis submitted is my/our own original work while completing a 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. I have acknowledged all main sources of help.

Student's Full Name & Signature:

_____

Student Full Name

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Approval

The thesis titled "Assessment of Airborne Bacterial Pathogens and Antimicrobial Resistance in Selected Urban Areas of Dhaka City"

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Ethics Statement

This research was conducted under proper supervision and is the original work of the author. The thesis does not contain any previously published or third-party material without appropriate citation and accurate referencing. Also, for this research, none of human or animal samples were not taken. For this reason, no ethical clearance is needed. Air samples were collected from a safe place inside different Dhaka cities. All procedures were carried out following proper safety protocols and in accordance with the rules and regulations of the Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University.

Abstract

Airborne microorganisms in urban environments pose a substantial but often neglected public health risk, particularly in densely populated cities such as Dhaka, where intense human activity, traffic congestion, and inadequate environmental controls can facilitate the spread of pathogenic bacteria. This study investigated the presence, spatial distribution, and antimicrobial resistance patterns of selected airborne bacterial pathogens across ten locations in Dhaka city, including Badda, Khilgaon, Aftabnagar, Mohakhali, Mirpur-10, Mirpur-12, Hatirjheel, Farmgate, Panthapath, and TB Gate Quarter. Air samples were analyzed for *Pseudomonas spp., Klebsiella spp., Staphylococcus aureus, Acinetobacter baumannii, and Escherichia coli* using selective culture methods followed by molecular confirmation through DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis, yielding characteristic amplicons of 618 bp, 130 bp, 279 bp, 353 bp, and 585 bp, respectively. The distribution of airborne bacteria varied notably among sites, with Khilgaon showing the highest diversity of detected pathogens, while Hatirjheel samples showed no detectable target organisms. Antibiotic susceptibility testing revealed an alarming prevalence of multidrug resistance among all isolates, as *Staphylococcus aureus* exhibited 100% resistance to all tested antibiotics, *E. coli and Klebsiella pneumoniae* showed complete resistance to most antibiotics with only partial sensitivity to nitrofurantoin and trimethoprim, and both *Pseudomonas spp.* and *A. baumannii* demonstrated extensive resistance, remaining mainly susceptible to last-resort antibiotics such as colistin and selected fluoroquinolones. Overall, the findings highlight the presence of multidrug-resistant airborne bacterial pathogens in Dhaka's urban air and underscore the urgent need for routine air quality surveillance and strengthened public health and antimicrobial resistance control strategies.

Keywords: Dhaka city; Hatirzhil; Mirpur10; Bacteria etc.

Dedication

I would also like to dedicate this thesis to my family and friends whose support, encouragement, and cherishment have been my biggest strength during this journey.

Acknowledgment

To begin with, I would like to extend my sincere thanks to Almighty Allah, providing me with the strength and endurance to accomplish my research thesis.

I would like to thank my parents with all my heart for supporting me, encouraging and believing in me all the time. I would also like to extend my profound thanks to the study participants who dedicated their time and knowledge to this research willingly and made it much more qualitative.

I would like to thank our respected supervisor, Dr. Professor Fahim Kabir Monjurul Haque, the most, because he agreed to our proposal and allowed us to do the research in his lab. I have benefited immensely through his mentorship and sponsorship.

I would also like to express extra gratitude to Ms. Amena Begum Rinji, Research Assistant, who was always helpful and cooperative at all steps of the research. I would like to thank the International Scholarship Office (ISO) of BRAC University for providing me with this opportunity to study and develop in such a caring academic atmosphere.

And the last thing I want to mention is the devotion and effort of my thesis group members. They all had a crucial role to play, and their presence and efforts deserved a lot of thanks.

Table of Contents

List of Figures:

List of Tables:

List of Acronyms

AST	Antibiotic Susceptibility Testing
AMR	Antimicrobial Resistance
BFSA	Bangladesh Food Safety Authority
BSTI	Bangladesh Standards and Testing Institution
CFU	Colony Forming Unit
DA	Clindamycin
E. coli	Escherichia coli
K. pneumoniae	Klebsiella pneumoniae
PCR	Polymerase Chain Reaction
UV	Ultraviolet
AK	Amikacin (Antibiotic Disc Code)
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
BFSA	Bangladesh Food Safety Authority
bp	Base Pair
CAZ	Ceftazidime
CIP	Ciprofloxacin
CPM	Cefepime
CRO	Ceftriaxone
DNA	Deoxyribonucleic Acid

E. coli	Escherichia coli
EDTA	Ethylenediaminetetraacetic Acid
F	Nitrofurantoin (Antibiotic Disc Code)
KPC	Klebsiella pneumoniae Carbapenemase
LE	Levofloxacin
MAC	MacConkey Agar
MEM	Meropenem
mm	Millimeter
mL	Milliliter
NOR	Norfloxacin
PCR	Polymerase Chain Reaction
TAE	Tris-Acetate-EDTA
TGC	Tigecycline
UTI	Urinary Tract Infection
UV	Ultraviolet
WHO	World Health Organization
°C	Degree Celsius

Chapter 1

Introduction

1.1 Background of the Study

Air quality in urban environments is a critical determinant of public health, particularly in densely populated cities such as Dhaka, where rapid urbanization, heavy traffic congestion, industrial activities, and inadequate environmental management have significantly deteriorated ambient air conditions. In addition to chemical pollutants and particulate matter, urban air also serves as a carrier for a wide range of microorganisms, including bacteria that may pose serious risks to human health. Airborne bacteria can remain suspended in the atmosphere for extended periods and may be inhaled by individuals during routine daily activities, thereby facilitating the transmission of infectious agents.

Dhaka is characterized by high population density, constant human movement, construction activities, and limited green spaces, all of which contribute to increased microbial load in the air. Public places such as busy roads, residential areas, commercial hubs, and transportation zones provide favorable conditions for the dissemination of pathogenic microorganisms. Exposure to contaminated air has been associated with respiratory tract infections, allergic reactions, and opportunistic infections, particularly among children, elderly individuals, and immunocompromised populations. Despite these risks, microbiological assessment of ambient air remains an underexplored area of environmental health research in Bangladesh.

Several clinically significant bacteria, including *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter baumannii*, and *Escherichia coli*, are known to survive in

airborne form and are frequently implicated in respiratory infections and hospital-acquired infections. These organisms are of particular concern due to their ability to develop resistance to multiple antibiotics, thereby complicating treatment outcomes and increasing healthcare burdens. Airborne transmission of such pathogens may act as an overlooked route for their spread within communities and healthcare settings.

Although numerous studies have focused on water, food, and surface contamination in Dhaka, limited attention has been given to the microbiological quality of ambient air across different urban locations. The lack of routine monitoring and regulatory frameworks for airborne microbial contamination further exacerbates the problem. Understanding the distribution and diversity of airborne bacteria in various parts of Dhaka is therefore essential for evaluating potential health risks and for developing strategies to improve urban environmental health.

1.2 Rationale and Problem Statement

The increasing prevalence of respiratory illnesses, combined with the growing threat of antimicrobial resistance (AMR), underscores the importance of investigating airborne bacterial contamination in urban settings. In Dhaka, constant exposure to polluted air is an unavoidable part of daily life, yet the microbial component of air pollution remains largely unaddressed. Airborne bacteria originating from human activities, waste disposal, vehicular emissions, and environmental disturbances may serve as reservoirs for pathogenic and drug-resistant organisms.

Hospital-acquired pathogens such as *Acinetobacter baumannii*, *Klebsiella* spp., and *Pseudomonas* spp. have been increasingly reported in clinical settings, raising concerns about their circulation beyond hospital environments. The presence of these organisms in ambient air could facilitate their

spread among the general population, increasing the risk of infection and contributing to the dissemination of antibiotic-resistant strains. Despite the seriousness of this issue, there is a scarcity of data regarding the occurrence of these pathogens in the outdoor air of Dhaka.

This study was undertaken to address this gap by investigating airborne bacterial contamination across selected areas of Dhaka city. By identifying pathogenic bacteria and assessing their distribution, the study seeks to highlight a significant but overlooked public health concern and to emphasize the need for improved environmental monitoring and preventive measures.

1.3 Objectives of the Study

The primary objective of this study was to investigate the presence of selected pathogenic bacteria in ambient air samples collected from different locations in Dhaka city and to assess their potential public health implications.

The specific objectives of the study were:

- To detect the presence of airborne bacterial pathogens, including *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli*, in selected urban areas of Dhaka.
- To confirm the identity of the isolated bacteria using molecular techniques such as polymerase chain reaction (PCR) and gel electrophoresis.
- To analyze the spatial distribution of airborne bacteria across different locations within the city.

- To raise awareness regarding the public health risks associated with airborne bacterial contamination, particularly in relation to respiratory infections and antimicrobial resistance.

1.5 Significance of the Study

This study holds significant importance in the context of public health, environmental microbiology, and urban air quality management in Dhaka. By providing baseline data on airborne bacterial contamination, the findings contribute to a better understanding of the potential health risks associated with daily exposure to polluted urban air. The detection of clinically important and potentially drug-resistant bacteria highlights the need for integrating microbiological parameters into routine air quality monitoring programs.

Moreover, the study draws attention to the role of ambient air as a possible transmission route for respiratory and hospital-associated pathogens, which is particularly relevant in densely populated cities. The outcomes of this research may assist policymakers, public health authorities, and researchers in developing preventive strategies, improving urban planning, and promoting awareness regarding airborne microbial hazards. Additionally, the study serves as a foundation for future research on antimicrobial resistance and environmental dissemination of pathogenic bacteria in Bangladesh.

1.6 Scope and Limitations

This study investigated the presence of selected pathogenic bacteria (*Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli*) in ambient air samples collected from ten locations in Dhaka city using culture-based and molecular techniques.

Sampling was conducted only during the winter season and within a fixed daytime period (approximately 11:00 AM to 2:00 PM). As a result, seasonal and diurnal variations in airborne bacterial distribution were not assessed. Despite these limitations, the study provides baseline data on airborne bacterial contamination in selected urban areas of Dhaka.

Chapter 2

Literature Review

2.1 Airborne Microorganisms and Urban Environments

Airborne microorganisms are an important but often underestimated component of urban air pollution. Bacteria, fungi, and viruses can become aerosolized through human activities, vehicular movement, construction, waste handling, and resuspension of contaminated dust particles (Bowers et al., 2011). In densely populated megacities such as Dhaka, high population density, inadequate sanitation, and heavy traffic contribute significantly to microbial loading in ambient air (Haque et al., 2020).

Airborne bacteria may originate from soil, water bodies, sewage, human skin, respiratory secretions, and waste disposal sites (Burrows et al., 2009). Once aerosolized, these microorganisms can remain suspended for extended periods and be transported across urban environments, increasing the risk of inhalation exposure. Several studies have demonstrated that outdoor air in urban settings frequently contains opportunistic and pathogenic bacteria capable of causing respiratory and systemic infections, particularly among immunocompromised individuals (Gandolfi et al., 2015).

2.2 Common Airborne Bacterial Pathogens

Among airborne bacterial pathogens, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas spp.*, and *Acinetobacter spp.* are frequently reported in urban air samples (Fang et al., 2018). Although *E. coli* is primarily an enteric organism, its presence in air indicates fecal contamination and poor environmental hygiene (World Health Organization [WHO], 2023). Similarly, *Klebsiella pneumoniae* is an opportunistic pathogen associated with pneumonia, urinary tract infections, and bloodstream infections and is increasingly detected in non-clinical environments (Podschun & Ullmann, 1998).

Studies conducted in South Asian cities have reported the presence of Gram-negative bacteria in outdoor air near markets, hospitals, and traffic intersections, highlighting environmental dissemination pathways (Begum et al., 2019). The ability of these organisms to survive environmental stress, including desiccation and ultraviolet radiation, enables them to persist in air and dust particles (Tong & Lighthart, 2000).

2.3 Antimicrobial Resistance in Environmental Air

Antimicrobial resistance (AMR) is no longer confined to healthcare settings and has become a growing environmental concern. Airborne dissemination of antibiotic-resistant bacteria allows resistance genes to spread beyond hospitals into the community (Allen et al., 2010). Environmental isolates of *E. coli* and *K. pneumoniae* have been shown to carry resistance against commonly used antibiotics, including β-lactams, fluoroquinolones, and aminoglycosides (Berendonk et al., 2015).

Urban air acts as a potential vector for resistant bacteria due to the mixing of emissions from hospitals, wastewater systems, and densely populated residential areas (Pal et al., 2016). The World Health Organization has identified environmental AMR surveillance as a critical component of the global strategy to combat antimicrobial resistance (WHO, 2022). The inhalation of resistant bacteria may contribute to asymptomatic colonization, which can later lead to difficult-to-treat infections.

2.4 Air Quality and Public Health in Dhaka

Dhaka consistently ranks among the most polluted cities in the world, with air quality affected by construction activities, traffic congestion, industrial emissions, and waste mismanagement (World Bank, 2022). While most air quality studies in Bangladesh focus on chemical pollutants such as particulate matter ($PM_{2.5}$ and PM_{10}), microbial air pollution has received limited attention.

Existing studies indicate that poor urban sanitation, open drains, and unmanaged waste significantly increase the microbial burden of air in Dhaka (Islam et al., 2017). Continuous exposure to contaminated air may increase the incidence of respiratory infections, allergic reactions, and the spread of opportunistic pathogens. The presence of antibiotic-resistant bacteria in ambient air further amplifies the public health risk, especially in a city with high antibiotic misuse and limited regulatory enforcement.

2.5 Research Gaps and Justification

Although several international studies have investigated airborne bacterial contamination and AMR, data from Bangladesh remain scarce. Most local studies focus on food, water, or clinical samples, with minimal emphasis on airborne transmission pathways. There is a significant lack of molecular-level confirmation of airborne pathogens and their resistance profiles in urban Bangladeshi environments.

This study addresses these gaps by isolating airborne bacterial pathogens from selected urban areas of Dhaka, confirming their identity using molecular techniques, and evaluating their antimicrobial resistance patterns. The findings aim to contribute baseline data for environmental AMR surveillance and support public health interventions targeting urban air quality and infection control.

Chapter 3

Materials and Methods

3.1 Study Area and Study Design

This study was conducted to investigate the presence of selected airborne bacterial pathogens in different areas of Dhaka city. An experimental laboratory-based study design was followed. Air samples were collected from selected urban locations using the settle plate technique and subsequently analyzed using selective culture media and molecular methods. All laboratory analyses were carried out under aseptic conditions in a microbiology laboratory following standard biosafety guidelines. Our Study area was Badda, Khilgaon, Aftabnagar, Mohakhali, Mirpur-10, Mirpur-12, Hatirjheel, Farmgate, Panthapath, and TB Gate Quarter.

3.2. Antifungal Stock Solution Preparation

The stock solution was prepared using Xpose (100 mg antifungal). One tablet was pulverized into a fine powder using a sterile mortar and pestle. The resulting powder was then dissolved in 10 mL of Dimethyl Sulfoxide (DMSO) to create a stock concentration of 10 mg/mL. This solution was stored in a sterile, light-protected container to maintain stability.

3.3. Media Supplementation and Plating

The antifungal stock was added to the growth media (e.g., Nutrient Agar or Tryptic Soy Agar) after sterilization but before the agar solidified (approximately 45–50°C). For every 20 mL of agar per plate, 0.5 mL of the antifungal stock solution was aseptically incorporated.

3.4 Air Sample Collection by Settle Plate Technique

Air sampling was performed using the passive settle plate technique. Sterile selective agar plates were exposed to the surrounding air at approximately 3 feet above ground level, which represents the average human breathing zone.

The following selective media were used for targeted isolation:

- Cetrimide agar for *Pseudomonas spp.*

- Mannitol Salt Agar (MSA) for *Staphylococcus aureus*

- MacConkey agar for *Klebsiella spp.* and *Escherichia coli*

- Leeds Medium for *Acinetobacter spp.*

Each agar plate was exposed to the air for 1 hour without disturbance. After exposure, plates were immediately covered, properly labeled, and transported to the laboratory for incubation.

3.5 Incubation and Preliminary Identification

All exposed plates were incubated at 37°C for 24 hours. After incubation, colonies were examined based on growth characteristics, color, shape, and morphology according to standard microbiological descriptions.

Presumptive identification was performed as follows:

- *Pseudomonas spp.* on cetrimide agar showed greenish or grey-colored colonies

- *Staphylococcus aureus* on MSA showed yellow, round colonies

- *Klebsiella spp.* on MacConkey agar showed large, round, pink colonies

- *Escherichia coli* on MacConkey agar showed small, dark pink colonies

- *Acinetobacter spp.* on Leeds agar showed orange-colored colonies

3.6 Subculturing on Selective Media

Suspected colonies were further processed by direct subculturing onto fresh selective media, without streak plate isolation.

The subculturing scheme was as follows:

- Suspected *Pseudomonas spp.* colonies were subcultured from cetrimide agar to cetrimide agar

- Suspected *Staphylococcus aureus* colonies were subcultured from MSA to MSA

- Suspected *Klebsiella spp.* colonies from MacConkey agar were subcultured onto KPC agar

- Suspected *Escherichia coli* colonies from MacConkey agar were subcultured onto EMB agar

- Suspected *Acinetobacter spp.* colonies were sub cultured from Leeds agar to Leeds agar

3.7 DNA Extraction

Genomic DNA was extracted from confirmed bacterial colonies using a boiling method, which was applied uniformly for all isolates. A single bacterial colony was transferred into a sterile microcentrifuge tube containing 400 µL of TE buffer. The suspension was mixed thoroughly by

vortexing. Centrifuge the mixture at 13000rpm at 10min. Discard the supernatant. Mixing the 400 µL TE buffer. Then vortex it for 15 seconds. After that the tubes were then put at 100°C for 10 minutes in water bath to lyse the bacterial cells. After heat treatment, the tubes were cooled to room temperature and centrifuged at 13,000 rpm for 10 minutes. The supernatant containing genomic DNA was carefully transferred 200µLinto a new sterile microcentrifuge tube. Extracted DNA was either used immediately for PCR or stored at −20°C until further use.

3.8 Polymerase Chain Reaction (PCR)

PCR was performed for molecular confirmation of the targeted bacteria using species-specific primers.

The primers used in this study were:

- KP F and R for *Klebsiella spp.*
- NUC F and R for *Staphylococcus aureus*
- A.B OXA F and R for *Acinetobacter spp.*
- PAGS F and R for *Pseudomonas spp.*
- Eco F and R for *Escherichia coli*

PCR reactions were carried out in appropriate reaction volumes following standard PCR protocols. Amplification was conducted using a thermal cycler under optimized cycling conditions suitable for each primer set.

Supplementary Table 1: Primers and PCR Conditions used in this Study:

Name of Bacteria	Primer Designation	Primer Sequence (5' to 3')	Product Size	PCR Conditions
Klebsiella pneumoniae	KPN-F KPN-R	TGCAGATAATTCACGCCCAG ACCCGCTGGACGCCAT	133 bp	Initial 94°C for 10 min; 30 cycles of (94°C for 30s, 60°C for 45s, 72°C for 45s); final extension 72°C for 10 min.

Staphylococcus aureus	nuc-F nuc-R	GCGATTGATGGTGGATACGGT AGCCAAGCCTTGACGAACTAAAGC	279 bp	Initial denaturation at 95°C for 5 min; 30 cycles of (95°C for 1 min, 55°C for 45s, 72°C for 1 min); final extension at 72°C for 10 min.
Acinetobacter baumannii	bla OXA-51-F bla OXA-51-R	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	353 bp	Initial denaturation at 94°C for 5 min; 30 cycles of (94°C for 30s, 58°C for 30s, 72°C for 30s); final extension 72°C for 10 min.
Escherichia coli	ECO-F ECO-R	GACCTCGGTTTAGTTCACAGA CACACGCTGACGCTGACCA	585 bp	Initial denaturation at 95°C for 5 min; 35 cycles of (94°C for 45s, 45°C for 45s, 72°C for 1 min); final extension at 72°C for 5 min.

Pseudomonas spp.	PA-GS-F PA-GS-R	GACGGTCAGGAGATGATCC CGCCCTCCCACACGGTGGTC	618 bp	Initial denaturation at 95°C for 5 min; 30 cycles of (94°C for 30s, 55°C for 30s, 72°C for 30s); final extension 72°C for 10 min.

3.9 Agarose Gel Electrophoresis

A 1% agarose gel was prepared by dissolving 1 g of agarose powder in 98 mL of distilled water and 2 mL of 1X TAE working solution. The mixture was heated until the agarose completely dissolved and then cooled to about 50–60°C. Ethidium bromide (4 µL) was added to the cooled agarose solution for DNA visualization. The agarose solution was poured into a gel casting tray with combs to form wells and allowed to solidify at room temperature for 30–45 minutes. The gel was then placed in the electrophoresis tank and covered with a 1X TAE buffer. DNA samples and a DNA ladder were loaded into the wells, and electrophoresis was run at 100 volts for 40 minutes. After completion, DNA bands were visualized under a UV Tran illuminator and documented.

3.10 Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility of the isolated strains was evaluated using the Kirby-Bauer disk diffusion method. A panel of antibiotics was used for both Escherichia coli and Klebsiella pneumoniae, with results recorded based on the diameter of inhibition zones.

Table 2: The following antibiotics were applied:

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
1	Aztreonam	Monobactam	Gram-negative rods (Enterobacterales, Pseudomonas)	ATM	30	≥21	18–20	≤17
2	Cefazolin	1st-gen Cephalosporin	Gram-positive cocci, limited Gram-negative rods	CZO	30	≥23	20–22	≤19
3	Cefepime	4th-gen Cephalosporin	Gram-positive and Gram-negative	FEP	30	≥25	19–24	≤18
4	Cefoxitin	Cephamycin	Gram-negative rods; MRSA screening	FOX	30	≥22	15–21	≤14
5	Ceftazidime	3rd-gen Cephalosporin	Pseudomonas, Gram-negative rods	CAZ	30	≥18	15–17	≤14
6	Ceftriaxone	3rd-gen Cephalosporin	Broad (Gram-negative & positive)	CRO	30	≥21	14–20	≤13
7	Clindamycin	Lincosamide	Gram-positive cocci, anaerobes	DA	2	≥21	15–20	≤14
8	Colistin	Polymyxin	MDR Gram-negative rods	CL	10	≥11	–	≤10
9	Erythromycin	Macrolide	Gram-positive cocci	E	15	≥23	14–22	≤13
10	Gentamicin	Aminoglycoside	Gram-negative & some Gram-positive	CN	10	≥15	13–14	≤12
11	Levofloxacin	Fluoroquinolone	Broad-spectrum	LEV	5	≥17	14–16	≤13
12	Linezolid	Oxazolidinone	Gram-positive (MRSA, VRE)	LZD	30	≥23	21–22	≤20
13	Nitrofurantoin	Nitrofuran	Urinary pathogens (E. coli, Enterococcus)	NIT-F	300	≥17	15–16	≤14
14	Piperacillin–Tazobactam	Ureidopenicillin + β-lactamase inhibitor	Gram-negative incl. Pseudomonas	TZP	100/10	≥21	18–20	≤17
15	Trimethoprim – Sulfamethoxazole	Folate pathway inhibitor	Gram-positive & Gram-negative	SXT	1.25/23.75	≥16	11–15	≤10
16	Vancomycin	Glycopeptide	Gram-positive cocci (Staph, Enterococcus)	VA	30	≥15	–	≤14

Chapter 4

Results

4.1 Sample taken from media

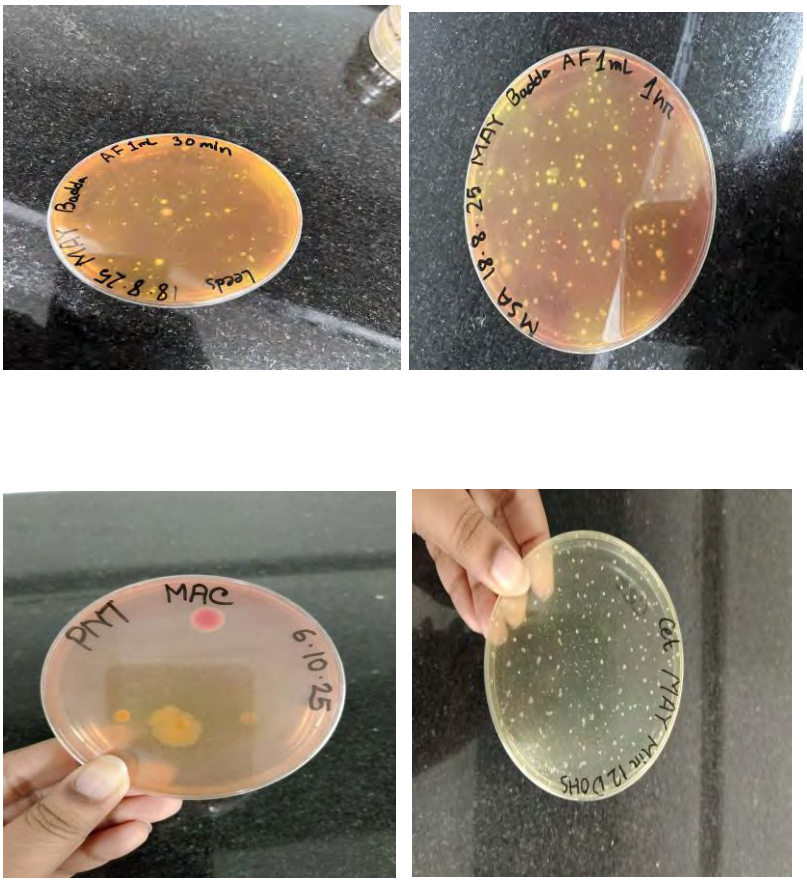
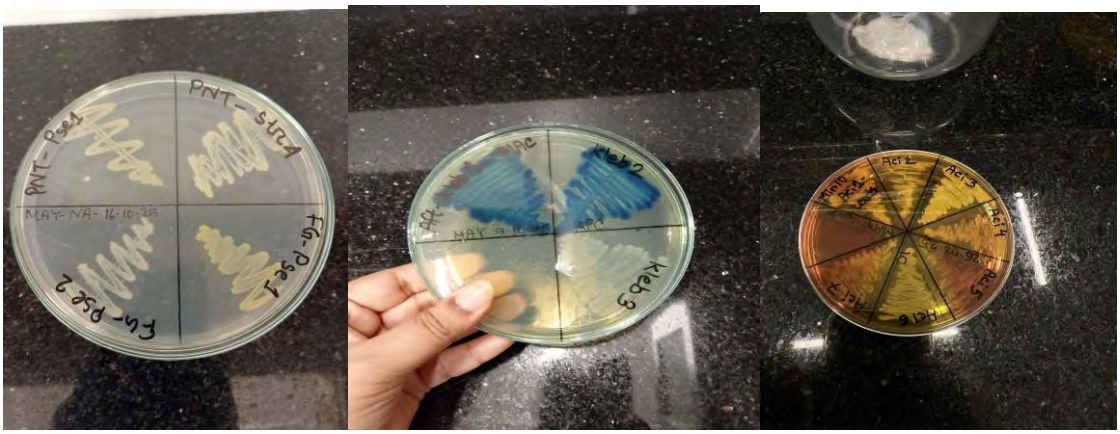


Figure 1: Mackonkey Agar (Mac), Manitol Salt Agar (Msa), Cetimide, Leeds agar plates for sample collection.

4.2 Colony Morphology and Selective Media Growth

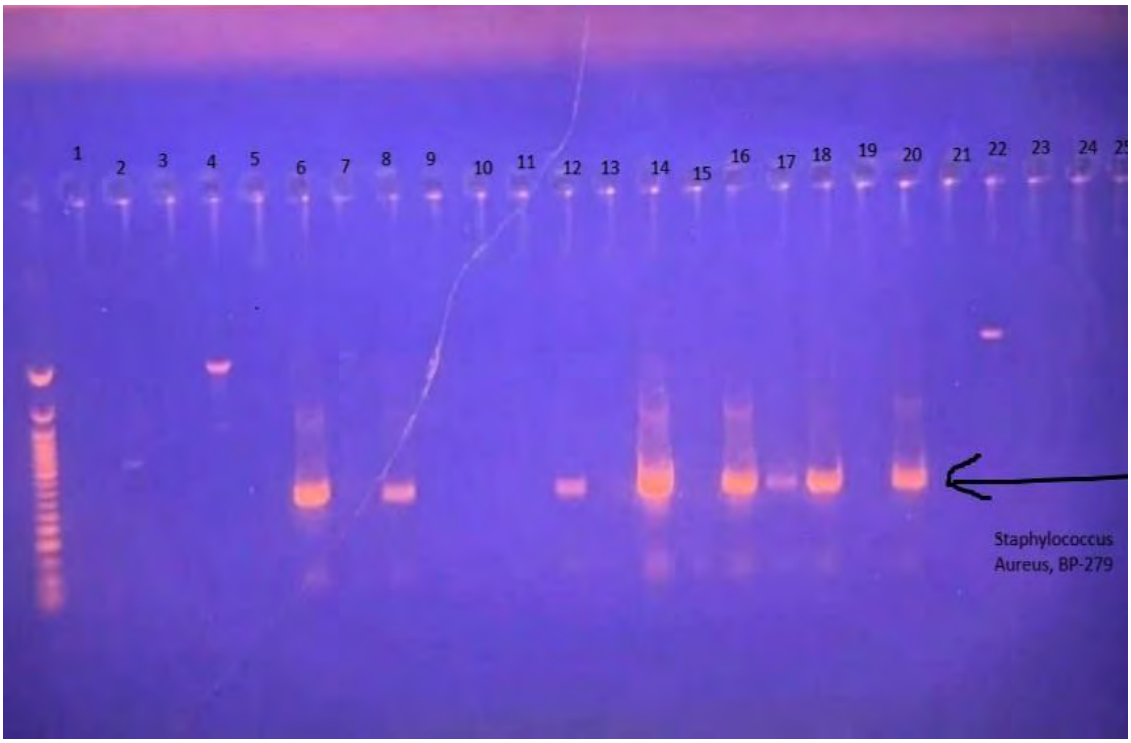


A **B** **C**

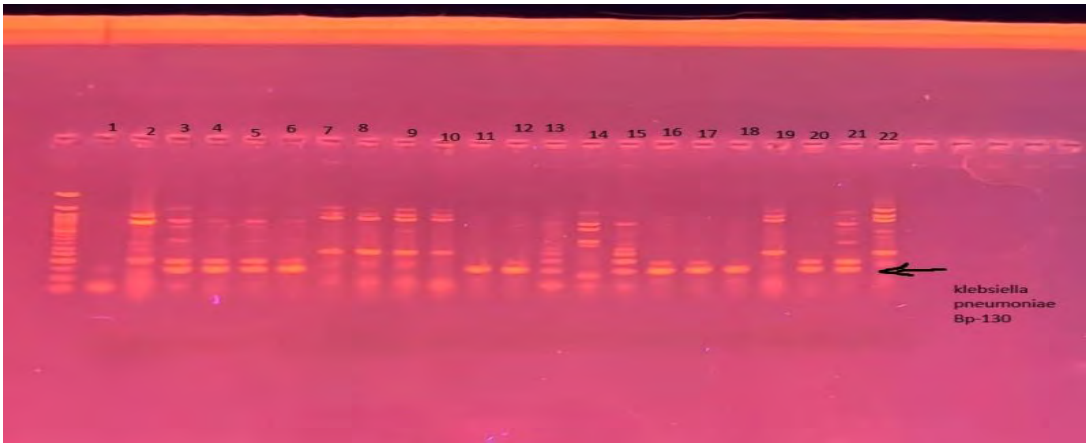
D **E**

Figure 2: Image A shows the Isolated *Pseudomonas Species* streaked on Na Media. Image B shows the isolated colonies of *K. pneumoniae* on Kpc media. Image C shows the isolated colonies of *Acinetobacter Baumannii* on Leeds media. Image D shows the isolated colonies of *E. coli* on EMB media. Image E shows the isolated colonies of *Staphylococcus Aureus* on MSA Media.

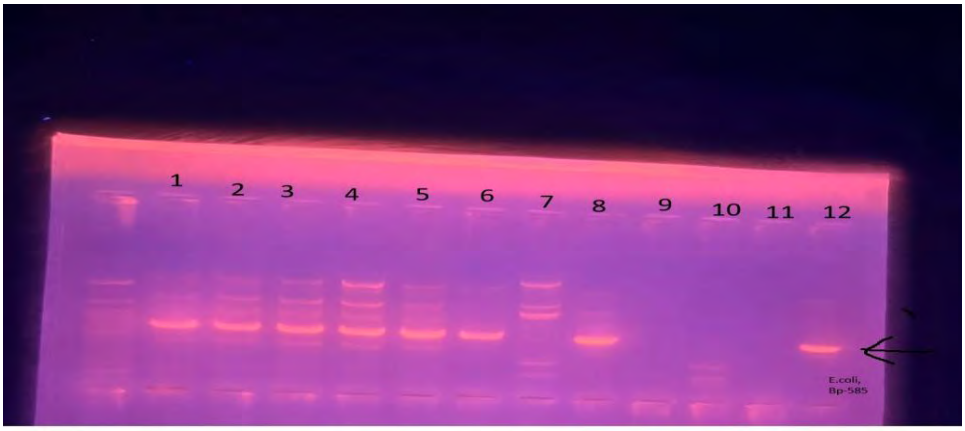
4.3 Identification of microbes (*K. pneumoniae* and *E. coli*, *Pseudomonas Species*, *Acinetobacter Baumannii, Staphylococcus Aureus*) molecular confirmation by Agarose Gel Electrophoresis



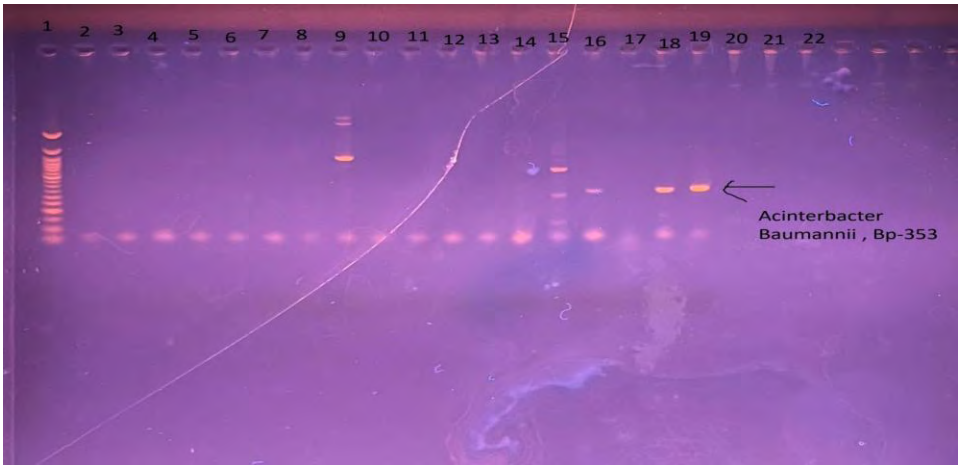
A


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
klebsiella
pneumoniae
Bp-130

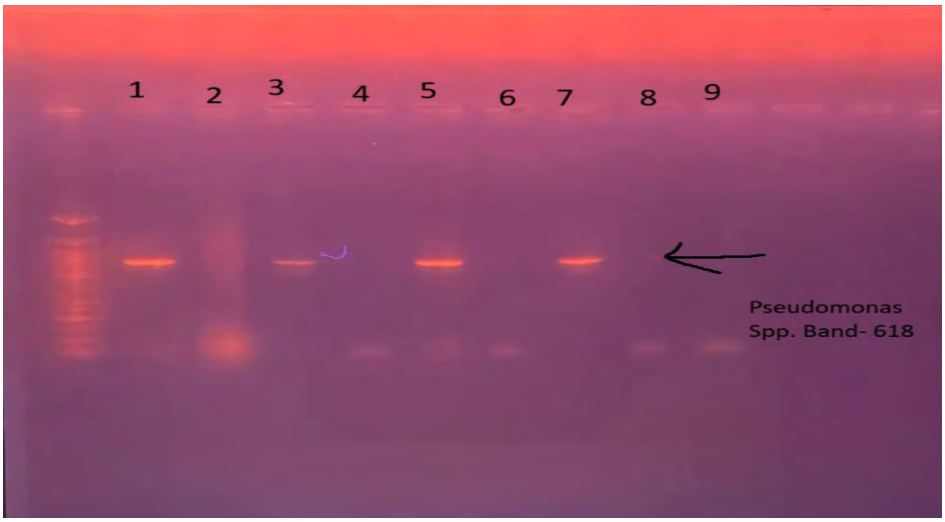
B


1 2 3 4 5 6 7 8 9 10 11 12
E.coli,
Bp-585

C



D



E

Figure 3: Image A shows *Staphylococcus Aureus* 279 bp, Image B shows *K. pneumoniae* 130 bp. Image C shows *E. coli* at 585bp, Image D shows *Acinetobacter Baumannii* at 353 bp. Image E shows *Pseudomonas spp.* at 618 bp.

Table1

Place	Pseudomonas (Pse)	Klebsiella (Klb)	Staphylococcus (Stp)	Acinetobacter (Aci)	E. coli
Badda		Present			
Khilgaon	Present	Present	Present		Present
Aftabnagar		Present	Present		Present
Mohakhali	Present				
Mir 10	Present			Present	
Mir 12			Present		
Hithirjhil					
Farmgate			Present		
Parthapath			Present		
TB Gate					Present

Figure 4: Bacteria that have been found from each Area**.**

4.4 Antibiotic Resistance Patterns

Analysis of Antibiotic Resistance Patterns

The antibiotic resistance patterns of five clinically important bacterial pathogens—*Klebsiella pneumoniae, Pseudomonas* spp., *Escherichia coli, Staphylococcus aureus,* and *Acinetobacter baumannii*—were analyzed based on susceptibility testing of three isolates per organism (n = 3). Resistance was scored as 1, intermediate susceptibility as 0.5, and sensitivity as 0. The results reveal organism-specific resistance profiles with notable multidrug resistance trends among Gram-negative pathogens.

1. *Klebsiella pneumoniae*

Klebsiella pneumoniae demonstrated a mixed resistance pattern. Complete resistance (score = 1) was observed against gentamicin, indicating poor efficacy of this aminoglycoside. Intermediate resistance (score = 0.5) was recorded for trimethoprim and nitrofurantoin, suggesting reduced susceptibility. In contrast, the isolates remained fully sensitive (score = 0) to cefepime, aztreonam, ceftriaxone, ceftazidime, levofloxacin, and piperacillin–tazobactam.

This pattern suggests that while several β-lactam antibiotics and fluoroquinolones remain effective, resistance to gentamicin may limit treatment options, especially in severe infections where aminoglycosides are commonly used in combination therapy.

2. *Pseudomonas* spp.

Pseudomonas spp. exhibited a concerning resistance profile. Full resistance (score = 1) was observed against gentamicin, cefepime, and piperacillin, reflecting the intrinsic and acquired

resistance mechanisms characteristic of this pathogen. Intermediate susceptibility (score = 0.5) was seen with aztreonam, while complete sensitivity (score = 0) was noted for piperacillin–tazobactam, ceftazidime, colistin, and levofloxacin.

The resistance to multiple first-line agents highlights the adaptive capacity of *Pseudomonas* spp. and underscores the importance of reserving effective agents such as colistin and combination therapies for confirmed resistant infections.

3. *Escherichia coli*

Escherichia coli isolates showed high resistance to several commonly used antibiotics. Complete resistance (score = 1) was observed against fosfomycin, gentamicin, aztreonam, and ceftazidime. Intermediate susceptibility (score = 0.5) was recorded for trimethoprim, while full sensitivity (score = 0) was seen with piperacillin–tazobactam, levofloxacin, ceftriaxone, and cefepime.

These findings indicate emerging resistance to both aminoglycosides and extended-spectrum cephalosporins, which may be suggestive of β-lactamase production. However, preserved sensitivity to fluoroquinolones and β-lactam/β-lactamase inhibitor combinations provides viable therapeutic options.

4. *Staphylococcus aureus*

Staphylococcus aureus demonstrated uniformly high resistance across most tested antibiotics. All tested agents showed complete resistance (score = 1), indicating a highly resistant phenotype. This resistance pattern is consistent with multidrug-resistant *S. aureus*, potentially reflecting methicillin-resistant strains (MRSA).

The absence of sensitive or intermediate responses highlights the clinical challenge posed by *S. aureus* infections and emphasizes the need for targeted therapy guided by susceptibility testing.

5. *Acinetobacter baumannii*

Acinetobacter baumannii exhibited significant resistance, consistent with its reputation as a problematic nosocomial pathogen. Full resistance (score = 1) was observed for ceftazidime and gentamicin, while cefepime showed intermediate susceptibility (score = 0.5). Complete sensitivity (score = 0) was recorded for colistin and levofloxacin.

The retained sensitivity to colistin is particularly notable, as it is often considered a last-resort antibiotic. However, reliance on such agents is clinically concerning due to toxicity and the risk of future resistance development.

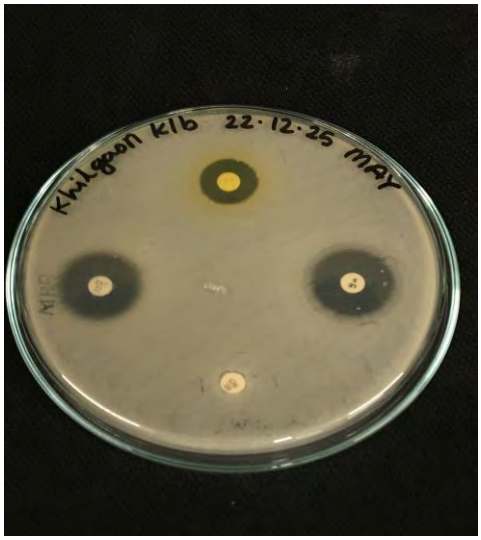
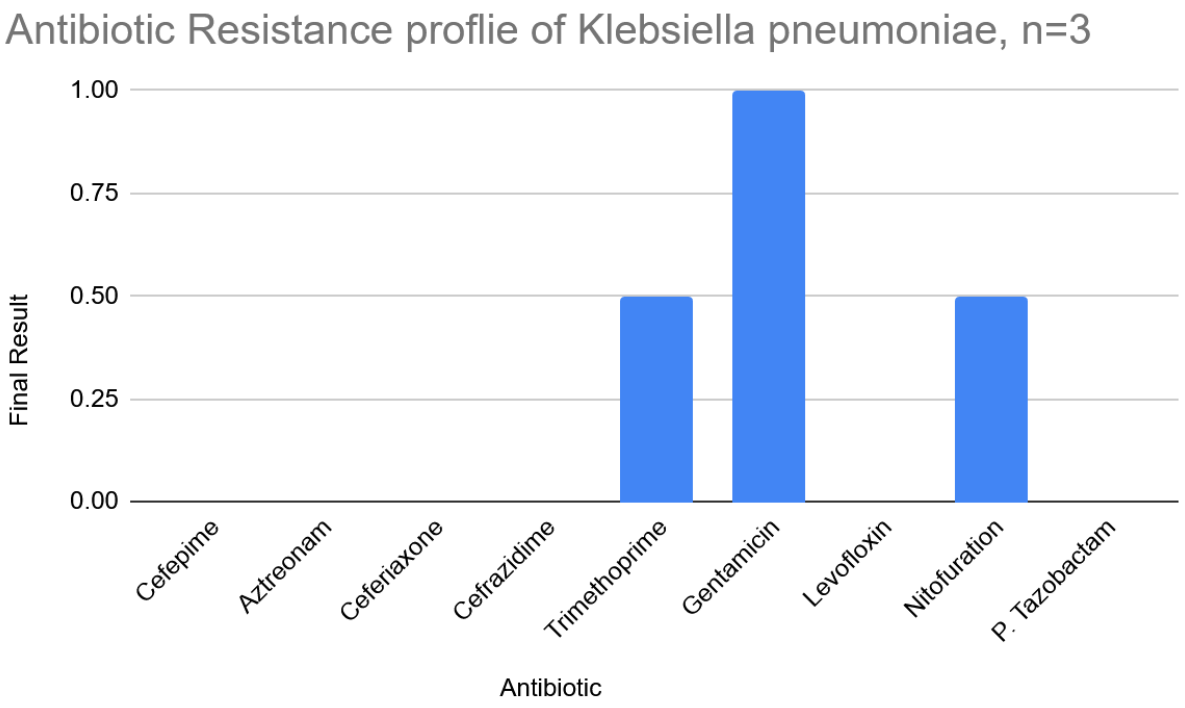
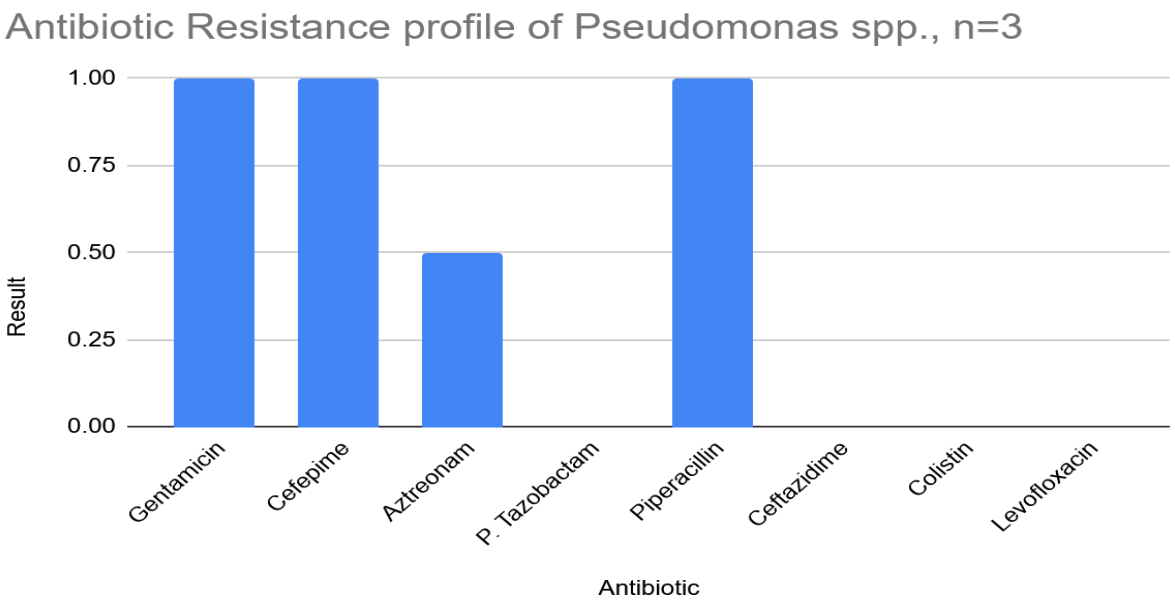
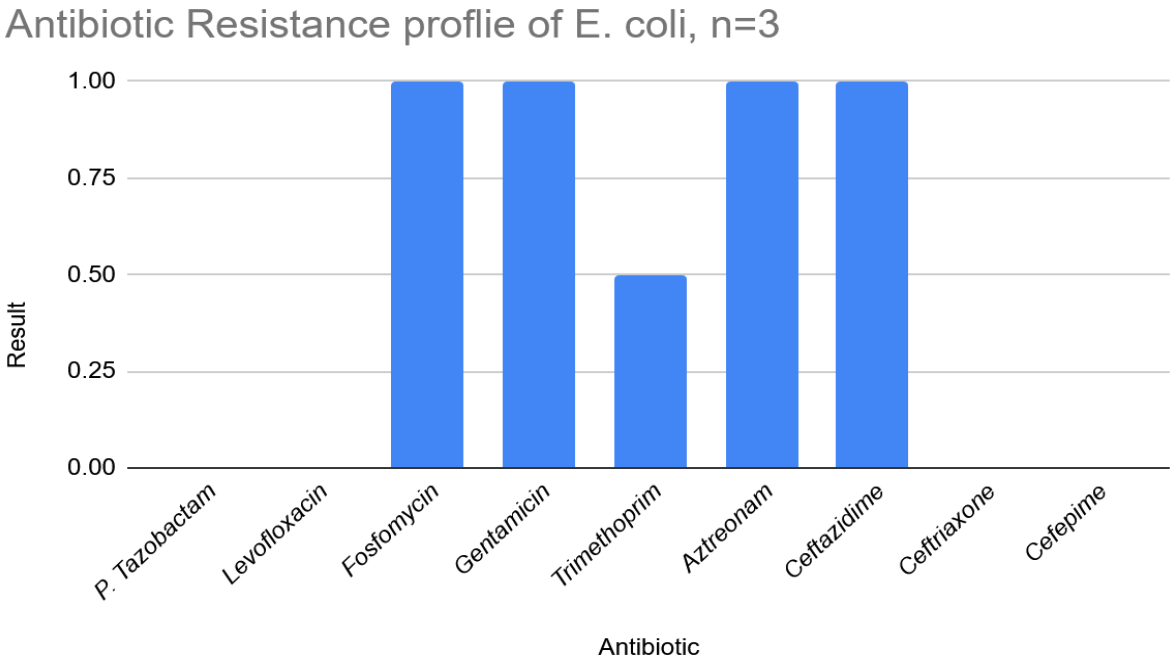
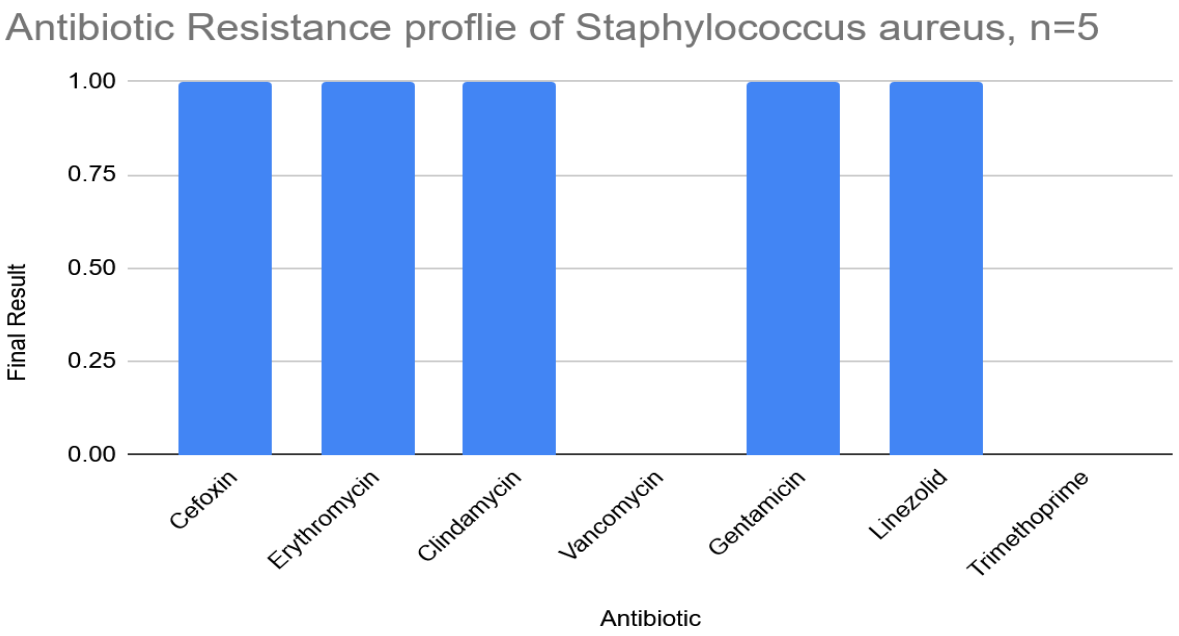


Figure 5: Antibiotic Susceptibility Test




Antibiotic Resistance profile of Pseudomonas spp., n=3
1.00
0.75
0.50
0.25
0.00
Result
Gentamicin
Cefepime
Aztreonam
P. Tazobactam
Piperacillin
Ceftazidime
Colistin
Levofloxacin
Antibiotic


Antibiotic Resistance proflie of E. coli, n=3
1.00
0.75
0.50
0.25
0.00
Result
P. Tazobactam
Levofloxacin
Fosfomycin
Gentamicin
Trimethoprim
Aztreonam
Ceftazidime
Ceftriaxone
Cefepime
Antibiotic


Antibiotic Resistance proflie of Staphylococcus aureus, n=5
1.00
0.75
0.50
0.25
0.00
Final Result
Cefoxin
Erythromycin
Clindamycin
Vancomycin
Gentamicin
Linezolid
Trimethoprime
Antibiotic

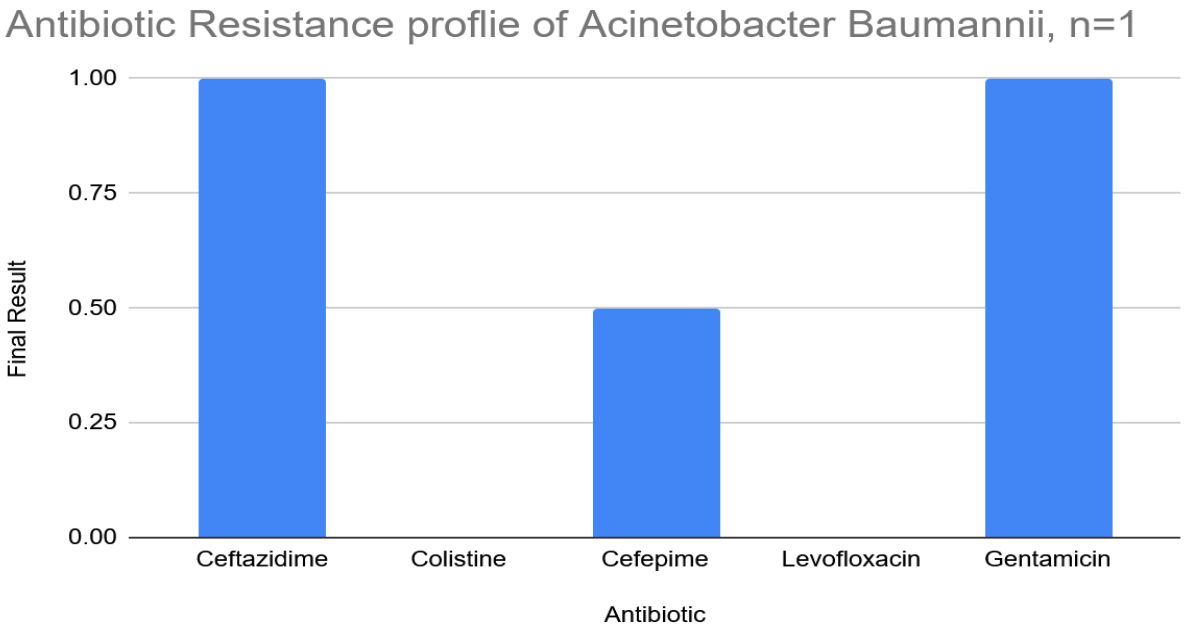

Antibiotic Resistance proflie of Acinetobacter Baumannii, n=1
1.00
0.75
0.50
0.25
0.00
Final Result
Ceftazidime
Colistine
Cefepime
Levofloxacin
Gentamicin
Antibiotic

Figure 5: *Antibiotic resistance profiles of Klebsiella pneumoniae* (n = 3), *Pseudomonas spp.* (n = 3), *Escherichia coli* (n = 3), *Staphylococcus aureus* (n = 5), *and Acinetobacter baumannii* (n = 1). The resistance patterns indicate a high prevalence of antimicrobial resistance among the tested isolates. S*taphylococcus aureus* exhibited complete resistance (score = 1) to all tested antibiotics, suggesting a multidrug-resistant phenotype. *Pseudomonas spp*. showed full resistance to gentamicin, cefepime, and piperacillin, with intermediate susceptibility to aztreonam, while remaining sensitive to colistin, ceftazidime, levofloxacin, and piperacillin–tazobactam.

Escherichia coli demonstrated 100% resistance to fosfomycin, gentamicin, aztreonam, and ceftazidime, with intermediate resistance to trimethoprim, while retaining sensitivity to piperacillin–tazobactam, levofloxacin, ceftriaxone, and cefepime. *Klebsiella pneumoniae* showed complete resistance to gentamicin and intermediate resistance to trimethoprim and nitrofurantoin, but remained sensitive to most β-lactam antibiotics and fluoroquinolones tested. *Acinetobacter baumannii* displayed high resistance to ceftazidime and gentamicin, intermediate susceptibility to cefepime, and sensitivity to colistin and levofloxacin.

Chapter 5

Discussion

5.1 Interpretation of Microbiological Findings

The present study provides important insights into the microbiological quality of ambient air in selected urban locations of Dhaka city. The detection of clinically significant bacterial species, including *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas spp., Acinetobacter baumannii,* and *Escherichia coli*, confirms that urban air can act as a reservoir and transmission

medium for potentially pathogenic microorganisms. The variation in bacterial distribution across sampling sites suggests that airborne microbial contamination is influenced by localized environmental and anthropogenic factors.

The frequent detection of *Staphylococcus aureus* across multiple locations is of particular concern, as this organism is commonly associated with respiratory tract infections, skin infections, and hospital-acquired diseases. Its presence in ambient air indicates continuous shedding from human carriers and resuspension from contaminated surfaces. Similarly, the identification of *Klebsiella pneumoniae* and *Escherichia coli*—organisms typically associated with the gastrointestinal tract—suggests possible fecal contamination of the environment, likely arising from poor sanitation, waste disposal practices, or aerosolization from open drains.

The detection of *Pseudomonas spp.* and *Acinetobacter baumannii* in outdoor air is especially noteworthy due to their well-documented role in opportunistic and nosocomial infections. These organisms are known for their ability to survive under harsh environmental conditions and to persist in aerosols, increasing the risk of inhalational exposure. The absence of detectable target bacteria in Hatirjheel may reflect comparatively better air circulation, open space, and reduced human crowding in that area.

Molecular confirmation using PCR strengthened the reliability of the findings by eliminating ambiguities associated with culture-based identification. The consistent amplification of species-specific gene targets confirms the authenticity of the isolated organisms and demonstrates the usefulness of molecular tools in environmental microbiological surveillance.

5.2 Comparison with Existing Studies

The findings of this study are consistent with previous research conducted in urban environments in Bangladesh and other developing countries. Earlier studies have reported the presence of *Staphylococcus aureus, Pseudomonas spp.,* and *Klebsiella spp.* in outdoor air samples from densely populated areas, particularly near roadsides and commercial zones (Begum et al., 2010; Fang et al., 2018). Similar to the present study, Begum et al. (2010) observed higher microbial loads in areas with heavy human activity and traffic congestion in Dhaka city.

The detection of *E. coli* in ambient air aligns with findings reported by Després et al. (2012), who highlighted that enteric bacterium can become aerosolized through environmental disturbances, sewage systems, and improper waste management. Studies conducted in India and China have also documented airborne dissemination of Gram-negative bacteria in urban settings, emphasizing the role of air as an underestimated transmission route (Ghosh et al., 2015; Li et al., 2020).

The high prevalence of antimicrobial resistance observed in this study mirrors global and regional trends. Previous investigations in Bangladesh have reported increasing multidrug resistance among environmental and clinical isolates of *Staphylococcus aureus, Klebsiella pneumoniae,* and *Acinetobacter baumannii* (Ahmed et al., 2019; Islam et al., 2021). The complete resistance of *Staphylococcus aureus* to tested antibiotics in the present study strongly suggests the circulation of multidrug-resistant strains in the community, potentially including MRSA, which has been increasingly reported in South Asia (Chowdhury et al., 2011).

5.3 Likely Sources of Contamination

The presence of pathogenic bacteria in ambient air can be attributed to multiple environmental and anthropogenic sources. In densely populated areas such as Badda, Khilgaon, and Mirpur, continuous human movement, coughing, sneezing, and direct shedding from skin and nasal cavities likely contribute to airborne dissemination of *Staphylococcus aureus*. Traffic-related turbulence and construction activities may further resuspend contaminated dust particles into the air.

The detection of enteric bacteria such as *E. coli* and *Klebsiella pneumoniae* suggests contamination from open sewage systems, waste dumping sites, and poorly managed drainage infrastructure, which are common in many parts of Dhaka. Aerosolization from contaminated water sources and solid waste can facilitate the spread of these organisms into the surrounding air.

Healthcare-associated pathogens like *Acinetobacter baumannii* and *Pseudomonas spp.* may originate from nearby hospitals, clinics, or laboratories, where these organisms are frequently present. Improper disposal of medical waste and inadequate infection control practices can allow these bacteria to escape into the environment, contributing to their presence in community air.

5.4 Implications for Public Health in Dhaka

The detection of pathogenic and multidrug-resistant bacteria in ambient air has significant public health implications for Dhaka city. Continuous exposure to contaminated air increases the risk of respiratory tract infections, allergic reactions, and opportunistic infections, particularly among

vulnerable populations such as children, elderly individuals, and immunocompromised patients. Airborne dissemination of antibiotic-resistant bacteria also raises concerns about the environmental spread of antimicrobial resistance, which may complicate treatment outcomes and increase healthcare costs.

The findings underscore the need to incorporate microbiological parameters into routine air quality monitoring programs, which currently focus primarily on chemical pollutants and particulate matter. Improved urban sanitation, waste management, and infrastructure development are essential to reduce environmental contamination. Public awareness campaigns and policy-level interventions are also necessary to address the overlooked role of air in the transmission of infectious agents.

5.5 Limitations of the Study

Despite its contributions, the study has several limitations. First, air sampling was conducted using the passive settle plate technique, which does not allow precise quantification of airborne bacterial concentrations and may underestimate smaller aerosolized particles. Second, sampling was restricted to a limited number of locations and conducted during a single season and time period, preventing assessment of seasonal and diurnal variations in microbial distribution.

Additionally, the study focused only on selected bacterial pathogens and did not investigate viral or fungal aerosols, which may also pose significant health risks. The relatively small number of isolates used for antibiotic susceptibility testing limits the generalizability of resistance patterns. Future studies should employ active air sampling methods, include a broader range of microorganisms, and cover multiple seasons to obtain a more comprehensive understanding of airborne microbial contamination in Dhaka.

Chapter 6

Conclusion And Recommendations

6.1 Conclusion

This study assessed the microbiological quality of ambient air in selected urban areas of Dhaka city by identifying clinically important airborne bacterial pathogens and evaluating their antimicrobial resistance patterns. The findings demonstrate that urban air in Dhaka can serve as a reservoir for pathogenic and multidrug-resistant bacteria, posing potential risks to public health.

The detection of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas spp., Acinetobacter baumannii,* and *Escherichia coli* across multiple locations highlights widespread environmental contamination. The variation in bacterial presence among sampling sites suggests that local environmental conditions, population density, traffic activity, sanitation infrastructure, and human movement significantly influence airborne microbial distribution. Molecular confirmation through PCR strengthened the accuracy of bacterial identification and validated the presence of these pathogens in outdoor air.

A particularly concerning finding was the high level of antimicrobial resistance observed among the isolated bacteria. Several isolates exhibited multidrug-resistant patterns, with *Staphylococcus aureus* showing complete resistance to all tested antibiotics. The presence of such resistant organisms in ambient air raises serious concerns about the environmental spread of antimicrobial resistance and the potential for community exposure through inhalation. Overall, this study

underscores the importance of considering microbial contamination as an integral component of urban air quality and public health assessment in Dhaka.

6.2 Key Findings Summary

- Pathogenic bacteria were detected in ambient air samples from multiple urban locations in Dhaka.

- *Staphylococcus aureus* was the most frequently identified organism across sampling sites.

- Enteric bacteria such as *E. coli* and *Klebsiella pneumoniae* were present, indicating possible fecal or environmental contamination.

- Opportunistic and nosocomial pathogens (*Pseudomonas spp.* and *Acinetobacter baumannii*) were detected in outdoor air.

- Molecular techniques (PCR) successfully confirmed bacterial identity and increased result reliability.

- A high prevalence of antimicrobial resistance was observed, with several isolates showing multidrug-resistant characteristics.

- Some areas showed no detectable target bacteria, suggesting the influence of environmental and infrastructural factors on air quality.

6.3 Recommendations for Vendors and Public Health Authorities

To reduce airborne microbial contamination and associated health risks, several practical measures are recommended:

- Improved sanitation and waste management: Proper disposal of solid and liquid waste should be enforced to minimize environmental sources of bacterial aerosolization.

- Regular cleaning of public spaces: Roadsides, markets, transport hubs, and residential areas should be cleaned frequently to reduce resuspension of contaminated dust.

- Awareness among vendors and workers: Street vendors and outdoor workers should be encouraged to maintain personal hygiene, use masks when necessary, and keep their surroundings clean.

- Incorporation of microbiological monitoring: Public health authorities should include microbial assessment in routine air quality monitoring programs alongside chemical pollutants.

- Strengthening antimicrobial stewardship: Policies regulating antibiotic use in healthcare and the community should be reinforced to limit the spread of resistant bacteria.

- Urban planning initiatives: Increasing green spaces and improving ventilation in crowded areas may help dilute airborne microbial concentrations.

6.4 Suggestions for Future Research

While this study provides valuable baseline data, further research is needed to gain a more comprehensive understanding of airborne microbial risks in Dhaka:

- Future studies should include seasonal and diurnal sampling to assess temporal variations in airborne bacterial distribution.

- Active air sampling methods should be used to quantify bacterial concentrations and particle size distribution.

- A broader range of microorganisms, including fungi and viruses, should be investigated.

- Advanced molecular approaches such as whole-genome sequencing could be employed to characterize resistance genes and track sources of contamination.

- Studies assessing health outcomes associated with long-term exposure to contaminated air would help establish stronger links between airborne pathogens and disease burden\

Chapter 7

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