Detection and Antimicrobial Resistance Profiling of Nosocomial Bacterial Pathogens in Two Tertiary Care Hospitals of Dhaka, Bangladesh

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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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February,2026

Declaration

It is hereby declared that -

1. The thesis submitted is our own original work while completing our degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. We have acknowledged all main sources of help.

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Approval

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Summer, 2021, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 26 February, 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

Nosocomial infections remain a significant global health burden, posing persistent challenges in hospital settings, including in Bangladesh. Prompt identification of causative agents and appropriate antimicrobial therapy are essential, as delayed or inadequate treatment increases patient morbidity and mortality. This study investigated the prevalence and antimicrobial resistance patterns of most ESKAPE pathogens- *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* —excluding *Enterococcus faecium* and *Enterobacter species*, known for high virulence and multidrug resistance in hospital-acquired infections worldwide. However, *Escherichia coli* was included in this study as it was one of the most prevalent bacteria found in the hospital environment. Environmental swabs were collected from high-touch surfaces, including bed rails, bed sheets, nursing tables, and saline stands, across ICU, NICU, and children's wards of two tertiary hospitals in Dhaka. Samples were serially diluted, cultured on selective media, and presumptively identified by colony morphology, with confirmation via polymerase chain reaction (PCR) and gel electrophoresis. Antimicrobial susceptibility was assessed using the Kirby–Bauer disc diffusion method. From 22 samples, *Klebsiella pneumoniae* was most prevalent (36.6%), followed by *Staphylococcus spp.* and *Escherichia coli* (18.18%). *Acinetobacter baumannii* (13.64%), and *Pseudomonas aeruginosa* (9.1%) with the lowest amount of prevalence. High resistance was noted against Beta-lactams (41.18% – 64.71%) and macrolides (47.06%), while lower resistance was found against carbopenem. MDR rates were highest in *Staphylococcus species* and *Escherichia coli* (100%), followed by *Klebsiella pneumoniae* (75%), *Pseudomonas aeruginosa* (50%), and *Acinetobacter baumannii* (33.3%). These findings underscore the urgent need for continuous surveillance, stringent infection control, and rational antibiotic use to mitigate multidrug-resistant pathogens in hospital environments.

Keywords: Nosocomial infections, Healthcare-acquired, MDR, High-touch surfaces.

Dedication

This work is dedicated to The Almighty, my family, friends, and lastly APDN for their constant encouragement and inspiration.

Acknowledgement

We would like to express our sincere gratitude to Amena Begum, our Research Assistant, for her dedicated support and invaluable technical assistance throughout this work, which played a significant role in its successful progression. We are also deeply grateful to our lab mate, Sananda Saha, for her constant support, camaraderie, and willingness to help whenever needed, and to our dear friend, Samiya Khan Neha, for standing by us through every challenge with unwavering encouragement. Finally, we extend our heartfelt appreciation to everyone who supported us, whether through academic guidance or personal encouragement; your collective belief in this work has been truly meaningful and instrumental in bringing this thesis to completion.

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Chapter 1

Introduction

1.1 Background

Healthcare-associated infections (HAIs) represent a critical failure in patient safety, transitioning the hospital from a place of healing to a silent reservoir for disease. As noted by Tobin & Zahra (2025), the impact of these infections extends beyond individual morbidity to a systemic crisis of antimicrobial resistance and escalating healthcare expenditures. While clinical interventions often focus on invasive procedures, the inanimate environment specifically high-touch surfaces such as bed rails and IV poles serves as a primary vector for multi-drug resistant (MDR) pathogens (CDC, 2024). Multidrug resistance (MDR) refers to the ability of a microorganism (especially bacteria) to resist the effects of two or more classes of antimicrobial agents that are structurally and functionally different, making standard treatments ineffective. For the immunocompromised patient, these surfaces are not merely furniture but are active 'fomites' that harbor pathogens capable of surviving standard disinfection protocols through biofilm formation (Weinstein, 1998; Cheung, 2025). Consequently, addressing the environmental bioburden is not just a cleaning task, but a vital clinical necessity to preserve the efficacy of modern medicine.

The physical environment in Bangladeshi public hospitals presents a unique challenge to standard infection control protocols. Due to acute bed shortages, it is clinically commonplace for patients to be managed in non-traditional care areas, including ward corridors, floor spaces, and areas behind nursing stations (Shishir Moral et al., 2025). This 'floor-level' care effectively transforms every low-lying surface into a primary clinical interface, increasing the surface-to-patient contact ratio to near-constant levels (Figure 1).

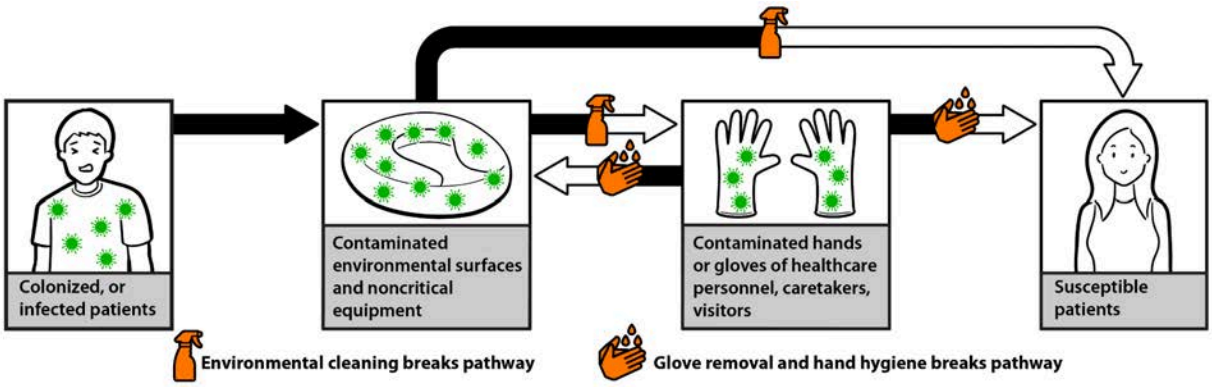

Figure 1: Contact transmission pathway showing role of environmental surfaces, role of environmental cleaning, and hand hygiene in breaking the chain of transmission

The environmental bioburden is compounded by the presence of numerous non-medical 'fomites' within the immediate patient zone. Household items including personal utensils, buckets, textiles, and unsealed food containers are often stored in close proximity to or directly on the floor-bedding (CDC, 2024). These items not only act as additional reservoirs for (MDR) pathogens but also create physical barriers that impede routine terminal cleaning, thereby facilitating the persistent transmission of nosocomial infections within the ward.

The study focused on hospital environmental samples, as contaminated surfaces and medical devices are recognized sources of hospital-acquired infections. Samples were collected from frequently touched and commonly used sites, including bed rails, saline stands, air conditioners (AC), floors, ventilators, and nebulizers. These locations were selected due to their frequent

exposure to patient contact and aerosols, which support the survival and spread of pathogenic bacteria in hospital environments (Otter et al., 2013; Dancer, 2014).

1.2 Literature review:

Studies in Bangladesh show that nosocomial infections are common, but the data are scattered and hospital-based, with no national surveillance system. Reported prevalence of hospital-acquired infections varies widely, from 3.7% to 9.4% among admitted patients, depending on the hospital and study design (Amin & Nahar, 2018; Rahman et al., 2019). This variation makes it difficult to estimate the true national burden.

Most studies report that Gram-negative bacteria are more prevalent than Gram-positive organisms. *Klebsiella pneumoniae* has been reported as the most common nosocomial pathogen in several studies (~30–33%), followed by *Escherichia coli* (13–32%) and *Pseudomonas aeruginosa* (10–14%) (Rahman et al., 2017; Hossain et al., 2020). In contrast, *Staphylococcus aureus* accounts for a smaller proportion of isolates, although MRSA (Methicillin-resistant *Staphylococcus aureus*) prevalence ranges from 60–85% among *Staphylococcus aureus* isolates, indicating serious resistance concerns (Islam et al., 2018).

Available data on antimicrobial resistance in Bangladesh suggests that nosocomial pathogens tend to be more resistant to later (third- and fourth-generation) classes of antibiotics compared to earlier (first- and second-generation) classes. This pattern has been observed in laboratory-based antimicrobial susceptibility reports, where resistance rates increase with antibiotic generation, especially among Gram-negative organisms isolated from clinical specimens (Hossain et al., 2020; Ahmed et al., 2021). However, because most studies are cross-sectional and conducted in single hospitals, there is no consistent, long-term data showing how resistance against different antibiotic generations has changed over time for nosocomial infections specifically.

In terms of research volume, only a small number of peer-reviewed publications (fewer than 10 hospital-based prevalence studies) have examined nosocomial infections and microbial resistance in Bangladeshi healthcare settings over the last decade (Amin & Nahar, 2018; Rahman et al., 2019; Hossain et al., 2020; Haque et al., 2023). For example, one study in a Dhaka tertiary hospital reported 8.33% HAI prevalence among admitted patients (Amin & Nahar, 2018), another documented 9.4% prevalence in general wards (Afroz et al., 2019), and a point-prevalence survey from Rajshahi Medical College Hospital found 3.7% prevalence (Haque et al., 2023). These individual studies are too few and too isolated to reflect the national scenario. Considering that Bangladesh has hundreds of hospitals with varying hygiene standards and infection control practices, the true prevalence of nosocomial infections is likely much higher than these limited figures suggest. Moreover, Bangladesh's ongoing challenges with hospital hygiene, overcrowding, and inadequate sanitation all known risk factors for HAIs further point to underrecognized and underreported infection burdens (Springer Nature, 2025). Therefore, there is an urgent need for more comprehensive, multi-center, and longitudinal microbial surveillance studies using standardized methods to accurately estimate the burden, temporal trends, and resistance profiles of nosocomial pathogens in Bangladesh. And filling in this knowledge gap about nosocomial bacteria in our country is the ultimate goal of our research.

Nosocomial infections, also known as Health care-associated infections, which can occur in any healthcare environment. Nosocomial infections not only lead to significant morbidity and mortality but also contribute to increased antimicrobial resistance and healthcare costs (Tobin & Zahra, 2025). Nosocomial infections are caused by MDR pathogens acquired via improper antibiotic use or other invasive procedures (Cheung, 2025). Usually, the patients who are immunocompromised because of age, underlying diseases, or undergoing invasive procedures are affected by nosocomial infections, according to Weinstein (1998). High touch surface areas and medical equipment play a critical role in persistence and transmission of pathogenic microorganisms, and among them, the areas are bed rails, bedside tables, IV poles, etc. (CDC, 2024). In the global phenomenon, the hospital is acting as a silent contributor to disease rather than a healing place. From a clinical standpoint, it is negatively contributing to the economy as well as the health of the patient.

In Bangladesh, it is very common for patients to stay on the floors of public hospitals when beds are not available. The floors and low-lying surfaces become the patient care areas, as a result, environmental surfaces are often in direct contact with patients 24/7. According to Shishir Moral et al. (2025), due to the shortage of beds in both the male and female wards, many patients are being treated on the floor, behind the nurses' desk, and in the ward corridors. Beside or above each patient's bed are household items like plates, glasses, buckets, water bottles, clothes and towels, food containers, medicine packets, and hand fans.

1.3 Objectives

The objectives of this study are as follows:

1. To investigate the prevalence and environmental presence of ESKAPE pathogens in two tertiary hospital settings located in Dhaka, Bangladesh, by isolating and identifying them using selective culture media, followed by confirmation through PCR-based molecular techniques to ensure accurate detection of clinically targeted organisms.

2. To assess the antibiotic susceptibility patterns of the identified isolates in order to determine their resistance profiles and understand the extent of multidrug resistance and susceptibility among the targeted nosocomial organisms.

Chapter 2

Materials And Methods

2.1 Work flow

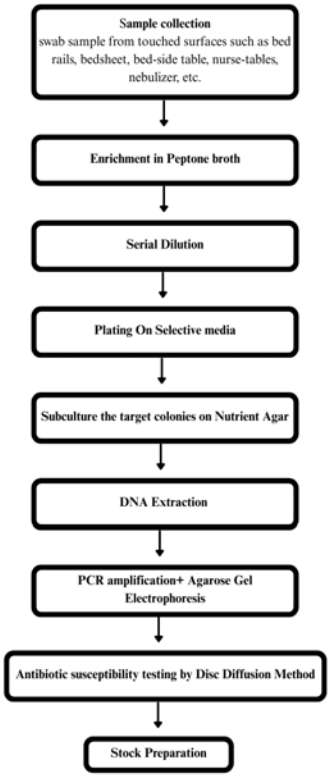

Figure 2: Work flow of this study

2.2 Description of the Study Area and Sample Type

This study was carried out in two tertiary-level hospitals in Dhaka, Bangladesh: Holy Family Red Crescent Medical College Hospital and Bangladesh Medical College Hospital. Both hospitals have high patient turnover and extensive use of shared medical equipment, making them high-risk settings for the transmission of nosocomial pathogens (WHO, 2011)

All collected samples were processed in the Microbiology Laboratory of BRAC University following standard laboratory biosafety and aseptic handling procedures. Standard aseptic techniques, personal protective equipment, and proper waste disposal procedures were followed to ensure biosafety and prevent contamination. Conducting laboratory analysis in a controlled academic setting allowed for reliable assessment of environmental contamination and supported the evaluation of hospital environments as reservoirs of nosocomial pathogens (WHO, 2020; Haque et al., 2023).

Site Selection and Environmental Sampling: A total of 22 environmental samples were purposely collected from high-contact surfaces within the Kidney Wards, Neonatal Intensive Care Unit (NICU), and Intensive Care Unit (ICU). Sampling sites were prioritized based on their high frequency of patient and healthcare worker interaction, which serves as a primary reservoir for cross-contamination. Specific surfaces included oxygen masks, bed linens (pillows and sheets), bedside tables, electrical switchboards, and bed rails. These sites were targeted to assess the prevalence of nosocomial pathogens in hospital environments (Figure 3). To maintain specimen integrity, all samples were collected using aseptic techniques, and swabs were immediately sealed in sterile transport tubes to prevent ambient contamination.

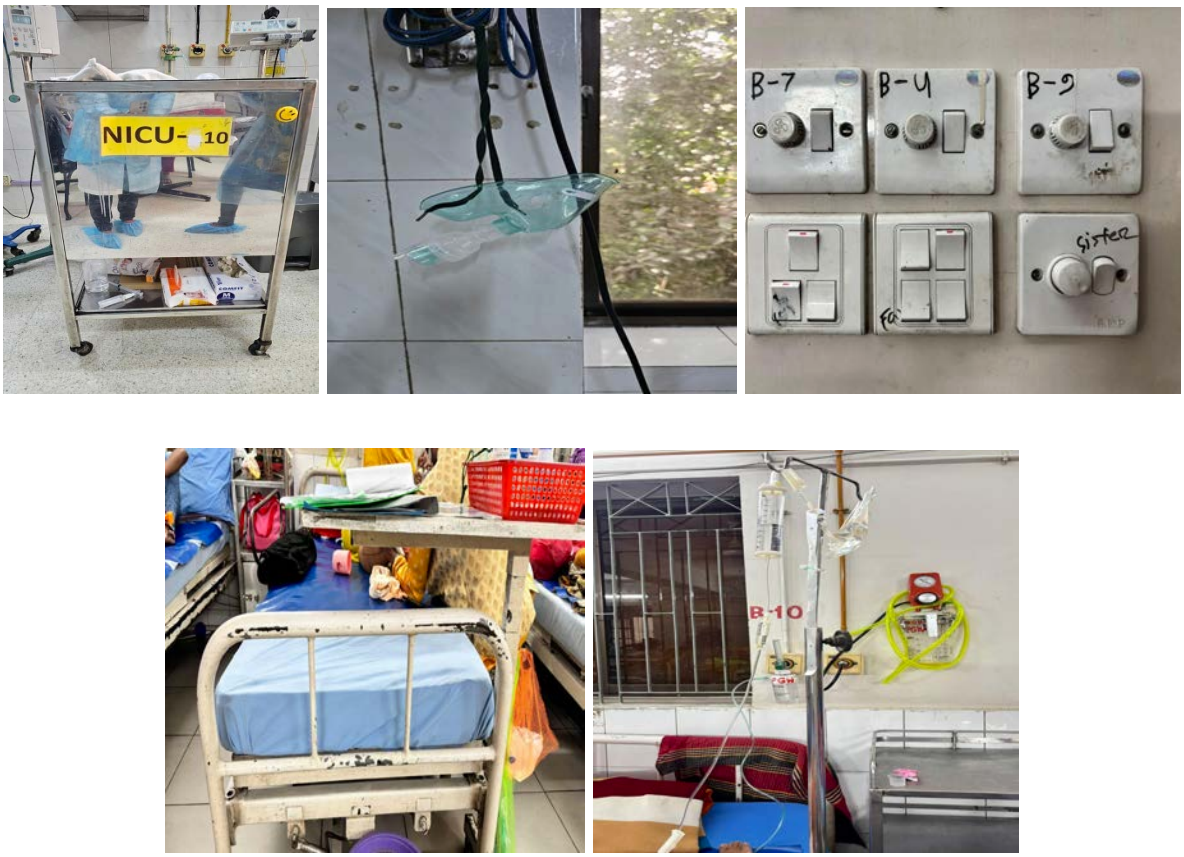

Figure 3: Sample collection sites

2.3 Sample Collection

Sampling Instrumentation and Biosafety

Field Sampling and Cold-Chain Maintenance To ensure standardized collection and prevent exogenous contamination, a specialized sampling kit was utilized. This included sterile cotton swabs and test tubes pre-filled with 6 mL of Peptone Broth as the primary transport medium. Personal Protective Equipment (PPE), including surgical face masks and sterile nitrile gloves, was strictly donned by the research team to maintain an aseptic environment and ensure researcher safety.

Using a sterile cotton swab approach, 22 environmental samples were taken from the surfaces of two chosen hospitals.
Using a sterile cotton swab, 22 environmental samples were taken from the surfaces of two chosen hospital. Before sampling, sterile cotton swabs were pre-moistened with sterile peptone water, which helped improve bacterial recovery from dry surfaces. The moistened swab was then rubbed firmly over the target area (such as bed rails, saline stands, ventilators, nebulizers, AC units, and floors) using a rotating motion to ensure maximum surface contact. Peptone water was used as an enrichment medium to support the survival and recovery of stressed or low-number bacteria commonly found on hospital surfaces (ISO, 2018; Cheesbrough, 2016).

After collection, each swab was immediately placed into a sterile tube containing peptone water, properly labeled, and transported to the laboratory in an ice box (4–8 °C) to prevent bacterial overgrowth or loss of viability. All samples were transported to the Microbiology Laboratory of BRAC University and processed within a short time frame following standard microbiological and biosafety guidelines. This method is widely recommended for environmental surveillance of hospital-acquired pathogens (WHO, 2011; Dancer, 2014).

2.4 Sample Processing

After collection, the swab samples in peptone broth were incubated in shaker incubator at 37 °C for 24 hours to enrich priority hospital-associated pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Acinetobacter baumannii*. After enrichment, we performed serial dilution and spread the samples on culture media using aseptic techniques and incubated to observe bacterial growth. This enrichment followed by subculture is a standard method for improving the detection of clinically important nosocomial pathogens from environmental samples (Cheesbrough, 2016; Dancer, 2014).

2.5 Serial Dilution and Culture on Selective Media

After enrichment, samples were subjected to serial dilution up to 10^{-6} using sterile normal saline to reduce bacterial load. During plating, dilutions from the raw sample to 10^{-3} were commonly used to avoid too numerous to count (TNTC) growth. From each selected dilution, 100ul of sample was spread evenly on selective and differential media using sterile spreaders under aseptic conditions, following standard microbiological practices (Cheesbrough, 2016).

Selective media were chosen based on the target organisms. UTI agar (HI Media) and Leeds Acinetobacter Medium (Leeds agar, HI Media) were used for isolation of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. Mannitol Salt Agar (MSA, Condalab) was used for selective isolation of *Staphylococcus aureus* and *Staphylococcus epidermidis*, while Cetrimide Agar (HI Media) was used specifically for *Pseudomonas aeruginosa.* All plates were incubated at 37 °C for 24 hours, and bacterial growth was observed and recorded based on colony morphology and media characteristics (Dancer, 2014; WHO, 2020).

2.6 Presumptive Identification of Bacteria

Presumptive identification was based on growth characteristics, metabolic traits, and chromogenic differentiation on selective and differential media. Acid production from carbohydrate fermentation, salt tolerance, and other physiological traits were used to differentiate bacteria, such as staphylococci on Mannitol Salt Agar and enteric bacteria on UTI agar (Cheesbrough, 2016).

For Leeds agar, an AC supplement was added (5 mL per 500 mL of medium) to enhance selective recovery of *Acinetobacter baumannii* by providing essential nutrients for growth. Features such as fermentation ability, salt tolerance, pigment production, and chromogenic differentiation on selective media were compared with published descriptions to predict organism identity (MacFaddin, 2000; Forbes et al., 2018).

2.7 Colony Morphology on Selective Media

Colony morphology on selective and differential media provides important initial clues for presumptive bacterial identification. Observations of colony shape, texture, pigmentation, and chromogenic differentiation were used to guide further confirmation tests (Table 1 &Figure 4).

Organism	Gram Reaction	Selective Media	Expected Colony Morphology / Notes
Escherichia coli	Gram-negative	HIMEDIA HI Crome UTI Agar, Modified	Lactose fermenter, smooth, round colonies; purple color; acid production (chromogenic differentiation)
Klebsiella pneumoniae	Gram-negative	HIMEDIA HI Crome UTI Agar, Modified	Lactose fermenter, mucoid, large colonies; blue color; acid production
Pseudomonas aeruginosa	Gram-negative	HIMEDIA Cetrimide agar	Pigment-producing colonies; green pigment, fluoresces under UV light.
Staphylococcus aureus	Gram-positive	Condalab Mannitol Salt Agar (MSA) (Chapman medium)	Mannitol fermenter, smooth, round colonies; yellowish appearance
Staphylococcus epidermidis	Gram-positive	Condalab Mannitol Salt Agar (MSA) (Chapman medium)	Non-mannitol fermenter, smooth colonies; pinkish/cream color
Acinetobacter baumannii	Gram-negative	HIMEDIA Leeds Acinetobacter agar + AC supplement	Non-lactose fermenter, smooth, convex colonies; pink/cream color; growth enhanced with AC supplement

Table 1: Colony Morphology of different bacteria on selective media

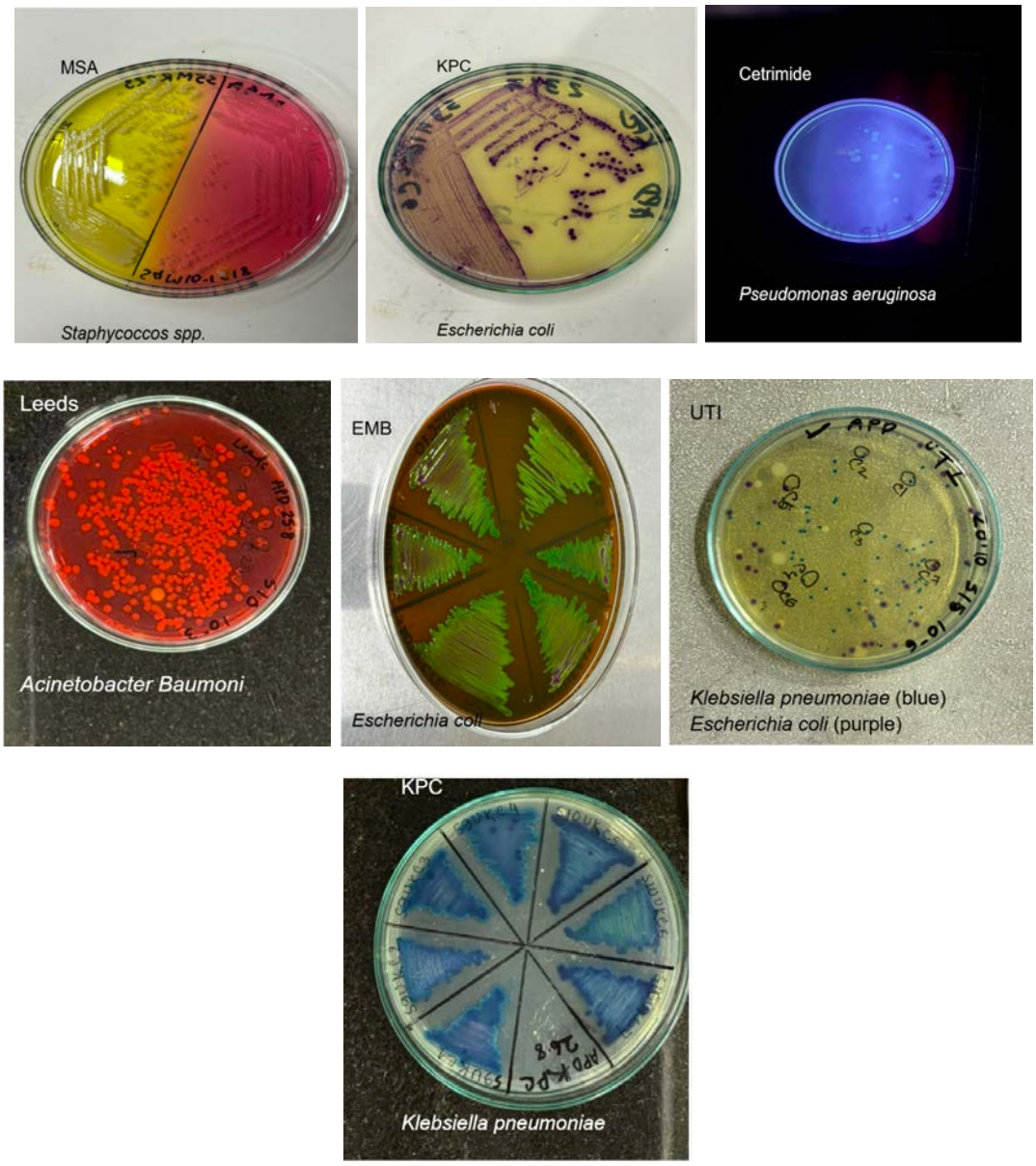

Figure 4: Different selective media used to isolate different organisms.

2.8 Further Screening of Suspected Colonies on Selective Media

Suspected colonies from the initial selective media were subcultured onto fresh selective and differential media to obtain pure cultures and enhance the accuracy of presumptive identification. Enteric bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, were subcultured onto Klebsiella Pneumoniae Selective Agar (KPC) and Eosin Methylene Blue (EMB) agar. On KPC agar, *Klebsiella pneumoniae.* formed mucoid blue colonies, while *E. coli* appeared as purple, metallic sheen colonies on EMB agar, reflecting lactose fermentation and acid production (Cheesbrough, 2016).

Gram-positive *Staphylococcus spp.* were sub cultured onto Mannitol Salt Agar (MSA) to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis*. *S. aureus* fermented mannitol, producing colonies with yellowish appearance, whereas *S. epidermidis* did not ferment mannitol, showing pinkish/cream colonies. *Acinetobacter baumannii* isolates were sub cultured onto Leeds agar with AC supplement (5 mL/500 mL medium) to ensure selective growth and recovery, producing pink/cream smooth colonies typical of this organism. *Pseudomonas aeruginosa.* colonies were further screened on Cetrimide agar, where they exhibited green pigment production and fluorescence under UV light (Forbes et al., 2018; MacFaddin, 2000) (Figure 4).

This subculturing step reduced contamination, provided pure and morphologically consistent colonies, and allowed for accurate downstream biochemical identification and antimicrobial susceptibility testing. Observing colony color, texture, and growth patterns on these selective media was essential for presumptive identification and differentiation of closely related species in hospital environmental samples.

2.9 DNA Extraction

Before DNA extraction, bacterial isolates were first subcultured on nutrient agar (NA) to obtain actively growing, stress-free cells, which improves DNA quality and yield (Sambrook & Russell,

2001). From these fresh cultures, a loopful (~1 µL) of bacterial colony was aseptically transferred into 400 µL of sterile 1X Tris-EDTA (TE) buffer in a microcentrifuge tube and vortexed for 15 seconds to ensure proper homogenization.

The suspension was then centrifuged at 13,000 RPM for 10 minutes to pellet the bacterial cells, after which the supernatant was gently discarded. Another 400 µL of 1X TE buffer was added to resuspend the pellet, and the mixture was thoroughly homogenized by pipetting and vortexing. The tubes were subsequently heated at 100 °C for 10 minutes using a heat block to lyse the cells and release genomic DNA. After heating, samples were allowed to cool at room temperature for 5–10 minutes, followed by a second centrifugation at 13,000 RPM for 10 minutes to remove remaining debris. The resulting supernatant, containing purified DNA, was carefully transferred to a fresh sterile tube and stored at –20 °C until further molecular analysis (Wilson, 2001; Ausubel et al., 2002).

2.10 Polymerase chain reaction

Polymerase Chain Reaction (PCR) was performed to amplify the genomic DNA of bacterial isolates for molecular identification. For each reaction, a total volume of 13µL was prepared in sterile PCR tubes, consisting of 6 µL of 2X PCR master mix, 2 µL of DNA template, 1 µL of forward primer, 1 µL of reverse primer, and 3 µL of nuclease-free water (NFW). All components were mixed gently and briefly centrifuged to collect the solution at the bottom of the tube.

The tubes were then placed in a thermal cycler for PCR amplification. The program included an initial denaturation at 95 °C for 5 minutes, followed by 25–35 cycles of denaturation at 94–95 °C for 30 seconds, primer annealing at 50–65 °C for 30 seconds, and extension at 72 °C for 1 minute per kilobase of expected product. A final extension at 72 °C for 5–10 minutes ensured complete synthesis of all DNA fragments. PCR products were stored at –20 °C until further analysis. It should be noted that the PCR program was optimized differently for each bacterial organism; the conditions mentioned here represent a generalized protocol (Table 2) (Ausubel et al., 2002; Sambrook & Russell, 2001).

Organism Name	Primer Name	Targeted gene	Band Size	Primer Sequence	PCR Condition	Reference
Escherichia coli	ECO 10x	16sRNA	585bp	ECO-F: GACCTCGGTTTAGTTCACAGA ECO-R: CACACGCTGACGCTGACCA	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s, and extension for 1 min,followed by a final extension at 72°C for 5 min	Odetoyin et al. (2022)
Pseudomonas aeruginosa	PA-GS	gyrB	618 bp	PA-GS_F: 5'-GACGGGTGAGTAATGCCTA-3' PA-GS_R: 5'-CACTGGTGTTCCTTCCTATA-3'	After an initial denaturation for 2 min at 95°C, 25 cycles were completed, each consisting of 20 s at 94°C, 20 s at 54°C annealing temperature, and 40 s at 72°C. A final extension of 1 min at 72°C was applied.	Spilker et al. (2004)
Klebsiella pneumoniae	KPN	16sRNA	130bp	KPN-F: TGCAGATAATTCACGCCCAG KPN-R: ACCCGCTGGACGCCAT	Initial 94℃ 10 minutes followed by 30 cycles of denaturation 94℃ at 30 sec, annealing 60℃ 45 sec, Extension 72℃ 45 sec, and final extension 72℃for 10 minutes.	Mahmudunnabi et al. (2018)
Staphylococcus aureus	nuc	thermonuclease	279bp	nuc-F: 5'-GCGATTGATGGTGGATACGGT-3' nuc-R: 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	Initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing temperature of the primers was 55°C for 45 sec and extension at 72°C for 1 min. The final extension was conducted at 72°C for 10 min	Humans.Txt (n.d.)
Staphylococcus epidermidis	gseA	Glutamate semialdehyde dehydrogenase	503bp	gseA F: 5'-ATGAAAAAGAGATTTTTATCT-3' gseA R: 5'GTTTGGTGACACTCTTAAG-3'	Cycling was performed as follows: an initial denaturation at 94 °C for 2 min; followed by 30 cycles for amplification, consisting of 94 °C for 10 s, 50 °C for 10 s, and 72 °C for 60 s; followed by a final extension step at 72 °C for 4 min.	Ikeda et al. (2004)
Acinetobacter baumannii	BLA-OXA	OXA-type carbapenemase enzyme	353bp	blaOXA51 F: 5'TAATGCTTTGATCGGCCTTG 3' blaOXA51 R: 5'TGGATTGCACTTCATCTTGG 3'	The PCR program was set up in a thermo cycler as follows: 94°C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min.	Falah et al. (2019)

Table 2: PCR protocol used for amplification

2.11 Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to visualize PCR-amplified DNA fragments and confirm successful amplification. 1.2% agarose (w/v) was prepared by dissolving the agarose powder in 98 ml of distilled water, and 2ml of 50X TAE buffer was also given. The mixture was heated until completely dissolved, then allowed to cool to approximately 45-40°C. Ethidium bromide (EtBr) was added to the molten agarose to a final concentration that allowed intercalation into the DNA for visualization under UV light. The agarose solution was poured into a casting tray with a comb inserted to create wells for sample loading. Once solidified, the comb was removed to reveal uniform wells.

The gel was placed in the electrophoresis tank and submerged in a 1X TAE running buffer. PCR products were not prepared by mixing them with any loading dye because the master mix that we use for PCR already contains the dye. Samples were loaded into the wells along with a DNA ladder as a molecular size marker and a positive control, and a negative control to ensure the PCR reaction worked correctly. An electric field (100 V) for 40 minutes was applied, and DNA molecules migrated towards the positive electrode due to their negative charge (Figures 5-10).

During electrophoresis, smaller DNA fragments migrate faster through the agarose matrix than larger ones, separating the fragments based on size. The EtBr dye intercalates between the bases of double-stranded DNA, enabling the DNA bands to fluoresce under UV light, which allows visualization. The positions of the PCR products relative to the DNA ladder and positive control were used to confirm the expected fragment sizes and validate the success of amplification (Sambrook & Russell, 2001; Ausubel et al., 2002).

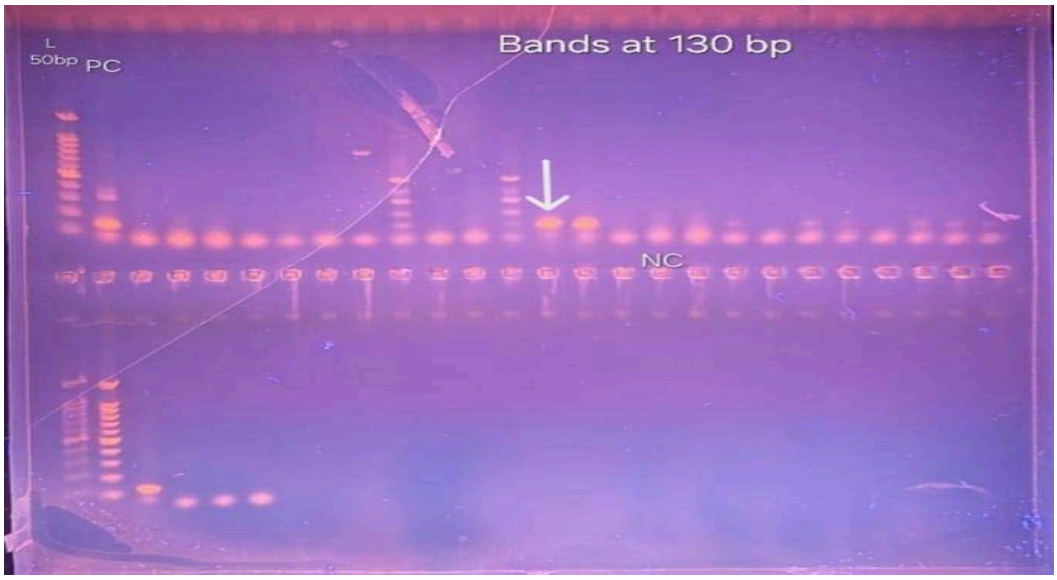

Figure 5: Gel electrophoresis results of *Klebsiella pneumoniae* at 130bp (PC for Positive control, NC for negative control)

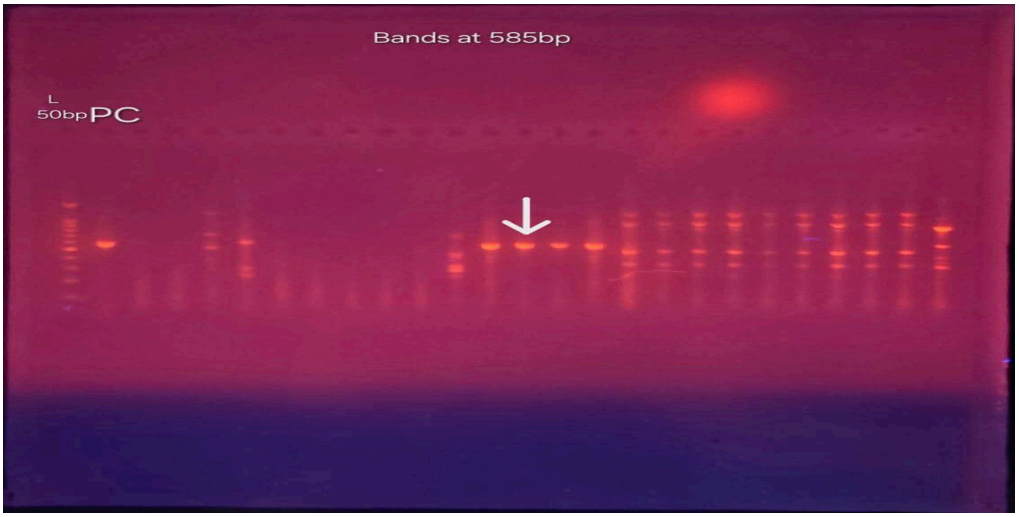

Figure 6: Gel Electrophoresis results of *E. coli* at 585bp (PC for Positive control, NC for negative control)

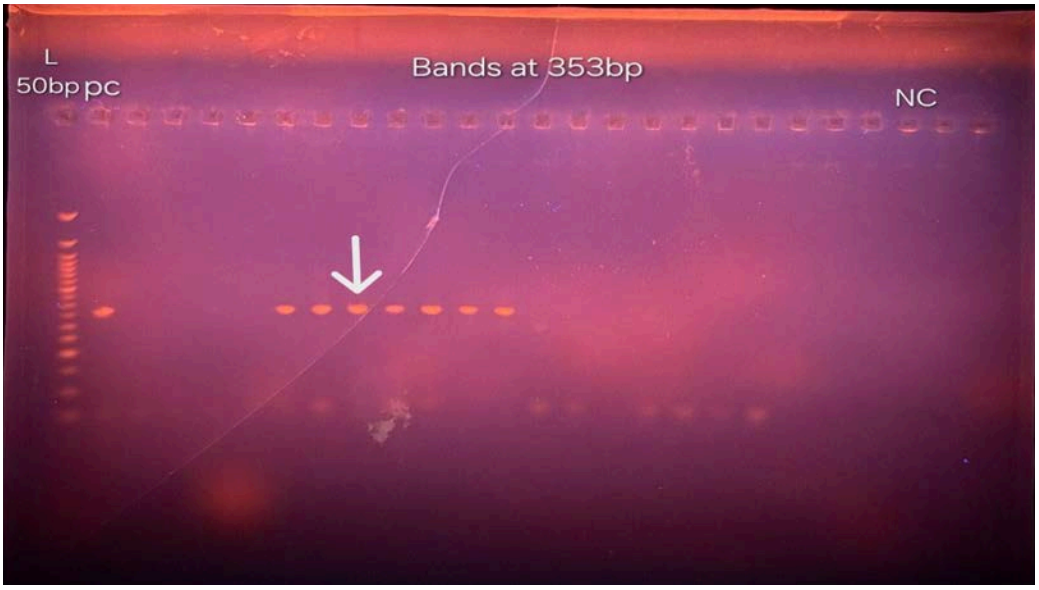

Figure 7: Gel Electrophoresis results of *Acinetobacter baumannii* at 353bp (PC for Positive control, NC for negative control)

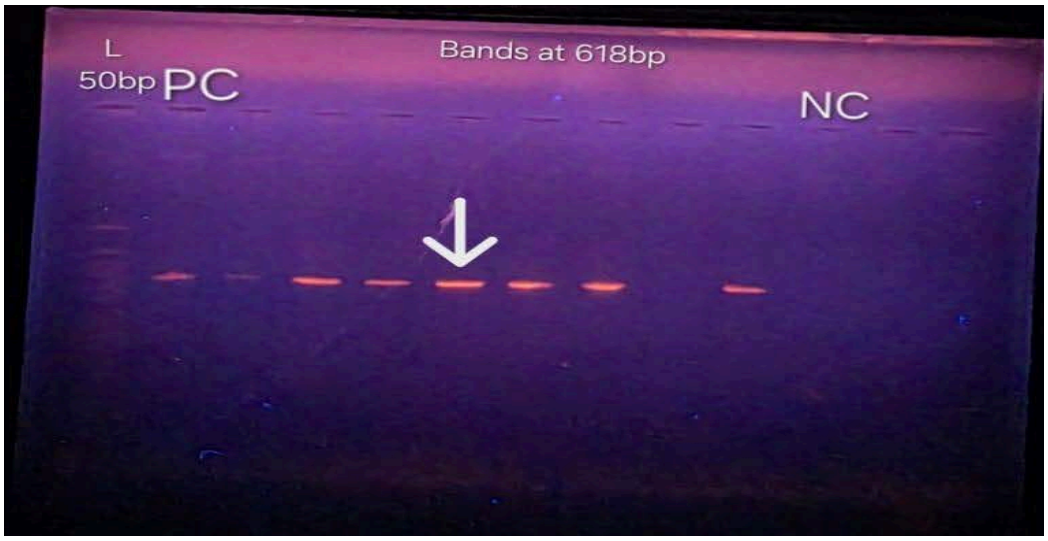

Figure 8: Gel Electrophoresis results of *Pseudomonas aeruginosa* at 628bp (PC for Positive control, NC for negative control)

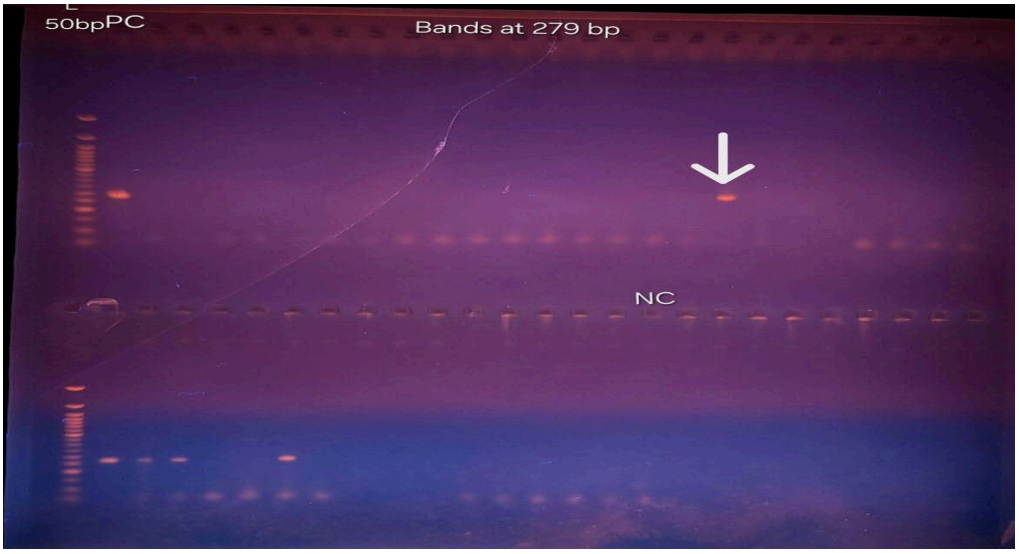

Figure 9: Gel Electrophoresis results of *Staphylococcus aureus* at 279bp (PC for Positive control, NC for negative control)

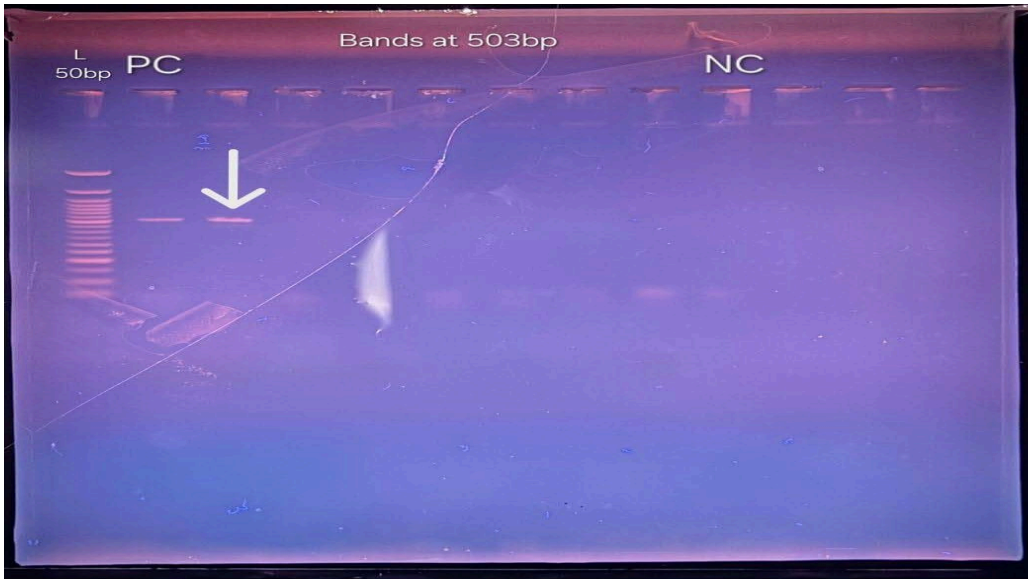

Figure 10: Gel Electrophoresis results of *Staphylococcus epidermidis* at 503bp (PC for Positive control, NC for negative control)

2.12 Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility testing (AST) determines how effectively antimicrobial agents inhibit bacterial growth, guiding clinical treatment decisions. In this study, the Kirby-Bauer disc diffusion method was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. This standardized technique uses Mueller-Hinton agar (MHA) as the medium because its uniform composition and low protein content enable consistent antibiotic diffusion, minimizing variability. MHA plates were prepared by autoclaving the medium and pouring it aseptically into sterile Petri dishes under a laminar flow hood to prevent contamination. Bacterial isolates were standardized by emulsifying a small loopful in 10 mL of sterile saline, vortexing to create a uniform suspension matched visually to a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). This ensures reproducible inoculum density, critical for reliable results.

Inoculation involved dipping a sterile cotton swab into the suspension, rotating it against the tube to remove excess liquid, and streaking it across the MHA surface in three directional rotations (60° apart) to achieve a confluent lawn. Antibiotic-impregnated discs were then placed on the agar using sterile forceps, spaced at least 24 mm apart (center-to-center) to prevent overlap of zones. Plates were inverted and incubated aerobically at 37∘C for 16-24 hours, depending on the organism.

Post-incubation, inhibition zone diameters were measured to the nearest millimeter using a ruler or caliper at the point of complete inhibition. Results were interpreted against CLSI breakpoint tables categorizing zones as "susceptible" (S; effective clinically), "intermediate" (I; possibly effective with higher dosing), or "resistant" (R; unlikely to succeed) and recorded in a spreadsheet for analysis.

This method is cost-effective, widely accessible, and correlates well with minimum inhibitory concentration (MIC) tests, though limitations include diffusion variability for fastidious organisms or certain antibiotics. Antibiotics names of our priority organisms (Tables 3-7)

Spectrum	Class of drug	Antibiotic	Content (µg)	Susceptible (mm)	intermediate (mm)	Resistant (mm)
Broad Spectrum	Carbapenem	Imipenem (IPM)	10	≥ 23	20-22	≤ 19
		Meropenem (MEM)	10	≥ 23	20-22	≤ 19
	Tetracycline	Tetracycline (TE)	30	≥ 15	12-14	≤ 11
	Cephems	Cefepime (FEP)	30	≥25	---------	≤18
		Ceftriaxone (CTR)	30	≥ 23	20-22	≤ 19
	Fluoroquinolone	Ciprofloxacin (CIP)	5	≥ 26	22-25	≤ 21
	Aminoglycosides	Amikacin (AMK)	30	≥ 17	15-16	≤ 14
		Gentamicin (CN)	10	≥ 15	13-14	≤ 12
	Phenicol	Chloramphenicol (C)	30	≥ 18	13-17	≤ 12
	Penicillin	Amoxicillin (AML)	10	≥ 17	14-16	≤ 13
		Ampicillin (AMP)	10	≥ 17	14-16	≤ 13
Narrow Spectrum	Macrolides	Erythromycin (E)	15	≥ 19	15-18	≤ 14

Table 3: Antibiotics used for *Escherichia coli*

Spectrum	Class of drug	Antibiotic	Content (µg)	Susceptible (mm)	intermediate (mm)	Resistant (mm)
Broad Spectrum	Carbapenem	Imipenem (IPM)	10	≥ 22	19-21	≤ 18
		Meropenem (MEM)	10	≥ 18	15-17	≤ 14
	Fluoroquinolone	Levofloxacin (LE)	5	≥ 17	14-16	≤ 13
		Ciprofloxacin (CIP)	5	≥ 21	16-20	≤ 15
	Cephems	Cefepime (FEP)	30	≥ 18	15-17	≤ 14
		Ceftazidime (CAZ)	30	≥ 18	15-17	≤ 14
	Aminoglycoside	Gentamicin (CN)	10	≥ 15	13-14	≤ 12

Table 4: Antibiotics used for *Acinetobacter baumannii*

Spectrum	Class of drug	Antibiotic	Content (µg)	Susceptible (mm)	intermediate (mm)	Resistant (mm)
Broad Spectrum	Lincosamides	Clindamycin (CD)	2	≥ 21	15-20	≤ 14
	Glycopeptide	Vancomycin (VA)	30	≥17	15–16	≤14
	Penicillin	Amoxicillin (AML)	10	≥29	---------	≤28
		Oxacillin (OX)	1	≥ 25	---------	≤ 24
	Oxazolidinones	Linezolid (LZ)	30	≥ 21	---------	≤ 20
	Folate Pathway Inhibitor	Sulfamethoxazole-Trimethoprim (SXT)	25	≥ 16	11-15	≤ 10
	Aminoglycoside	Gentamicin (CN)	10	≥ 15	13-14	≤ 12
	Tetracycline	Tetracycline (TE)	30	≥ 19	15-18	≤ 14
Narrow Spectrum	Macrolides	Erythromycin (E)	15	≥ 23	14-22	≤ 13

Table 5: Antibiotics used for *Staphylococcus species*

Spectrum	Class of drug	Antibiotic	Content (µg)	Susceptible (mm)	Intermediate (mm)	Resistant (mm)

Broad Spectrum	Carbapenem	Imipenem (IPM)	10	≥ 19	16-18	≤ 15
		Meropenem (MEM)	10	≥ 19	16-18	≤ 15
	Fluoroquinolone	Levofloxacin (LE)	5	≥ 22	15-21	≤ 14
		Ciprofloxacin (CIP)	5	≥ 25	19-24	≤ 18
	Cephems	Cefepime (FEP)	30	≥ 18	15-17	≤ 14
		Ceftazidime (CAZ)	30	≥ 21	---------	≤ 20
	Aminoglycoside	Gentamicin (CN)	10	≥ 15	13-14	≤ 12

Table 6: Antibiotics used for *Pseudomonas aeruginosa*

Spectrum	Class of drug	Antibiotic	Content (µg)	Susceptible (mm)	intermediate (mm)	Resistant (mm)

Broad Spectrum	Carbapenem	Imipenem (IPM)	10	≥ 23	20-22	≤ 19
Broad Spectrum		Meropenem (MEM)	10	≥ 23	20-22	≤ 19
Broad Spectrum	Cephems	Cefepime (FEP)	30	≥ 25	19-24	≤ 18
	Cephems	Ceftriaxone (CRO)	30	≥ 23	20-22	≤ 17
		Ceftazidime (CAZ)	30	≥ 21	18-20	≤ 17
	Fluoroquinolone	Levofloxacin (LE)	5	≥ 25	17-24	≤ 16
		Ciprofloxacin (CIP)	5	≥ 26	22-25	≤ 21
	Oxazolidinones	Linezolid (LZ)	30	≥21	18–20	≤17
	Tetracycline	Tetracycline (TE)	30	≥ 15	12-14	≤ 11
	Aminoglycoside	Gentamicin (CN)	10	≥ 15	13-14	≤ 12
	Glycopeptide	Vancomycin (VA)	30	---------	---------	---------
Broad Spectrum	Folate Pathway Inhibitor	Sulfamethoxazole-Trimethoprim (SXT)	25	≥ 16	11-15	≤ 10
Narrow Spectrum	Macrolides	Erythromycin (E)	15	---------	---------	---------

Table 7: Antibiotics used for *Klebsiella pneumoniae*

2.13 Stock Preparation of Bacterial Isolates

For long-term preservation of the confirmed bacterial isolates, glycerol stock cultures were prepared using Nutrient Broth (NB) and sterile glycerol. Freshly grown bacterial colonies were first inoculated into sterile Nutrient Broth and incubated at 37 °C for 18–24 hours to obtain an actively growing culture. This step ensured that viable and healthy bacterial cells were used for stock preparation.

After incubation, the broth culture was mixed with sterile glycerol to prepare glycerol stocks. Typically, 600 µL of overnight NB culture was mixed with 400 µL of sterile glycerol (80–100%) to obtain a final glycerol concentration of approximately 15–20%, which is optimal for cryopreservation.

Glycerol acts as a cryoprotectant, preventing the formation of ice crystals that can damage bacterial cell membranes during freezing. The prepared stock cultures were then transferred into sterile cryovials, properly labeled, and stored at -80 °C for long-term preservation. These stocks were later used for revival and further experimental procedures as needed.

Chapter 3

Results

3.1 Target bacteria in all samples

Among 22 samples, *Klebsiella pneumoniae* had the highest prevalence (36.36%), making it the most dominant organism. Then *E. coli* and *Staphylococcus spp.* were the second most prevalence

with (18.18%). *Acinetobacter baumannii* (13.64%), while *Pseudomonas aeruginosa* had the lowest presence (9.09%). Overall, a few bacteria were highly dominant, whereas others appeared less frequently in the samples. The (figure 11) shows the prevalence of different bacterial species in the samples, with clear differences among them.

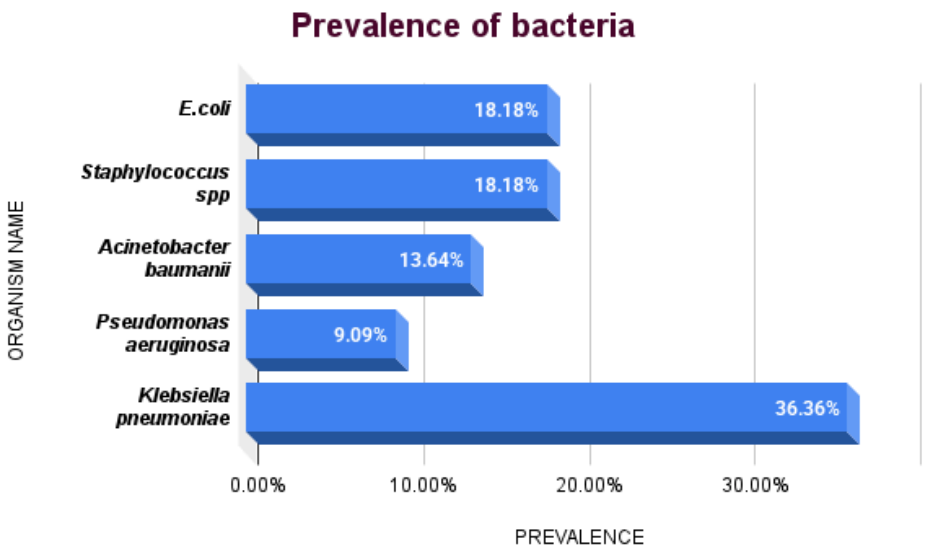

Figure 11: Prevalence of bacteria

3.2 Antibiotic Susceptibility Test Result of *Klebsiella pneumoniae*

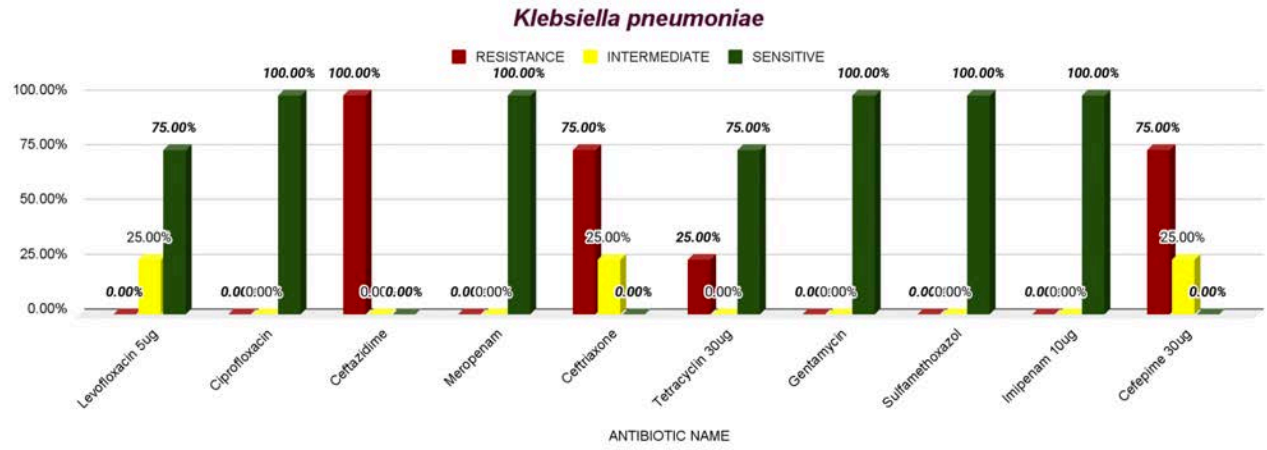

Figure 12: AST of *Klebsiella pneumoniae* (Total isolates 8)

The antibiotic susceptibility profile of *Klebsiella pneumoniae* in this study demonstrates a distinct resistance pattern, particularly against cephalosporins. Complete resistance (100%) was observed to Ceftazidime, indicating strong resistance to third-generation cephalosporins. High resistance was also noted for Ceftriaxone and Cefepime, each showing 75% resistance, suggesting reduced effectiveness of both third- and fourth-generation cephalosporins. Additionally, Tetracycline exhibited 25% resistance. Levofloxacin and Ceftriaxone showed 25% intermediate susceptibility, indicating partial effectiveness in a subset of isolates.

In contrast, several antibiotics retained high efficacy. Ciprofloxacin, Meropenem, Gentamycin, Sulfamethoxazole, and Imipenem demonstrated 100% sensitivity, indicating strong activity against the isolates tested. These findings suggest that while cephalosporin resistance is prominent, carbapenems, aminoglycosides remain highly effective therapeutic options. Overall, the data reflect a polarized susceptibility pattern, with significant resistance confined mainly to the cephalosporin class, while alternative antibiotic classes continue to show reliable activity against *Klebsiella pneumoniae* in this study.

3.3 Antibiotic Susceptibility Test Result of *Escherichia coli*

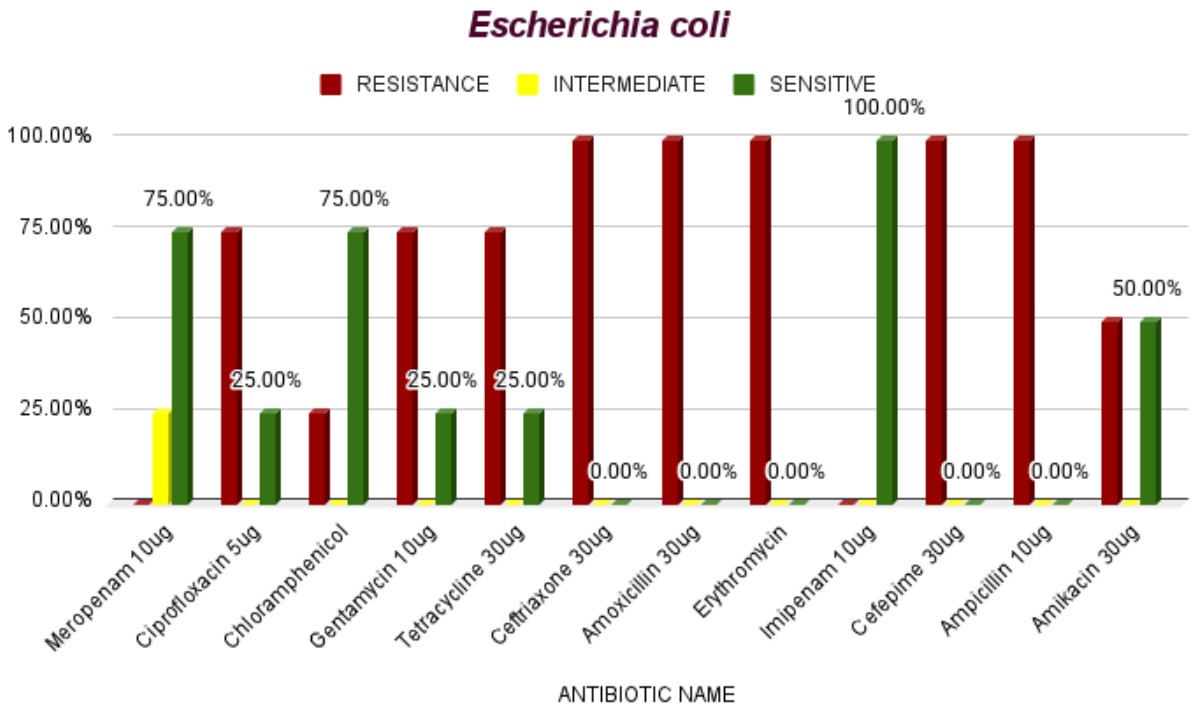

Figure 13: AST of *Escherichia coli* (Total isolates 4)

The antibiotic susceptibility profile for *Escherichia coli* isolates demonstrated a higher resistance pattern, characterized by extensive resistance to several common therapeutic agents and total susceptibility to others. High-level resistance reached 100% for Ceftriaxone (30 ug), Amoxicillin (30 ug), Erythromycin, Cefepime (30 ug), and Ampicillin (10 ug). This total resistance across multiple classes, including third and fourth-generation cephalosporins and penicillin's, strongly suggests the presence of Extended-Spectrum Beta-Lactamases (ESBLs) within the isolates. Furthermore, substantial resistance of 75% was observed against Ciprofloxacin (5 ug), Gentamycin (10 ug), and Tetracycline (30 ug). In significant contrast, Imipenem (10 ug) showed a

100% sensitivity rate, and Meropenem (10 ug) exhibited a 75% sensitivity rate (with 25% intermediate susceptibility), highlighting carbapenems as the most effective remaining options. Additionally, Chloramphenicol remained highly effective with 75% sensitivity, while Amikacin (30 ug) showed a balanced distribution with 50% sensitivity and 50% resistance. These findings underscore a critical multidrug-resistant (MDR) profile for *E. coli* in this study, necessitating strict adherence to susceptibility-guided prescribing (Figure 13).

3.4 Antibiotic Susceptibility Test Result of *Staphylococcus spp.*

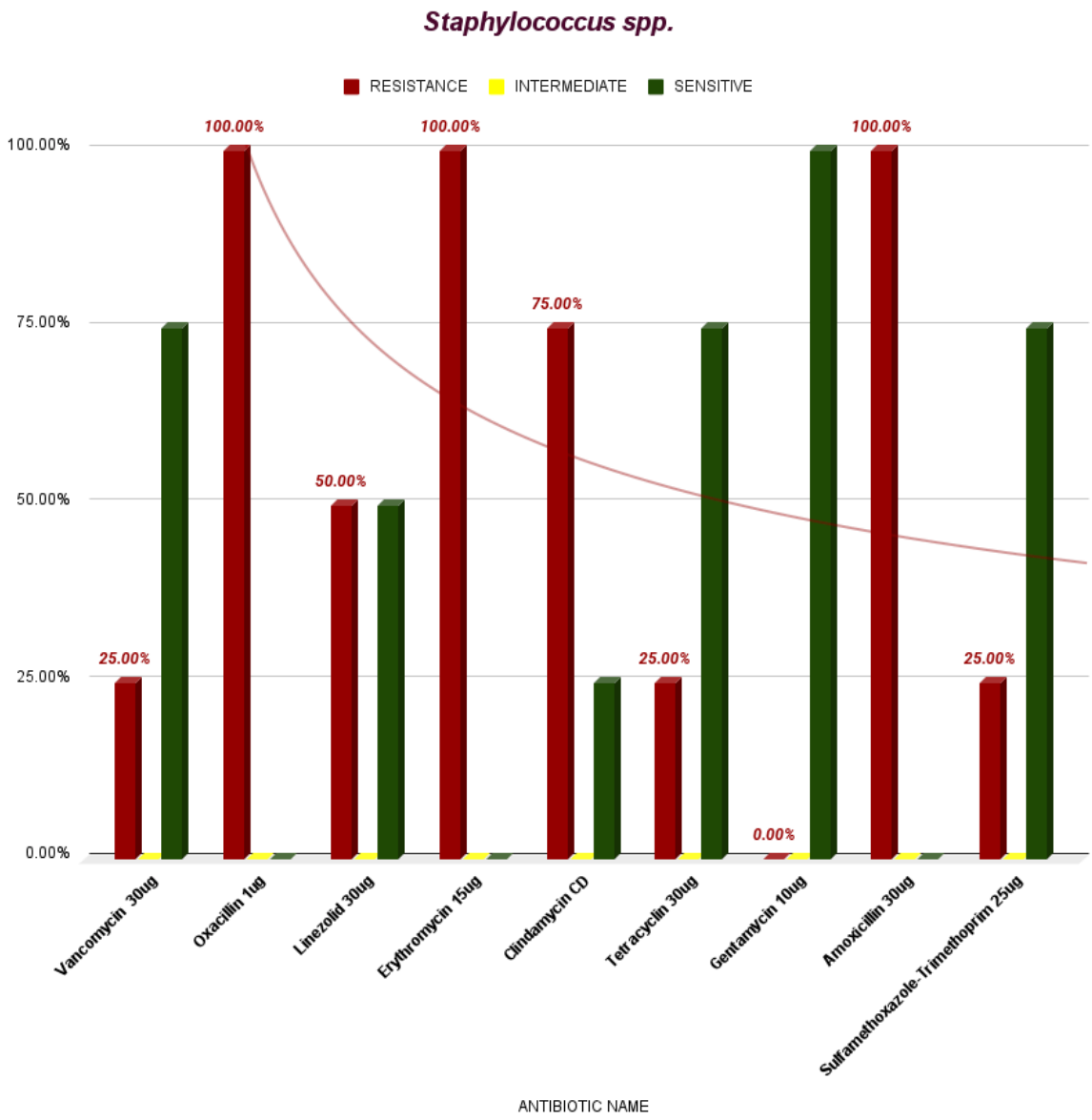

Figure 14: AST of *Staphylococcus spp.* (Total isolates 4)

The antibiotic susceptibility profile for Staphylococcus spp. isolates revealed a concerning level of resistance to several conventional antimicrobial agents, particularly within the penicillin and macrolide classes. The isolates demonstrated 100% resistance to Oxacillin (1 μg), Erythromycin (15 μg), and Amoxicillin (30 μg), suggesting a high prevalence of methicillin resistance and cross-resistance to related β-lactams and macrolides. Furthermore, substantial resistance was observed for Clindamycin CD at 75.00%, while Linezolid (30 μg) showed a balanced profile with 50.00% resistance and 50.00% sensitivity. Conversely, Gentamicin (10 μg) emerged as the most effective agent with a 100% sensitivity rate, followed by Vancomycin (30 μg), Tetracycline (30 μg), and Sulfamethoxazole-Trimethoprim (25 μg), all of which maintained a 75.00% sensitivity rate. These findings indicate that while multidrug resistance is prominent among these *Staphylococcus spp* isolates, aminoglycosides and glycopeptides remain viable therapeutic options for clinical management (Figure 14).

3.5 Antibiotic Susceptibility Test Result of *Acinetobacter baumannii*

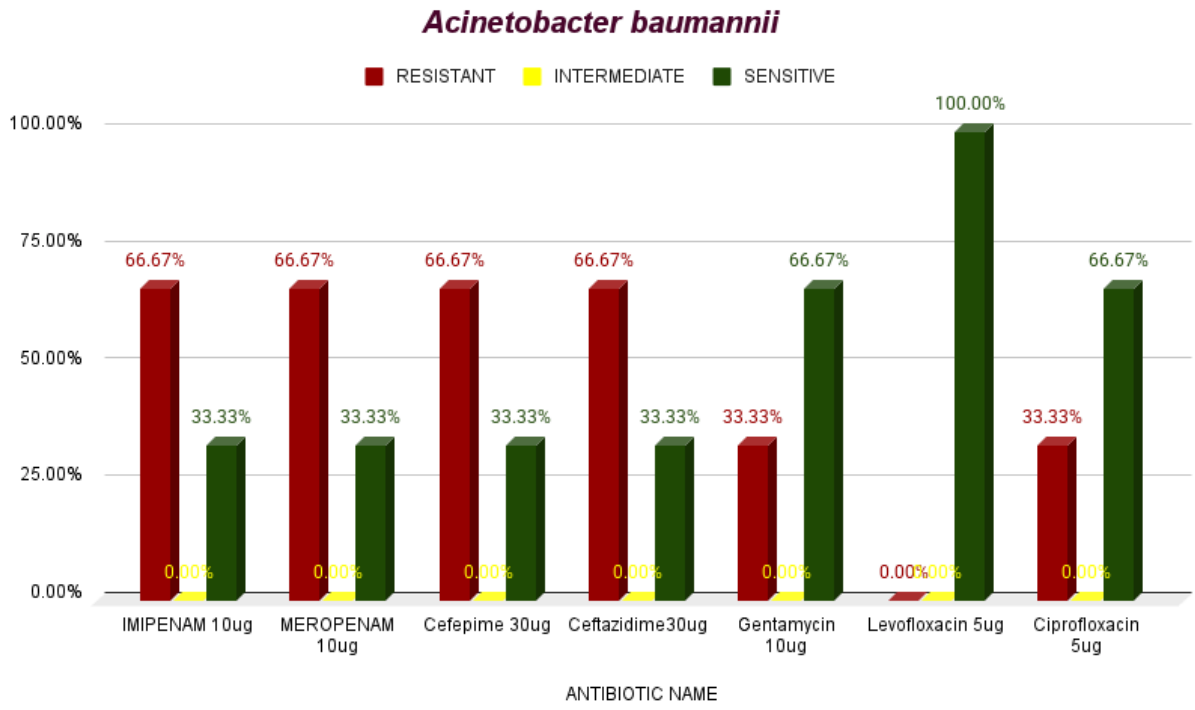

Figure 15: AST of *Acinetobacter baumannii* (Total isolates 3)

The antibiotic susceptibility profile of *Acinetobacter baumannii* revealed a concerning trend of multidrug resistance, particularly among the carbapenem and cephalosporin classes, where Imipenem (10 ug), Meropenem (10 ug), Cefepime (30 ug), and Ceftazidime (30 ug) all exhibited a high resistance rate of 66.67%. This significant resistance to primary beta-lactam treatments suggests the presence of robust enzymatic degradation mechanisms, such as carbapenemases, within the isolates. Conversely, the fluoroquinolone class demonstrated the highest therapeutic potential, with Levofloxacin (5 ug) achieving a 100% sensitivity rate, followed by Ciprofloxacin (5 ug) and the aminoglycoside Gentamycin (10 ug), both of which showed a sensitivity of 66.67%. Notably, the absence of intermediate susceptibility 0% across all tested agents indicates a

polarized resistance pattern, identifying Levofloxacin as the most effective *in vitro* candidate for treating the specific *A. baumannii* strains analyzed in this study (Figure 15).

3.6 Antibiotic susceptibility test result of *Pseudomonas aeruginosa*

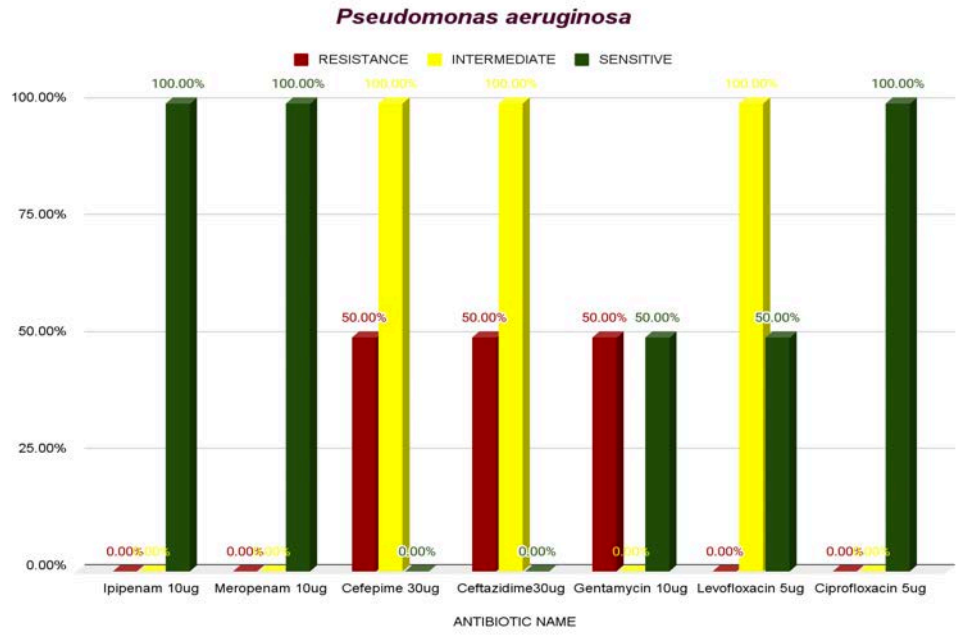

Figure 16: AST of *Pseudomonas aeruginosa* (Total isolates 2)

The antibiotic susceptibility profile for *Pseudomonas aeruginosa* isolates revealed a distinct pattern of susceptibility to carbapenems and fluoroquinolones, contrasted by significant intermediate resistance in other classes. The isolates demonstrated 100% sensitivity to Imipenem (10 ug), Meropenem (10 ug), and Ciprofloxacin (5 ug), identifying these as the most potent therapeutic options among the agents tested. However, a highly unusual and concerning trend of 100% intermediate susceptibility was observed for Cefepime (30 ug), Ceftazidime (30 ug), and Levofloxacin (5 ug). Within these specific categories, 50% of the isolates were also classified as resistant to Cefepime and Ceftazidime, indicating a severe reduction in the efficacy of these third and fourth-generation cephalosporins. Furthermore, Gentamicin (10 ug) showed a divided profile

with 50% sensitivity and 50% resistance. These findings underscore the critical nature of *P. aeruginosa* as an opportunistic pathogen with a high propensity for developing complex resistance profiles, necessitating the prioritization of carbapenems for effective clinical management in this study population (Figure 16).

3.7 Total resistance profile of AST results

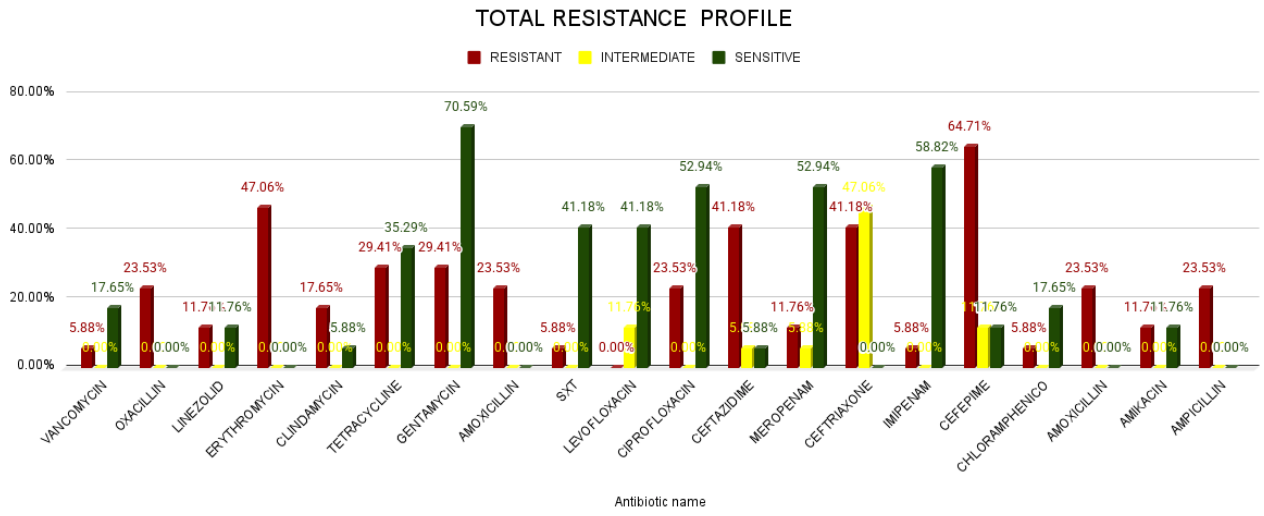

Figure 17: Total resistance profile

The antimicrobial susceptibility pattern of the isolates revealed a concerning level of resistance, particularly against Beta-lactam antibiotics. High resistance was observed for cefepime (64.71%), erythromycin (47.06%), ceftazidime (41.18%), and ceftriaxone (41.18%), indicating substantial resistance toward third- and fourth-generation cephalosporins and macrolides. Moderate resistance was noted for tetracycline (29.41%), gentamicin (29.41%), amoxicillin (23.53%), and ampicillin (23.53%). In contrast, carbapenems, including imipenem (58.82%) and meropenem (52.94%), demonstrated comparatively higher sensitivity, suggesting that they remain among the

most effective therapeutic options. Fluoroquinolones such as ciprofloxacin and levofloxacin showed moderate effectiveness, while vancomycin and linezolid exhibited good activity, reflecting lower resistance rates. Overall, these findings indicate a high burden of multidrug resistance, emphasizing the urgent need for strict antibiotic stewardship and routine resistance surveillance. The observed resistance trends align with previous regional and international studies. For instance, a multicenter study reported cephalosporin resistance exceeding 60% and macrolide resistance over 45%, with carbapenems retaining the highest sensitivity (Ahmed et al., 2023). Similarly, WHO global surveillance data documented widespread resistance to third-generation cephalosporins, especially among Gram-negative pathogens (WHO, 2023). Regional studies also report rising resistance to ceftriaxone (40–65%) and ciprofloxacin (30–50%), consistent with the present findings (Rahman et al., 2022), while carbapenems continue to serve as last-resort treatments, although emerging resistance remains a growing concern (Nordmann & Poirel, 2019) (Figure 17).

3.8 Multi-drug resistant

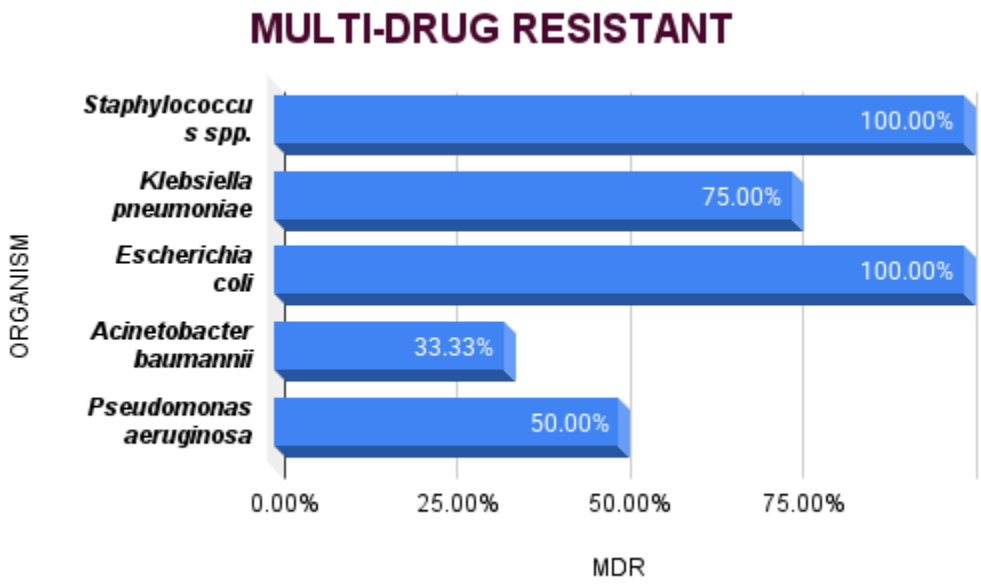

Figure 18: MDR patterns of the total isolates of 21.

The graph demonstrates a high prevalence of multidrug resistance among the isolated bacterial pathogens. *Staphylococcus spp.* and *E. coli* exhibited the highest MDR rates at 100%, while *Klebsiella pneumoniae* showed substantial resistance at 75%. Moderate MDR was observed in *Pseudomonas aeruginosa* (50%), and *Acinetobacter baumannii* had the lowest rate (33.33%) (Figure 18). These findings highlight a significant burden of multidrug-resistant organisms, posing challenges for empirical therapy and infection control.

These MDR patterns are consistent with both regional and global trends. Previous hospital-based studies have reported MDR rates of 85–100% in *E. coli* and *Staphylococcus spp.* and 70–80% in *Klebsiella pneumoniae*, closely matching the present findings. Globally, surveillance data indicate

that over 50% of *E. coli* and *Klebsiella pneumoniae* isolates in South-East Asia are multidrug resistant, with neighboring countries reporting rates between 50–90%. This underscores the widespread and persistent threat of multidrug resistance in healthcare settings. (Ondigui et al., 2022)

Chapter 4

Discussion

The present study provides important insights into the microbial contamination of hospital environments and the distribution of priority pathogens associated with nosocomial infections in Bangladesh. The isolation of *Klebsiella spp.*, *Escherichia coli*, *Pseudomonas spp.*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* from frequently touched hospital surfaces highlights the persistent risk of environmental reservoirs contributing to hospital-acquired infections. These findings are consistent with several studies conducted in Bangladesh, where similar organisms have been repeatedly identified as dominant nosocomial pathogens (Tania et al., 2023; Rahman et al., 2020).

The clinical impact of these pathogens is profound. The Gram-negative members of this group- *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, are primary causative agents of catheter-associated urinary tract infections (CAUTIs) and ventilator-associated pneumonia (VAP). Meanwhile, *A. baumannii* is notorious for its survival in dry conditions, often leading to difficult-to-treat outbreaks in intensive care units (ICUs). Gram-positive *Staphylococcus* spp., particularly methicillin-resistant strains, are easily shed from the skin of healthcare workers and patients, contaminating surfaces and subsequently entering the bloodstream or surgical sites.

The transition of these bacteria from environmental surfaces to patients is a major driver of Healthcare-Associated Infections (HAIs). Because these organisms often harbor mobile genetic

elements, such as plasmids and transposons, the hospital environment becomes a "melting pot" for the exchange of antibiotic resistance genes. Understanding the prevalence and susceptibility profiles of these specific bacteria on environmental surfaces is therefore essential for developing effective infection control strategies and reducing the morbidity associated with hospital-acquired superbugs. (García-Solache & Rice, 2019)

Among the isolates in our study, *Klebsiella pneumoniae* emerged as the most prevalent organism, which aligns with previous Bangladeshi studies reporting prevalence rates of 25–32% in hospital samples (IJRSI, 2024). *Klebsiella pneumoniae* species are widely recognized as critical nosocomial pathogens due to their remarkable ability to survive desiccation and persist on inanimate surfaces such as bed rails, saline stands, ventilators, and floors. Their high prevalence in our samples may be attributed to this resilience, which allows them to persist in dry, nutrient-limited environments more effectively than *E. coli*, which prefers nutrient-rich clinical specimens. Large-scale cross-sectional studies in Bangladesh have shown that *Klebsiella pneumoniae* accounted for 15.5% of over 230,000 clinical isolates, second only to *E. coli* among major pathogens (Mdpi Resist. Patterns, 2025). Similarly, environmental surveillance from intensive care units has reported *Klebsiella* as one of the top three organisms recovered from hospital surfaces (Bangladesh J Med Microbiol ICU data, 2014), highlighting its capacity to act as a persistent environmental reservoir. This resilience has significant implications for nosocomial transmission, as contaminated surfaces can serve as a vector for patient infections, particularly in high-risk areas such as ICUs.

E. coli, in contrast, shows more variable prevalence depending on the type of sample and the hospital environment. In Bangladesh, hospital-based studies report rates ranging from 13%, ranking after *Staphylococcus aureus* (28%) and *Pseudomonas aeruginosa* (17%) (Farzin et al., 2024), to 32–49% in urinary and clinical samples (BMC Infectious Diseases, 2025). In our study, *E. coli* was one of the prevalent organisms, suggesting that while it is still a significant contaminant, it does not survive dry surfaces as effectively as *Klebsiella*. Environmental survival studies indicate that *E. coli* is less resilient to desiccation and often requires organic residues or moisture to persist, which may explain its lower recovery from surfaces such as bed rails, ventilators, and floors. Despite this, its presence remains clinically relevant, as environmental reservoirs of *E. coli* can contribute to hospital-acquired infections through contact with contaminated surfaces or medical equipment.

Internationally, *E. coli* is often the dominant nosocomial pathogen, particularly in ICUs and urinary tract infections, with prevalence rates exceeding 40–60% (WHO, 2017). However, in Bangladesh, environmental prevalence is more variable, sometimes ranking lower due to higher relative abundances of opportunistic pathogens such as *Pseudomonas* and *Acinetobacter*, which thrive in moist, contaminated hospital areas. Furthermore, antimicrobial resistance among *E. coli* isolates is a significant concern. Meta-analyses show that around 21% of *E. coli* isolates in Bangladesh are ESBL producers (Hossain et al., 2023), emphasizing that resistant strains may persist longer in the environment and increase the risk of nosocomial transmission.

The co-occurrence of *Klebsiella* and *E. coli* in hospital surfaces highlights the mixed environmental-clinical contamination pattern characteristic of Bangladeshi healthcare settings.

While *Klebsiella* dominates environmental samples due to its resilience, *E. coli* remains a significant pathogen, reflecting both clinical shedding and environmental contamination. Their presence on frequently touched surfaces, medical equipment, and high-risk hospital zones underscores the need for integrated infection control measures, including routine cleaning of surfaces, hand hygiene reinforcement, and environmental monitoring. Additionally, the contrast between environmental and clinical prevalence of these organisms emphasizes that relying solely on clinical surveillance may underestimate environmental reservoirs, which play a critical role in pathogen transmission and hospital-acquired infections. The presence of these organisms in environmental samples in the current study reinforces the role of hospital surfaces as continuous sources of infection transmission.

Gram-positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* were detected in our environmental samples. In Bangladesh, *S. aureus* is consistently reported among the leading clinical pathogens, with prevalence rates ranging from 20–25% in hospital isolates (Tania et al., 2023). *Staphylococci* like *Staphylococcus epidermidis* are frequently associated with device-related infections due to their ability to form biofilms on medical equipment (Islam et al., 2019). Their presence on surfaces such as bed rails, ventilators, and other hospital equipment suggests that inadequate disinfection and environmental persistence contribute to their contamination.

Compared with earlier studies in Bangladesh, pre-2015 research often reported *E. coli* and *S. aureus* as the dominant isolates; however, more recent studies indicate that Gram-negative bacteria are becoming increasingly prevalent in environmental samples (Islam et al., 2019). This

trend highlights that while Gram-positive organisms are clinically important, they are often less dominant on hospital surfaces, particularly those associated with urinary contamination.

Internationally, *Staphylococcus aureus* remains a major nosocomial pathogen implicated in bloodstream, surgical site, and device-related infections (WHO, 2017). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a notable concern, with prevalence in Bangladesh reported as high as 42% in some cross-sectional surveillance studies (Mdpi national AMR, 2025). *S. epidermidis*, although less virulent, contributes to hospital ecology by colonizing indwelling medical devices and forming biofilms, serving as a reservoir for nosocomial infections (Rahman et al., 2020).

The relatively low proportion of Gram-positive organisms in our environmental samples aligns with global observations that Gram-negative bacteria dominate hospital surfaces. Nonetheless, the presence of *Staphylococcus spp.* underscores their continuous role in nosocomial transmission and highlights the need for stringent environmental cleaning and infection control practices in hospitals

Acinetobacter baumannii was detected in our environmental sample through Leeds media, which is consistent with its preference for ICU and respiratory-associated niches. In this study, Leeds media, which favors growth of *Acinetobacter baumannii* but may not fully recover organisms from dry surfaces. Environmental studies in Bangladesh have reported *Acinetobacter baumannii* prevalence on ICU surfaces ranging from 5% to over 20%, depending on the hospital area and sample type (Rahman et al., 2020; Islam et al., 2019). The very low recovery in our samples

likely reflects both the sampling strategy focusing on urinary-associated surfaces and the limited environmental persistence of this organism outside moist or biofilm-prone niches.

Acinetobacter baumannii remains a critical nosocomial pathogen due to its ability to survive harsh conditions and rapidly acquire resistance. In Bangladesh, *Acinetobacter baumannii* shows high rates of multidrug resistance, particularly to carbapenems and aminoglycosides, with prevalence in ICU isolates reported at 70–80% (NMCJ, 2024). Internationally, *Acinetobacter baumannii* is recognized as a persistent environmental contaminant in ICU settings, capable of surviving on dry surfaces for extended periods and forming biofilms that facilitate nosocomial transmission (Peleg et al., 2008). These findings highlight that environmental detection is highly niche-dependent and may not fully reflect clinical importance.

Pseudomonas aeruginosa was infrequently encountered in our samples, consistent with its preference for moist environments and respiratory or wound-associated surfaces. Selective isolation was performed using Cetrimide media, which favors *Pseudomonas aeruginosa* growth but may under-represent organisms on dry, urinary-associated surfaces. ICU and wound surveillance studies in Bangladesh report *Pseudomonas aeruginosa* prevalence ranging from 10% to 21%, particularly on sinks, ventilators, and wound-care equipment (Rahman et al., 2020). Its low frequency in our samples illustrates how selective media choice and environmental niche significantly influence recovery rates.

Globally, *Pseudomonas aeruginosa* is a major opportunistic pathogen, frequently implicated in ventilator-associated pneumonia, catheter-related bloodstream infections, and wound infections. Its ability to form biofilms and survive in water-rich hospital environments explains its limited

detection on dry surfaces like bed rails or floors in our study. This underscores the importance of targeting appropriate sampling sites and media to fully understand environmental reservoirs of *Pseudomonas aeruginosa* in hospital settings.

The analysis of antibiotic susceptibility in our environmental isolates highlights a concerning trend of widespread resistance among commonly encountered nosocomial pathogens. In our dataset, *Staphylococcus spp*. and *Escherichia coli* displayed complete resistance to multiple classes of commonly used antibiotics, while *Klebsiella pneumoniae* showed resistance in 75% of isolates, and even the least resistant species, *Acinetobacter baumannii*, demonstrated resistance in approximately one-third of cases. This pattern indicates that a significant proportion of hospital-associated bacteria are no longer susceptible to many standard therapies, posing a substantial challenge for effective infection control and treatment (Rahman et al., 2020; NMCJ, 2024).

The underlying cause of this high resistance appears to be selective pressure from frequent antibiotic use, combined with environmental persistence and adaptability of these organisms. For instance, the elevated resistance observed against commonly prescribed cephalosporins and macrolides, with Cefepime and Erythromycin showing resistance rates of 64.71% and 47.06%, respectively, and Ceftriaxone at intermediate sensitivity of 47.06%, suggests that repeated exposure to these drugs has rendered them increasingly ineffective. In contrast, antibiotics that are typically reserved as last-line agents, such as aminoglycosides (Gentamycin) and carbapenems (Imipenem), still maintain moderate efficacy, with sensitivity rates of 70.59% and 58.82%, respectively. These findings are consistent with national surveillance reports from Bangladesh,

which indicate that multidrug-resistant Gram-negative bacteria now dominate hospital environments, particularly in high-risk areas such as intensive care units (Islam et al., 2019; NMCJ, 2024).

Comparing these findings with prior Bangladeshi studies reveals a temporal increase in resistance levels. Studies conducted between 2010 and 2015 reported comparatively lower resistance rates for *E. coli* and *Klebsiella pneumoniae*, whereas recent surveillance demonstrates a 10–15% increase in resistance over a few years (Islam et al., 2019). This escalation mirrors the growing misuse and over-prescription of broad-spectrum antibiotics in hospitals, inadequate infection control, and the persistence of bacteria on environmental surfaces such as bed rails, ventilators, and saline stands. Internationally, similar patterns are observed in low- and middle-income countries, where high rates of resistance in *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* species are linked to hospital-acquired infections and limited availability of last-line antibiotics (WHO, 2017; Allegranzi et al., 2018).

Interestingly, the pattern of resistance in our study also reflects the differences between environmental and clinical isolates. Gram-negative organisms such as *Klebsiella pneumoniae* and *E. coli* dominate the environmental samples due to their ability to survive desiccation and persist on hospital surfaces, which allows them repeated exposure to sub-inhibitory antibiotic residues and disinfectants, fostering resistance development. In contrast, *Staphylococcus spp.,* though highly resistant in our study, were present at moderate frequencies, reflecting their typical niche on skin and mucosal surfaces rather than environmental reservoirs. Similar findings have been reported in both national and international environmental surveillance studies, emphasizing that

persistence in the environment is a key factor influencing resistance patterns (Tania et al., 2023; Allegranzi et al., 2018).

The limited recovery of *Acinetobacter baumannii* (33.33% resistance) aligns with its known preference for ICU and respiratory surfaces rather than urinary tract-associated environmental sites. While its overall presence was low, the observed resistance indicates a clinically significant risk, as even a minority of resistant isolates can serve as reservoirs for horizontal gene transfer, further spreading resistance genes among other hospital pathogens (Rahman et al., 2020).

Overall, these results underscore a critical need for comprehensive environmental surveillance in Bangladesh, alongside rational antibiotic stewardship. Despite the alarming resistance levels observed, the continued susceptibility to aminoglycosides and carbapenems provides an opportunity for targeted therapy; however, reliance on these last-line options must be carefully managed to prevent further emergence of pan-resistant strains. Expanding multi-center studies, improving hygiene and disinfection practices, and monitoring environmental reservoirs are essential steps toward controlling the spread of these highly resistant nosocomial pathogens, aligning Bangladesh's efforts with global infection control standards (WHO, 2017; Allegranzi et al., 2018).

4.1 Limitations:

This study has several limitations that should be acknowledged. First, the research was conducted in only two hospitals, and obtaining formal permission restricted the number and types of sampling sites, which may not fully represent the microbial diversity and prevalence of

nosocomial pathogens across other healthcare settings in Bangladesh. Hospital environments vary greatly in hygiene practices, patient load, and infection control measures, so the findings may not be fully generalizable. Second, the study focused on selective media targeting priority pathogens, primarily *Klebsiella pneumoniae*, *E. coli, Staphylococcus spp., Acinetobacter baumannii, and Pseudomonas aeruginosa*. As a result, other clinically relevant bacteria may have been missed, potentially underestimating the overall microbial burden and diversity in hospital environments.

4.2 Future Prospects:

Future research should aim to expand environmental surveillance to more hospitals across different regions and include a broader range of bacterial species. Incorporating molecular techniques such as PCR-based detection of resistance genes and whole-genome sequencing, along with long-term monitoring, can enhance detection accuracy and provide better insight into the prevalence of resistant strains. These strategies will support more effective infection control practices, inform rational antibiotic use, and ultimately contribute to safer hospital environments, reducing the burden of nosocomial infections and improving patient outcomes in Bangladesh.

Chapter 5

Conclusion

This study highlights that hospital environments in Bangladesh act as significant reservoirs for clinically important pathogens. The findings align with national and international literature, showing that Gram-negative organisms generally dominate nosocomial pathogens, while Gram-positive bacteria, though present, are less frequent in environmental reservoirs. Contamination of high-touch surfaces such as bed rails, ventilators, and medical equipment plays a critical role in pathogen persistence and transmission within healthcare settings.

The analysis also underscores the growing concern of antimicrobial resistance, with many isolates showing reduced susceptibility to multiple drug classes, which limits treatment options and complicates infection management. While the study provides valuable insight into environmental reservoirs and their potential contribution to hospital-acquired infections, it also emphasizes the limited scope of comprehensive surveillance in Bangladesh, revealing a substantial knowledge gap. Addressing this gap will require expanded environmental monitoring, stricter hygiene and disinfection protocols, and the incorporation of molecular detection methods to track and control resistant strains more effectively. Implementing these measures is essential for reducing the prevalence of hospital-acquired infections, improving patient outcomes, and strengthening infection control strategies in the country.

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