Analysis of Antimicrobial Resistance in *Acinetobacter baumannii* and *Klebsiella pneumoniae* from Patients with Wound Infections

By

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

Department of Mathematics and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

Permission to collect samples for conducting this study was obtained from the departmental review board at National Institute of Traumatology and Orthopaedic Rehabilitation (NITOR) hospital Dhaka, Bangladesh. Swab samples from post-traumatic wound infection from patients were collected and consent was taken in agreement to use the samples for thesis purposes. Patients' privacy and data’s confidentiality were strictly protected during data collection.

Abstract/ Executive Summary

This study assessed the antimicrobial resistance profiles, key resistance genes, and hemolytic phenotypes of the organisms *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from 61 pre-surgical traumatic leg wound samples in Dhaka. Of the specimens, 34 (55.7%) yielded target organisms: 15 *A. baumannii* and 19 *K. pneumoniae*. Both species demonstrated extremely high multidrug non-susceptibility across all tested antibiotic classes, with *A. baumannii* showing complete resistance to several agents. Colistin and Tigecycline remained the only antibiotics with comparatively lower resistance. All isolates exhibited γ-hemolysis, indicating the absence of RBC lysis. Molecular testing confirmed the presence of class 1 and class 2 integron integrase genes in *A. baumannii*, supporting the observed co-resistance patterns. These findings underscore a severe local resistance burden that compromises empiric therapy, highlight the need for routine culture-guided management, and emphasize the importance of strengthened antimicrobial stewardship and infection-control measures in trauma care settings.

Keywords: *Acinetobacter baumannii*; *Klebsiella pneumoniae*; Multidrug resistance; integron integrase genes; Traumatic pre-surgical leg wound infections.

Dedication (Optional)

Let this stand as a testament to my own determination and growth.

May I never falter.

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List of Acronyms

bp	Base Pair(s)
CLSI	Clinical and Laboratory Standards Institute
CR	Carbapenem-resistant
dNTPs	Deoxynucleotide Triphosphates
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ESBL	Extended Spectrum β-Lactamase
HAIs	Healthcare-Associated Infections
kb	Kilobase
MIC	Minimum Inhibitory Concentration
MDR	Multidrug-resistant
PCR	Polymerase Chain Reaction
SSIs	Surgical Site Infections
TBE	Tris-borate-EDTA

Glossary

Thesis:	An extended research paper that is part of the final exam process for a graduate degree. The document may also be classified as a project or collection of extended essays.
Glossary:	An alphabetical list of key terms This is an optional page and can be removed if not used. Use one table row for each item to allow sorting using Word's table tools. Apply the style **1_Para_NoSpace** to table rows as shown here.

Chapter 1

Introduction

1.1 Background

Wound infections generally occur when microorganisms colonize and proliferate in the areas of damaged tissues, which in turn transforms a moist, warm, and nutritious environment into a breeding ground for pathogens. These infections have the tendency to arise from injuries, surgeries, or various underlying conditions such as vascular diseases or diabetes, leading to inevitable complications, delayed healing, increased patient trauma, and higher treatment costs, in some cases (Bowler et al., 2001). Moreover, microorganisms can enter wounds through direct contact, airborne dispersal, or general contamination.

In an ideal clinical or healthcare setting, pre-surgical traumatic wounds become important not only as a primary infection site but also because they act as focus for nosocomial transmission, also categorized as healthcare-associated infections or HAIs. HAIs are defined as infections that manifest either within 48 hours of hospital admission, three days post-discharge, or 30 days after a surgical procedure (Cleveland Clinic, 2025). Surgical site infections, or SSIs, are a prevalent type of HAI, which contributes significantly to the overall patient morbidity, mortality, prolonged hospital stays, and economic burden globally (Mateescu et al., 2023).

This study focuses on two prominent and clinically significant bacteria – *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. *Acinetobacter baumannii* is a Gram-negative, aerobic bacterium that frequently causes opportunistic nosocomial infections, particularly in immunocompromised patients, those with severe burn or surgical wounds, cancer, or cystic fibrosis. It accounts for approximately 20-22% of hospital acquired infections worldwide, including wound infections. The inherent versatility and advanced antibiotic resistance

mechanisms of *A. baumannii* makes its infections exceptionally challenging to treat, contributing to substantial morbidity and mortality rates (Dubey et al., 2025).

Klebsiella pneumoniae is another important Gram-negative bacterium, recognized as one of the ESKAPE pathogens, and they are notorious for their role in hospital-acquired infections (Karner et al., 2020). In surgical intensive care units, *K. pneumoniae* has been identified to be among the most common causative microorganisms in nosocomial infections, including skin and soft tissue infections (Aiesh et al., 2023). It is frequently isolated from surgical patients upon admission, highlighting its prevalence in environments prone to wound contamination, such as pressure ulcers near the sphincter, which are susceptible to intestinal bacteria (Karner et al., 2020).

Traumatic pre-operative wound samples in Dhaka face a high practical risk because the pathogens—*Acinetobacter baumannii* and *Klebsiella pneumoniae*—are now well established as frequent, multidrug-resistant causes of hospital-associated wound and surgical-site infections in local settings. Genomic and surveillance studies from Dhaka report rising frequencies of MDR and carbapenem-resistant *A. baumannii* in clinical wound and hospital isolates, with isolates often carrying multiple resistance determinants and biofilm-associated traits that complicate wound clearance and decontamination (Rahman et al., 2022). *K. pneumoniae* in Bangladesh likewise demonstrates alarming levels of ESBL production and the local emergence/spread of carbapenemase and hypervirulent lineages, with genomic work showing international high-risk clones circulating in clinical and environmental reservoirs—findings that directly threaten the effectiveness of peri-operative empiric regimens for trauma patients (Kawser et al., 2025). Local retrospective wound surveillance from tertiary hospitals in Dhaka corroborates these genomic data, reporting *K. pneumoniae* and *A. baumannii* among the common wound pathogens with high rates of resistance to frontline antibiotics—an epidemiologic context that makes documenting phenotypic AST, resistance genes, and

virulence traits in pre-surgical wound isolates essential for guiding peri-operative therapy and infection-control policies.

In Dhaka's trauma and surgical settings, high and rising rates of multidrug resistance among wound-associated Gram-negative pathogens mean empirical peri-operative therapy is increasingly unreliable; local genomic and surveillance studies document frequent ESBL- and carbapenemase-producing *K. pneumoniae* and widespread MDR *A. baumannii*, which directly threaten the effectiveness of standard prophylaxis and empiric regimens (Kawser et al., 2025). Phenotypic AST therefore remains essential to guide immediate treatment choices, while molecular detection of key resistance genes (e.g., *bla_CTX-M*, *bla_NDM*, carbapenemase loci) rapidly identifies high-risk clones, informs targeted therapy, and supports infection-control actions such as cohorting and source-investigation (Hattab et al., 2024).

Moreover, assessing hemolytic phenotype and hemolysin genes (for example the *khe* locus described in *K. pneumoniae*) provides complementary virulence information that can help stratify isolates by pathogenic potential and prioritize aggressive management or surveillance of strains more likely to cause severe or persistent wound infections (Wu et al., 2024). Because SSIs markedly increase morbidity, re-operations and length of stay, integrating AST profiles, molecular resistance-gene detection, and simple virulence assays (such as hemolysis) is a pragmatic, evidence-based strategy in Dhaka to optimize peri-operative therapy, curb nosocomial spread, and strengthen antimicrobial stewardship.

In short, this study aimed to contribute actionable, locally relevant data on the phenotypic antimicrobial susceptibility, key resistance genes, and hemolytic virulence traits of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from pre-surgical traumatic leg wounds in Dhaka.

1.2 Objectives

To determine the phenotypic antimicrobial susceptibility, presence of key resistance genes, and hemolytic virulence traits of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from pre-surgical traumatic leg wounds in Dhaka, to inform peri-operative antibiotic selection and hospital infection-control measures.

Chapter 2

Materials and Methods

2.1 Study Design

This study was carried out between March 2023 and December 2024 at the National Institute of Traumatology & Orthopaedic Rehabilitation (NITOR). Samples were obtained from patients with post-traumatic leg injuries who showed clinical signs of bacterial infection prior to surgery.

2.2 Population

A total of 61 wound specimens were collected from hospitalized patients. Eligible participants were those who exhibited clinical signs of bacterial infection in leg wounds resulting from traumatic injuries. Samples were obtained from both male and female patients, aged 20 to 70 years.

2.3 Sample Collection

Sterile 0.9% NaCl was used as the collection/transport medium. The wound exudates were sampled with sterile cotton-tipped swabs using a rotating (Levine) technique across the wound surface to express fluid, after which each swab was placed into a test tube containing sterile saline. Samples were maintained under refrigeration and transferred to the laboratory within 24 hours while preserving the cold chain (Song et al., 2021). This procedure followed standard wound-swab collection techniques and current guidance on microbiological specimen transport.

2.4 Bacterial Culture

For identification of the target organism, the wound swabs were cultured immediately upon receipt. Each swab was rolled over the surface of the primary plates and then streaked on different specific selective media for isolation using the quadrant streak method to obtain single colonies. For both *Acinetobacter baumannii* and *Klebsiella pneumoniae*, the plates were incubated at 37°C for 18 to 24 hours (Karah et al., 2020). The morphology of the colonies was observed afterwards, in order to identify the microorganisms primarily.

Table 1: Expected Colony Morphology of Specific Bacteria on Selective Media

Organism	**Gram Postive/Negative**	**Media**	**Expected Colony Morphology**
Acinetobacter baumannii	Gram negative	MacConkey (MAC) Agar (without added antibiotic)	Pale, colourless, or slightly pink
Klebsiella pneumoniae	Gram negative	HiCrome KPC Agar (without added antibiotic)	Metallic bluish green

2.5 DNA Isolation

Genomic DNA from selected isolates was obtained using a crude boiling lysis method. Overnight cultures grown on nutrient agar at 37 °C were suspended in 150 µL Tris-EDTA buffer. The suspensions were vortexed thoroughly and heated at 95 °C for 20 minutes to disrupt cells and release nucleic acids. Lysates were centrifuged at 10,000 rpm for 10 minutes at 4 °C, and the resulting supernatants containing DNA were collected and stored at –20 °C for Polymerase Chain Reaction (Brown, 2016).

2.6 Molecular Detection via Polymerase Chain Reaction

Molecular confirmation of isolates was performed by PCR. Each 13 µL reaction contained 6.5 µL 2× PCR Master Mix (dNTPs, $MgCl_2$, and Taq polymerase), 0.5 µL each of forward and reverse primers, 3.5 µL nuclease-free water, and 2 µL template DNA. Amplification was carried out in a thermal cycler under optimized cycling conditions for the target genes.

Table 2: Bacteria-Specific Gene Information for PCR

Name of Bacteria	**Target Gene**	**Primer Sequence (5' - 3')**	**Amplicon Size (bp)**	**References**
Acinetobacter baumannii	blaOXA-51	F: 5'-TAATGCTTTGATCGGCCTTG-3'	353	Falah et al., 2019.
		R: 5'-TGGATTGCACTTCATCTTGG-3'		
Klebsiella pneumoniae	Diguanylate Cyclase	F: 5′-TGCAGATAATTCACGCCCAG-3′	133	Diaz et al., 2013.
		R: 5′-ACCCGCTGGACGCCAT-3′		

The **tables 3** and **4** given below were followed to set the PCR conditions for microorganism detection.

Table 3: PCR Conditions for *Klebsiella pneumoniae*

Organism	**PCR Conditions**	**Temperature**	**Time**	**Number of Cycles**
Klebsiella pneumoniae	Initial Denaturation	94 °C	10 min	30 cycles
	Denaturation	94 °C	30 sec	
	Annealing	60 °C	45 sec	
	Elongation	72 °C	45 sec	
	Final Elongation	72 °C	10 min	

Table 4: PCR Conditions for *Acinetobacter baumannii*

Organism	PCR Conditions	Temperature	Time	Number of Cycles
Acinetobacter baumannii	Initial Denaturation	94 °C	5 min	30 cycles
	Denaturation	94 °C	30 sec	
	Annealing	55 °C	45 sec	
	Elongation	72 °C	45 sec	
	Final Elongation	72 °C	7 min	

2.7 Agarose Gel Electrophoresis

PCR products were resolved on 1.5% agarose gels prepared in 1× TBE buffer (cast as ~100 mL gels), stained with 2 µL ethidium bromide, and electrophoresed at 110 V for 40–45 minutes. A 1 kb DNA ladder was run alongside samples to size amplicons, enabling identification of the expected bands for *Klebsiella pneumoniae* (130 bp) and *Acinetobacter baumannii* (353 bp). Gels were visualized on a UV transilluminator and documented (Brown, 2016).

2.8 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing (AST) was to evaluate the resistance patterns of the isolates and to identify multidrug-resistant (MDR) strains. Testing was performed using the Kirby–Bauer disk diffusion method in accordance with the standards and interpretive criteria outlined by the Clinical and Laboratory Standards Institute (CLSI M100). Briefly, 24-hour bacterial cultures were suspended in sterile 0.9% saline and adjusted to match the 0.5 McFarland turbidity standard. A sterile cotton swab was then used to inoculate the suspension uniformly across the surface of Mueller–Hinton agar (MHA) plates. After drying for 3–5 minutes, commercially prepared antibiotic discs were applied using sterile forceps. After incubation,

inhibition zone diameters were measured and interpreted according to CLSI guidelines to determine susceptibility categories.

Table 5: List of antibiotics used in the study

Serial	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretive criteria		
						Sensitive mm or more	Intermediate mm	Resistant mm or less
1	Gentamicin	Aminoglycoside	Gram-positive and Gram-negative	GEN	10	15	13-14	12
2	Ampicillin	β-lactam	Gram-positive and Gram-negative	AMP	10	17	14-16	13
3	Meropenem	Carbapenem	Gram-positive and Gram-negative	MEM	10	23	20-22	19
4	Cefepime	Cephalosporin	Gram-positive and Gram-negative	CPM	30	25	19-24	18
5	Piperacillin tazobactam	Penicillin and β-lactamase inhibitor	Gram-positive and Gram-negative	PIT	100/10	21	18-20	17
6	Imipenem	Carbapenem	Gram-positive and Gram-negative	IMP	10	23	20-22	19
7	Azithromycin	Macrolide	Gram-positive and Gram-negative	AZM	15	18	14-17	13
8	Amikacin	Aminoglycoside	Gram-positive and Gram-negative	AK	30	17	15-16	14
9	Ciprofloxacin	Fluoroquinolone	Gram-positive and Gram-negative	CIP	5	21	16-20	15
10	Tigecycline	Glycylcycline	Gram-positive and Gram-negative	TGC	15	19	15-18	14
11	Cefixime	Cephalosporin	Gram-positive and Gram-negative	CFM	5	18	16-17	15
12	Nalidixic Acid	Fluoroquinolone	Gram-positive and Gram-negative	NA	30	19	14-18	13
13	Aztreonam	Monobactam	Gram-negative	AT	30	21	18-20	17
14	Colistin	Polymyxin E	Gram-negative	CT	10	15	13-14	12

2.9 Hemolysis Test

The hemolysis test was performed to assess the capability of bacterial isolates to lyse red blood cells. Blood agar containing 5% sheep blood was prepared as the growth medium. A 24-hour pure bacterial culture was streaked onto the blood agar plates, which were then incubated at 37 °C for 24 hours.

Hemolytic activity was observed afterwards as clear (β-hemolysis), greenish (α-hemolysis), or no (γ-hemolysis) zones around the colonies (Cleveland Clinic, 2025).

2.10 Gene-Specific Polymerase Chain Reaction

Class 1 and class 2 integrons of *Acinetobacter baumannii* were amplified by conventional PCR using integrase-specific primers targeting the intI1 and intI2 genes, respectively.

Table 6 below states the information for class 1 and class 2 integrons respectively for PCR.

Table 6: Information for intI1 and intI2 integrons for PCR

Integron Class	**Primer Name**	**Targeted Sequences (5' - 3')**	**Amplicon Size (bp)**	**References**
Class 1	intI1-F	CAGTGGACATAAGCCTGTTC	160	Europe PMC. (n.d.).
	intI1-R	CCCGAGGCATAGACTGTA		
Class 2	intI2-F	TTGCGAGTATCCATAACCTG	288	(Mirnejad et. al., 2013)
	intI2-R	TTACCTGCACTGGATTAAGC		

Tables 7 and 8 respectively demonstrates the PCR conditions to be followed for class 1 and class 2 Integrons.

Table 7: PCR Conditions for Class 1 Integrons

PCR Conditions	Temperature	Time	Number of Cycles
Initial Denaturation	94 °C	10 min	35 cycles
Denaturation	94 °C	30 sec	
Annealing	55 °C	30 sec	
Elongation	72 °C	30 sec	
Final Elongation	72 °C	10 min	

Table 8: PCR Conditions for Class 2 Integrons

PCR Conditions	Temperature	Time	Number of Cycles
Initial Denaturation	94 °C	10 min	35 cycles
Denaturation	94 °C	30 sec	
Annealing	55 °C	30 sec	
Elongation	72 °C	30 sec	
Final Elongation	72 °C	10 min	

Chapter 3

Results

3.1 Population Demographic

The study included 61 patients, of whom 8 were female and 53 were male, all within the age range of 20 to 70 years. The dynamics are demonstrated in Table 9 below.

Table 9: Population Characteristics

Attributes	Categories	Frequency	Percentage (%)
Gender	Male	53	87
	Female	8	13
Age	20-25	9	15
	26-30	8	13
	31-35	12	20
	36-40	9	15
	41-45	6	10
	46-50	5	8
	51-55	4	6
	56-60	3	5
	61-65	3	5
	66-70	2	3

3.2 Colony Characteristics

To identify the target bacterial isolates, each sample was inoculated onto specific selective media to observe characteristic colony morphology. MacConkey agar was used for the isolation and presumptive identification of *Acinetobacter baumannii*, while HiCrome KPC agar was employed for the detection of *Klebsiella pneumoniae*.

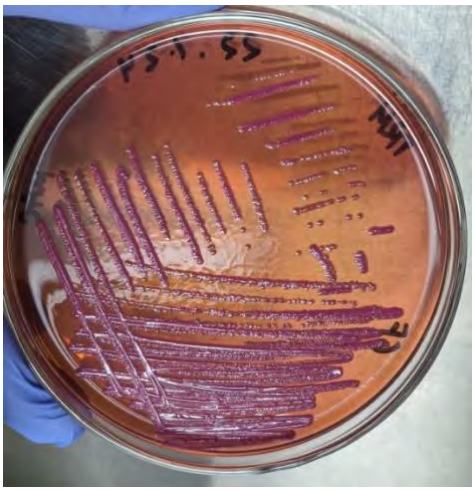

Figure 1: Acinetobacter baumannii colonies on MacConkey agar.

Figure 2: Klebsiella pneumoniae colonies on HiCrome KPC agar.

Following incubation, small, mucoid pink colonies appeared on MacConkey agar, indicative of *A. baumannii* (Figure 1), while metallic blue colonies were observed on HiCrome KPC agar, confirming the presence of *Klebsiella pneumoniae* (Figure 2).

3.3 Polymerase Chain Reaction

Species confirmation was performed through PCR using species-specific primers. The amplified products were analyzed on a 1.5% agarose gel to verify successful amplification by comparing the observed band sizes. A 1 kb DNA ladder served as the molecular size marker, and both positive (P) and negative (N) controls were included to validate the PCR results.

Figure 3 below illustrates the gel electrophoresis results of *Acinetobacter baumannii* PCR products. A 353 bp fragment corresponding to the blaOXA-51 gene was successfully amplified, confirming the species identity.

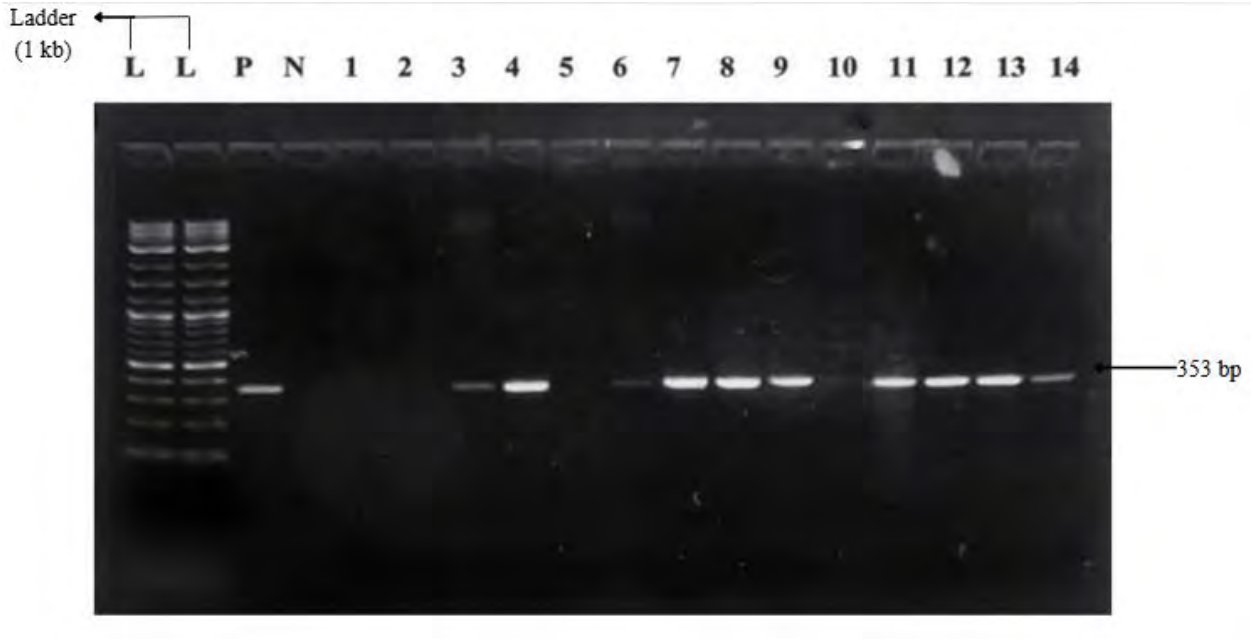

Figure 3: Agarose gel electrophoresis of PCR assay of Acinetobacter baumannii isolates. Here Lanes (L) are 1 kb DNA marker, and Lane (1-14) are some positive samples at 353 bp.

Figure 4 below presents the gel electrophoresis results of *Klebsiella pneumoniae* PCR products, where a 133 bp fragment corresponding to the diguanylate cyclase gene was successfully amplified, confirming species identification.

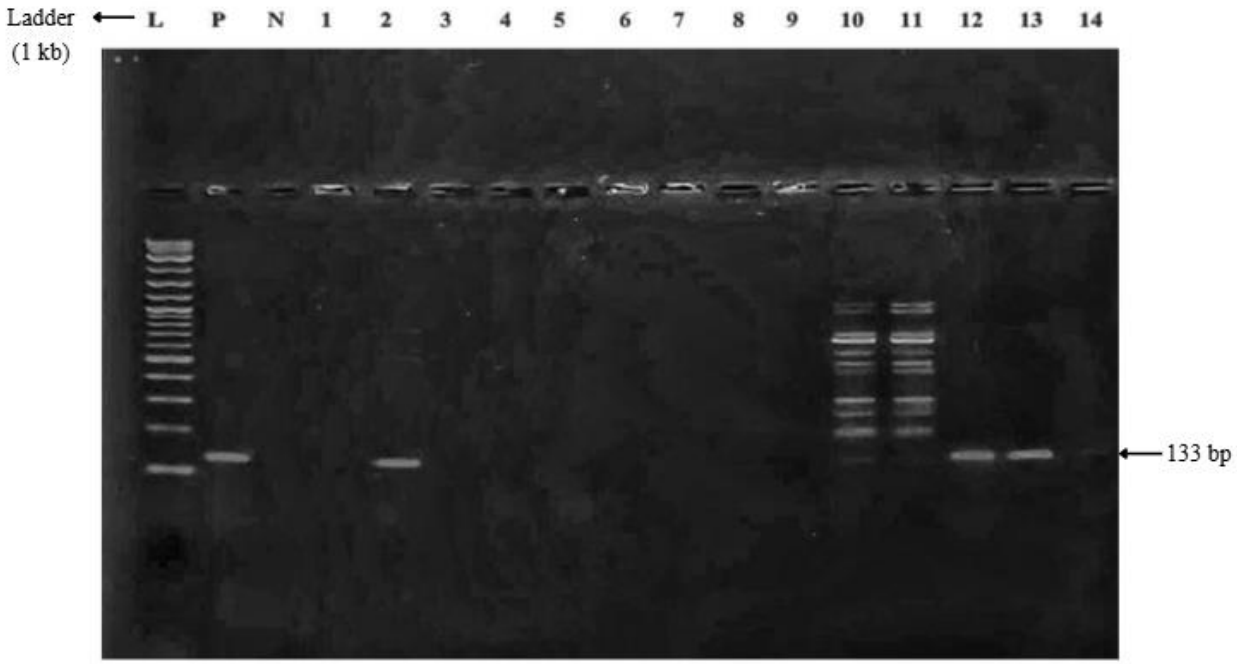

Figure 4: Agarose gel electrophoresis of PCR assay of Klebsiella pneumoniae isolates. Here Lane (L) is 1 kb DNA marker, and Lane (1-14) are some positive samples at 133 bp.

3.4 Bacteria Detection

After performing Polymerase Chain Reaction and Agarose Gel Electrophoresis of the aforementioned 61 wound samples collected from patients with traumatic leg injuries prior to surgery, it was seen that 34 isolates, or 55.7% of the samples showed desired bacterial growth. The remaining 27, or 44.3% of the samples did not yield the desired bacterial growth. Out of these 34 confirmed isolates, 44.1% were identified as *Acinetobacter baumannii*, that is 15 in number, while 19 isolates, or 55.9% of the samples were confirmed as *Klebsiella pneumoniae*. These findings indicate that *K. pneumoniae* was slightly more prevalent than *A. baumannii* among the wound infection cases analyzed in this study.

Table 10: Detected Bacteria from Wound Samples

Bacterial Profiles	Name of Bacteria	Frequency	Percentage (%)
Bacterial Isolates	*Acinetobacter baumannii*	15	44.1
	Klebsiella pneumoniae	19	55.9

3.5 Antibiotic Susceptibility Testing

In this study, all PCR-confirmed isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* were subjected to antibiotic susceptibility testing to assess their resistance patterns and identify multidrug-resistant (MDR) strains. The testing was performed using the Kirby–Bauer disk diffusion method, and zone diameters were interpreted in accordance with CLSI guidelines. A range of antibiotics from different therapeutic classes was included to evaluate multidrug resistance among the isolates. The number of antibiotic resistance pattern is depicted in figures 5 and 6 below for *Acinetobacter baumannii* and *Klebsiella pneumoniae* respectively.

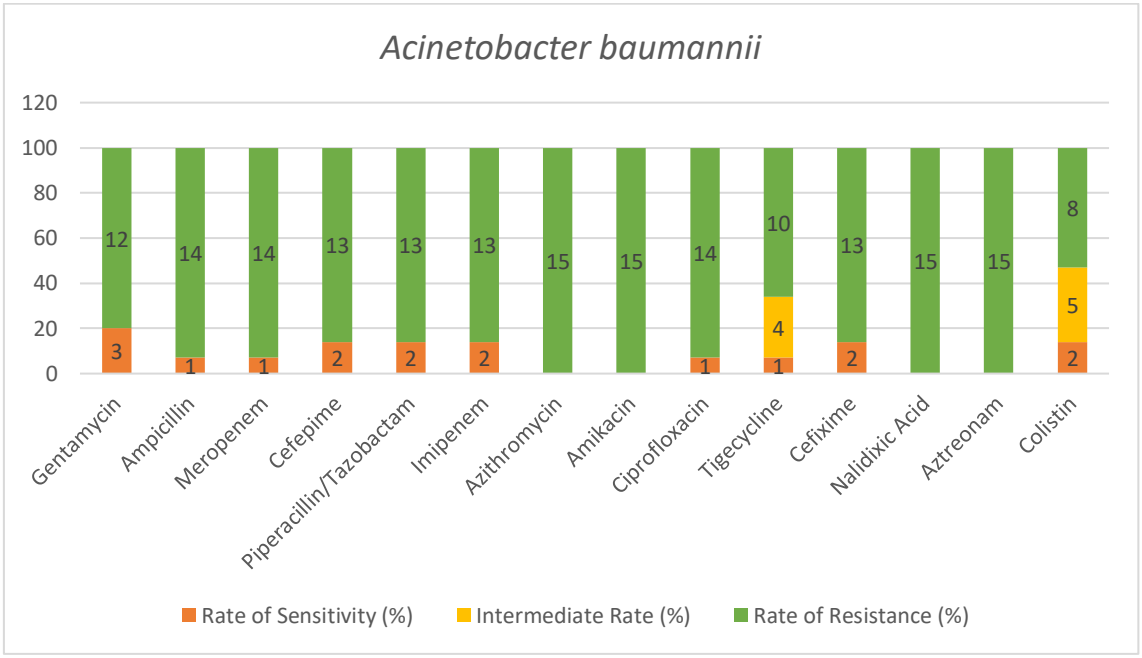

Figure 5: Antibiotic resistance pattern in Acinetobacter baumannii. At the center of the

figure, the number of isolates that showed sensitivity, intermediate resistance, and resistance respectively are mentioned.

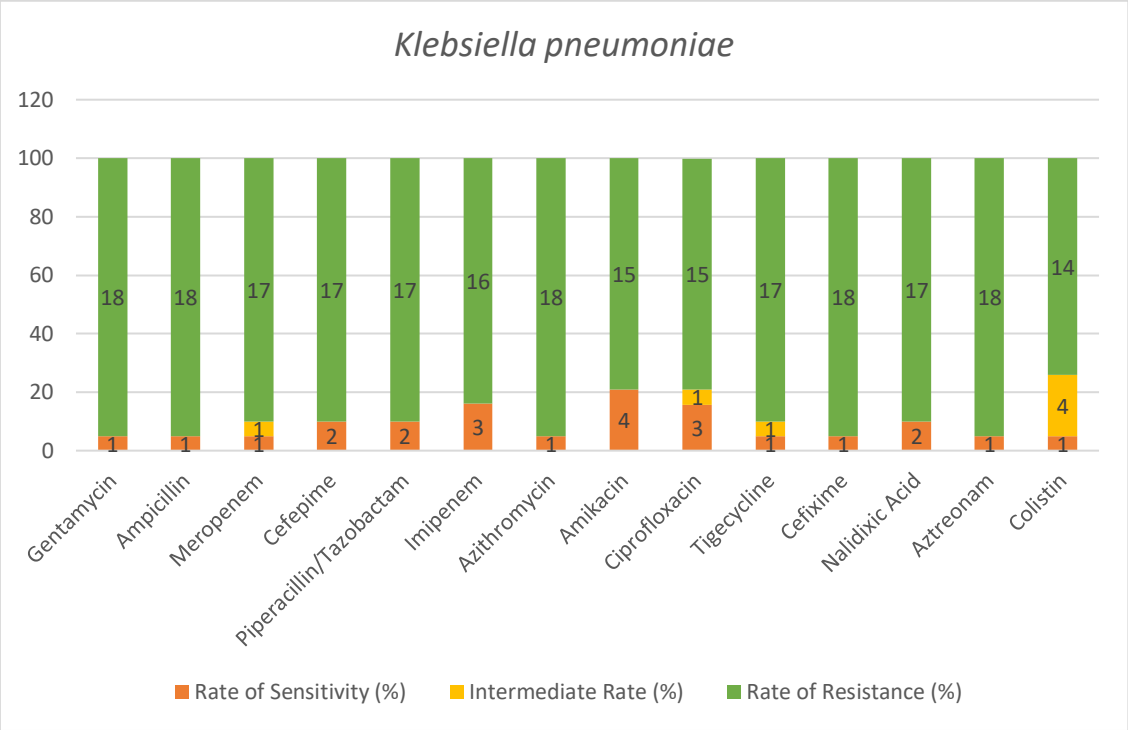

Figure 6: Antibiotic resistance pattern in Klebsiella pneumoniae. At the center of the figure, the number of isolates that showed sensitivity, intermediate resistance, and resistance respectively are mentioned.

From figures 5 and 6, *Acinetobacter baumannii* exhibits complete (100%) resistance to Aztreonam, Nalidixic acid, Amikacin, and Azithromycin, with marked resistance to most other tested antibiotics, including Cefixime, Ampicillin, Ciprofloxacin, and Meropenem. It shows the highest sensitivity to Gentamicin (20%) and retains notable susceptibility to several antibiotics, except those with complete resistance. Both *Acinetobacter baumannii* and *Klebsiella pneumoniae* demonstrate the lowest resistance to Colistin and Tigecycline, with intermediate susceptibility to these antibiotics, making them among the few effective treatment options. For *A. baumannii*, no intermediate susceptibility is observed for antibiotics other than Colistin and Tigecycline.

In contrast, *Klebsiella pneumoniae* shows no instances of complete resistance but displays substantially elevated resistance levels across nearly all antibiotics, particularly Aztreonam, Cefixime, Azithromycin, Ampicillin, and Gentamicin. It exhibits the greatest sensitivity to Amikacin (21%) and retains partial sensitivity to all tested antibiotics. Additionally, *K. pneumoniae* demonstrates intermediate susceptibility to Ciprofloxacin and Meropenem, alongside Colistin and Tigecycline, but shows no intermediate responses for other antibiotics. Both species highlight Colistin and Tigecycline as key therapeutic options due to their lower resistance profiles.

Table 11: Analysis of Non-Susceptibility Across 10 Antimicrobial Groups

Groups	**Agents Tested**	***A. baumannii* Max Non-Susceptibility (%)**	***K. pneumoniae* Max Non-Susceptibility (%)**
Aminoglycoside	Gentamycin, Amikacin	100	95
Macrolide	Azithromycin	100	95
Monobactam	Aztreonam	100	95
Cephalosporin	Cefepime, Cefixime	86	90
Polymyxin E	Colistin	86	95
Fluoroquinolone	Ciprofloxacin, Nalidixic Acid	100	90
Carbapenem	Meropenem, Imipenem	93	95
Penicillin and β-lactamase inhibitor	Piperacillin/Tazobactam	86	90
Penicillin	Ampicillin	93	95
Glycylcycline	Tigecycline	93	95
Average Non-Susceptibility (%)		**93.7**	**93.0**

Upon further analysis of the aggregated resistance data, *A. baumannii* is marginally more MDR compared to *Klebsiella pneumoniae*. Both populations exhibit extremely high levels of non-susceptibility (Resistance + Intermediate) in all 10 antimicrobial categories, thereby classifying the entire study population as highly MDR. However, *A. baumannii* displayed a slightly higher average non-susceptibility rate of 93.7% across the categories (compared to 93.0% for *K. pneumoniae*). Crucially, *A. baumannii* also exhibited complete (100%) resistance to four individual antibiotics (Aztreonam, Nalidixic acid, Amikacin, and Azithromycin), a level of absolute resistance not observed in *K. pneumoniae*.

Furthermore, based on the clinical MDR definition (non-susceptibility to ≥3 antimicrobial categories), and given that both organisms exhibit non-susceptibility (R + I ≥ 86%) in all 10 categories tested, it is highly probable that every single isolate in the study populations would be classified as MDR.

Therefore, the ratio of MDR to non-MDR isolates is estimated to be approximately 100% : 0% for both organisms, reflecting the extreme, multi-category resistance observed in these populations.

Figures 7 and 8 demonstrate the MDR dynamics of *Acinetobacter baumannii* and *Klebsiella pneumoniae* respectively.

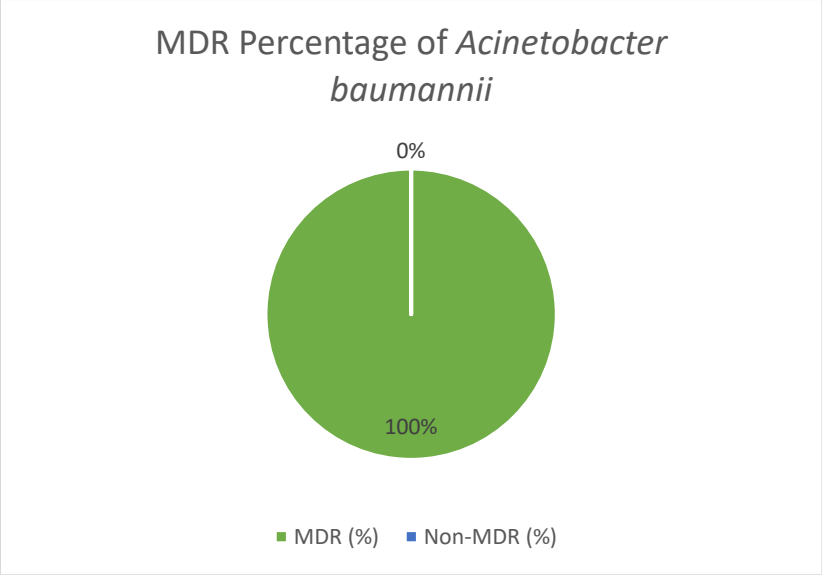

Figure 7: MDR dynamics of Acinetobacter baumannii.

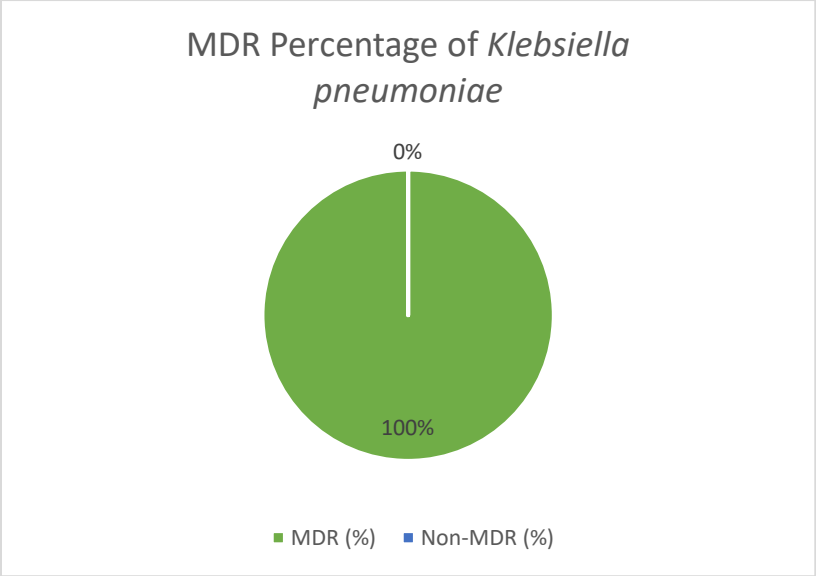

Figure 8: MDR dynamics of Klebsiella pneumoniae.

3.6 Hemolysis

Hemolytic activity was assessed using blood agar plates containing 5% sheep blood. Following incubation, each plate was examined for visible hemolysis surrounding the bacterial colonies.

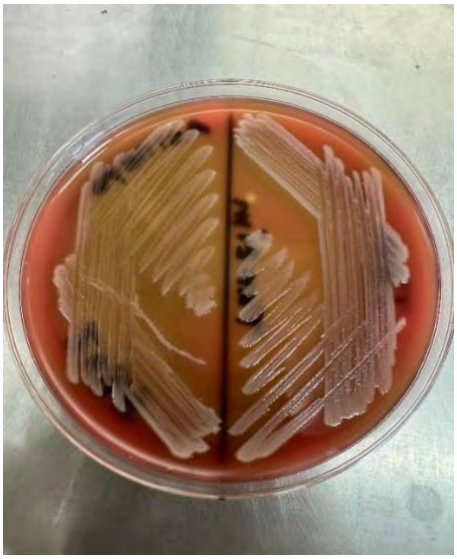

Figure 9: Acinetobacter baumannii on Blood agar indicating γ-hemolysis, with no visible clear zones or discoloration surrounding their colonies.

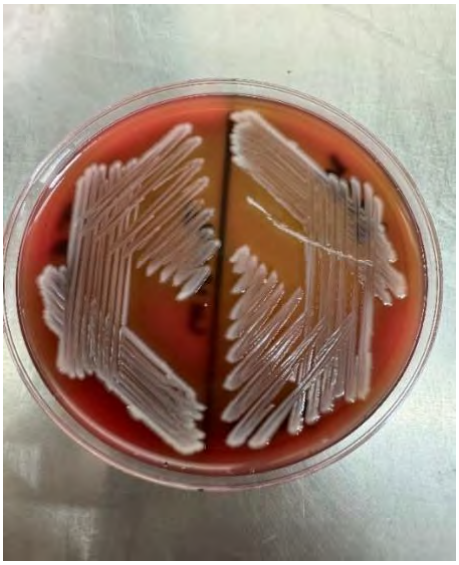

Figure 10: Klebsiella pneumoniae on Blood agar indicating γ-hemolysis, with no visible clear zones or discoloration surrounding their colonies.

Following incubation, the *Acinetobacter baumannii* isolates (figure 9) and *Klebsiella pneumoniae* isolates (figure 10) demonstrated an absence of any visible clear zones or discoloration surrounding their colonies on blood agar plates, an indication of γ-hemolysis, or non-hemolytic reaction.

The PCR-confirmed isolates were further evaluated for their hemolytic activity on blood agar. Figure 11 demonstrates the percentage of hemolytic patterns observed in *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates.

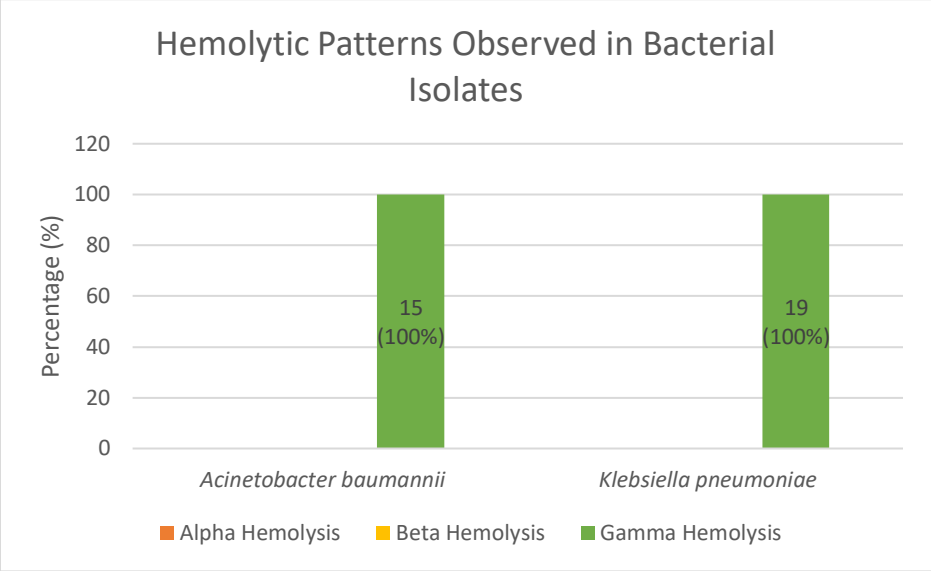

Figure 11: Hemolytic patterns observed in bacterial isolates. At the center of the figure, the number and percentage of isolates that showed hemolysis are mentioned.

From the figure, among the 34 bacterial isolates, all 15 *Acinetobacter baumannii* strains exhibited γ-hemolysis (100%), indicating the absence of red blood cell lysis. To add to that, all 19 *Klebsiella pneumoniae* isolates also demonstrated γ-hemolysis (100%), also characterized by the absence of red blood cell lysis.

3.7 Gene-Specific Polymerase Chain Reaction

Polymerase Chain Reaction for resistant gene detection was performed after species-specific PCR, the amplifications of both class 1 and class 2 integron integrase genes in the *A. baumannii* isolates were recorded.

In figure 12, a sharp band at 160 bp verified the presence of the class 1 integrase gene, while in figure 13, a distinct band at 288 bp verified the presence of the class 2 integrase gene.

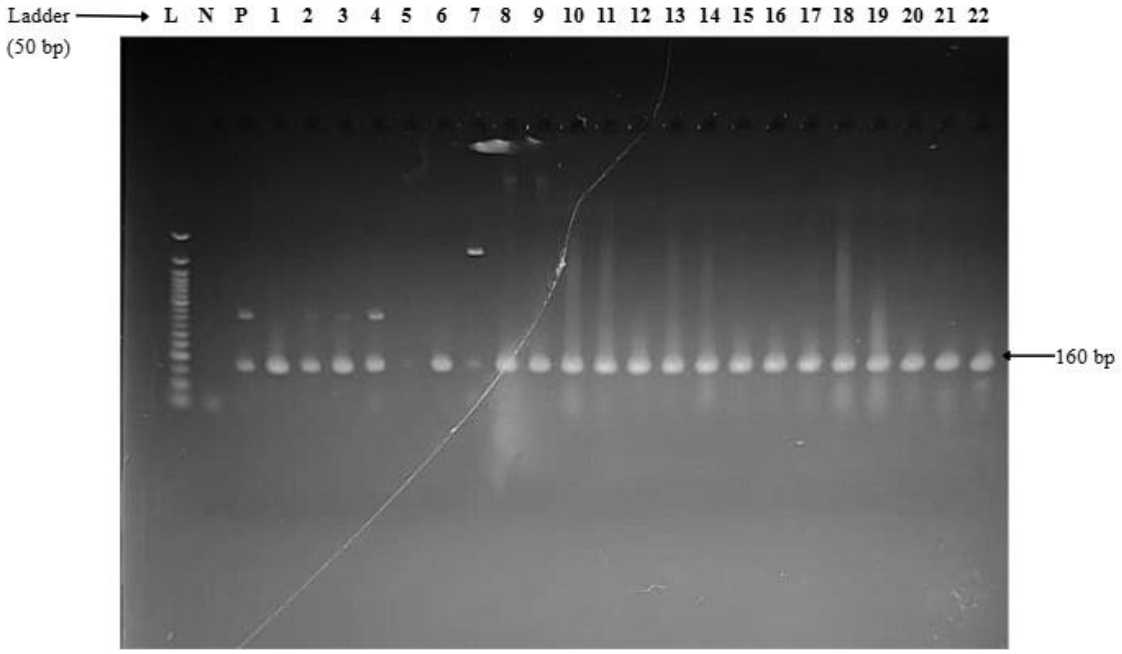

Figure 12: Agarose gel electrophoresis of class 1 integron PCR products from Acinetobacter baumannii isolate. Here Lane (L) is 50 bp DNA ladder, and lanes (1-22) are some positive samples at 160 bp.

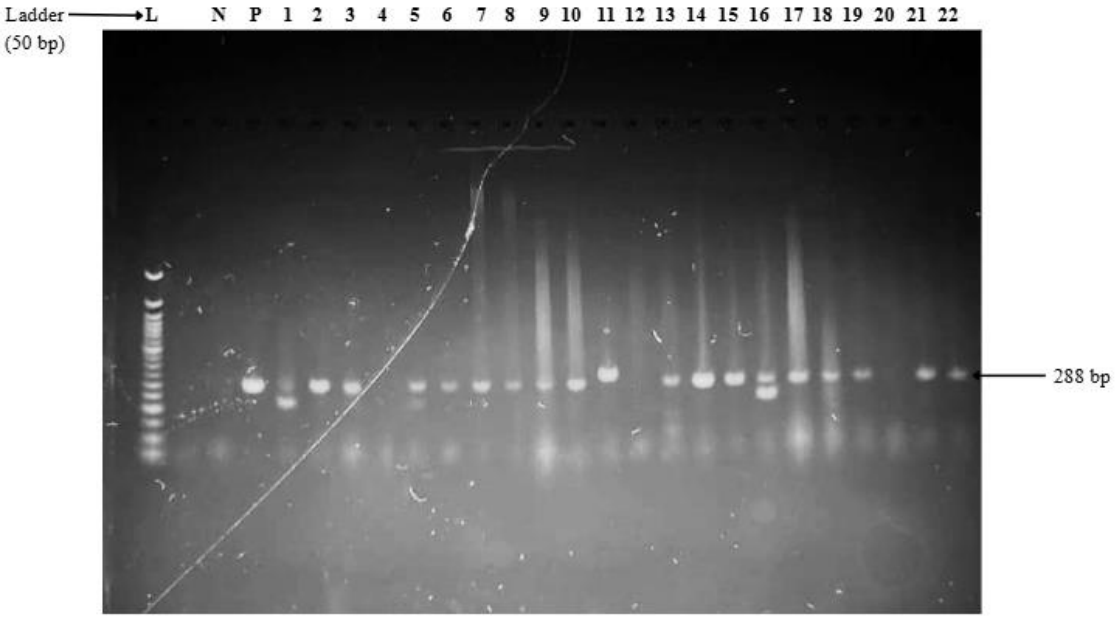

Figure 13: Agarose gel electrophoresis of class 2 integron PCR products from Acinetobacter baumannii isolate. Here Lane (L) is 50 bp DNA ladder, and lanes (1-22) are some positive samples at 288 bp.

After careful observation of the band sizes of the Agarose Gel Electrophoresis results, it can safely be affirmed that the bands at 160 bp and 288 bp sharp in figures 12 and 13 respectively denotes the integrase-integron gene of *A. baumannii*. The integron-integrase genes detected were respectively intI1, which corresponded to Gentamicin resistance (Tosini et al., 1998), and intI2, which corresponded to resistance to Gentamicin (Sáenz et al., 2004). This however, doesn't infer that the intI1 and intI2 genes themselves are resistant to the antibiotic Gentamicin. Rather, they contributed to *A. baumannii* acquiring antibiotic resistant genes for themselves, so in a way, an indirect contribution to resistance.

Chapter 4

Discussion

This study characterized organisms recovered from 61 pre-surgical traumatic leg wound swabs at a tertiary trauma centre in Dhaka. Thirty-four samples (55.7%) yielded the target organisms, of which 15 (44.1%) were confirmed as *Acinetobacter baumannii* and 19 (55.9%) as *Klebsiella pneumoniae*. Both species displayed an alarmingly high burden of non-susceptibility across the antibiotic panel: aggregated non-susceptibility (resistant + intermediate) averaged approximately 93.7% for *A. baumannii* and 93.0% for *K. pneumoniae*. Practically, these results indicate that the isolates circulating in this trauma setting are overwhelmingly multidrug-resistant by commonly used clinical definitions (non-susceptibility to ≥3 antimicrobial categories) and that empiric peri-operative protocols that do not account for local susceptibility patterns are likely to fail in a large proportion of cases.

The resistance profiles observed in this study mirror and reinforce recent local and regional surveillance. Multiple Bangladeshi studies and genomic reports document rising Carbapenem resistance and widespread multidrug resistance among *A. baumannii* and *K. pneumoniae* isolates in Dhaka and surrounding areas (Khan et al., 2024). The present dataset is consistent with that pattern: *A. baumannii* reached 100% resistance to several antibiotics tested (Aztreonam, Nalidixic acid, Amikacin, Azithromycin), while *K. pneumoniae* showed very high resistance across the panel yet retained low levels of sensitivity to a few agents such as Amikacin in some isolates. Taken together, the findings offer robust local confirmation that trauma wards in Dhaka are currently challenged by highly resistant Gram-negative pathogens, and that local empiric policies must be informed by up-to-date institutional data rather than by older or external guidelines alone (Kawser et al., 2025).

The molecular findings give a plausible and clinically important explanation for the extensive co-resistance documented phenotypically. Detection of class 1 (intI1) and class 2 (intI2) integron integrase genes in the *A. baumannii* isolates indicates the presence of mobile genetic platforms known to capture and express diverse resistance gene cassettes (Adeniji et al., 2022). Simply put, the presence and abundance of integrons increase the likelihood of multi-drug resistance across many antibiotic classes because multiple resistance determinants can co-exist and be transferred. This link helps explain why so many isolates are co-resistant to Aminoglycosides, β-lactams, Carbapenems and other classes in the present study, and it aligns with prior reports that link integron carriage to high MDR rates in clinical Gram-negative populations (Mirnejad et al., 2013).

Hemolytic phenotype testing showed γ-hemolysis (no visible RBC lysis) for all isolates of both species. This is an important but limited virulence observation: absence of hemolysis on blood agar does not imply low pathogenic potential, because hemolytic activity is not a universal virulence determinant for these organisms and virulence arises from multiple factors (capsule, siderophores, biofilm, etc.). The observed γ-hemolysis is therefore a simple phenotypic result that is consistent with prior clinical isolates of these species and should be interpreted as complementary information rather than as a primary indicator of clinical severity (Wu et al., 2024). Future molecular screening for specific virulence loci could clarify whether locally circulating strains harbor additional virulence determinants, but the lack of hemolysis alone does not diminish the clinical concern posed by the resistance patterns.

From a therapeutic perspective the data present a narrow set of remaining options. Colistin and Tigecycline showed comparatively lower resistance rates in this panel and therefore remain among the few active agents against many isolates. However, reliance on these last-line drugs carries clear caveats: Polymyxins and Tigecycline testing by disk diffusion is less reliable than MIC methods, Colistin dosing and toxicity issues complicate clinical use, and plasmid-

mediated and chromosomal mechanisms of resistance to Polymyxins (e.g., *mcr* genes or lipid A modification pathways) have been described elsewhere and can emerge under selection pressure (Hattab et al., 2024). Consequently, when these agents are considered, confirmatory MIC testing and stewardship oversight are recommended rather than empiric use.

Operationally and for infection control, the results support three actions:

1. The result ensures routine culture and susceptibility testing for wound infections in trauma patients rather than purely empiric prescribing;
2. It reports and periodically reviews institutional antibiograms so peri-operative prophylaxis and empiric therapy are aligned with local data; and
3. The result further strengthens basic infection prevention and control (IPC) practices in trauma units to limit nosocomial transmission of MDR clones.

These steps are practical, evidence-based, and achievable in many hospital settings and they directly follow from the high MDR rates documented here (Kawser et al., 2025).

This study was done with a moderate sample size, limiting the ability to generalize beyond the local institution. The use of disk diffusion without MIC confirmation for last-line drugs limits precision in interpreting susceptibility for Colistin and Tigecycline. To add to that, the study did not correlate microbiological findings with patient outcomes, which would be valuable in future work.

Chapter 5

Conclusion

In this study of 61 pre-surgical traumatic leg wound samples in Dhaka, 34 yielded bacterial growth; *Klebsiella pneumoniae* and *Acinetobacter baumannii* were the predominant pathogens. Both species exhibited alarmingly high levels of multidrug non-susceptibility across multiple antibiotic classes, demonstrating a local resistance burden that substantially limits empiric therapeutic options for trauma-related wound infections.

The combined phenotypic and molecular approach implemented in this study provides immediately actionable data for clinical teams: it clarifies organism prevalence, documents virulence-associated phenotypes, and identifies genetic platforms that likely facilitate the accumulation and spread of resistance. Together, these results should inform peri-operative antibiotic decision-making, prompt enhanced infection-control responses in trauma units, and support targeted antimicrobial-stewardship measures at the institutional level.

Taken together, the findings highlight an urgent need to prioritize routine, culture-directed diagnostics, to align empiric protocols with local susceptibility data, and to strengthen surveillance of resistance determinants within the hospital. Implementing these steps, coupled with timely reporting to surgical teams and coordinated stewardship efforts, will be essential to limit nosocomial transmission, preserve remaining effective agents, and improve outcomes for trauma patients in this setting.

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