Isolation, Identification, and Antibiotic Susceptibility Pattern of *Klebsiella pneumoniae* from Sputum Sample.

Submitted by-

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A thesis submitted to the Department of Biotechnology in partial fulfillment of the requirements for the degree of Bachelor of Science in Biotechnology.

Department of Biotechnology

BRAC University

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.

Student's Full Name & Signature:

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Approval

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Ethics Statement

The ethical permission to conduct this study was obtained from the National Institute of Diseases of the Chest and Hospital (NIDCH), Mahakhali, Dhaka**.**

Abstract

This study was aimed to isolate, identify and determine the antibiotic susceptibility pattern of *Klebsiella pneumoniae* from sputum samples, as lower respiratory tract infections (LRTIs) remain a major public health concern worldwide, particularly in developing countries like Bangladesh. Clinical sputum samples were collected from patients suspected of lower respiratory tract infections. The samples were cultured on MacConkey agar and further processed using selective media for the isolation of suspected *Klebsiella* species.

Molecular confirmation of the isolates was carried out using Polymerase Chain Reaction (PCR) targeting the species-specific 16S-23S rDNA region. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. PCR amplification confirmed the presence of *Klebsiella pneumoniae* in 15 isolates with the appearance of a distinct 130 bp band during agarose gel electrophoresis. The antibiotic susceptibility results revealed a high level of resistance among the isolates. Complete resistance was observed against cefixime (100%), while high resistance was also found for ceftriaxone (71.42%) and tetracycline (64.28%). Moderate resistance was detected for cefepime (53.84%) and meropenem (58.34%). In contrast, comparatively better susceptibility was observed for imipenem (42.85%) and amoxicillin-clavulanic acid (35.71%). Additionally, multidrug resistance was identified in 6 out of 15 confirmed *Klebsiella pneumoniae* isolates, indicating resistance to at least three different classes of antibiotics.

The findings indicate that higher-generation antibiotics, particularly carbapenems, remain more effective against *Klebsiella pneumoniae*. It also highlights the growing concern of antibiotic resistance in *Klebsiella pneumoniae* and emphasize the need for continuous surveillance and appropriate antibiotic use for effective infection management.

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List of Acronyms

AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
bp	Base Pair
CLSI	Clinical and Laboratory Standards Institute
CRKP	Carbapenem-Resistant *Klebsiella pneumoniae*
DNA	Deoxyribonucleic Acid
ESBL	Extended-Spectrum Beta-Lactamase
EtBr	Ethidium Bromide
HAP	Hospital-Acquired Pneumonia
LPS	Lipopolysaccharide
LRTIs	Lower Respiratory Tract Infections
MDR	Multidrug-Resistant
MHA	Mueller-Hinton Agar
NaCl	Sodium Chloride
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
TAE	Tris-Acetate-EDTA
UTI	Urinary Tract Infection
VAP	Ventilator-Associated Pneumonia
WHO	World Health Organization

Chapter 1: Introduction

One of the most serious public health problems that is seen globally is lower respiratory tract infections (LRTIs). Millions of people are affected significantly every year and it remains a leading cause of illness and death especially in developing countries (World Health Organization, 2024). These infections are dangerous for the vulnerable people such as the elders, young children, hospitalized patients and individuals with weak immune systems. Different types of microorganisms including viruses, bacteria and fungi cause lower respiratory tract infections, and *Klebsiella pneumoniae* has especially come out as one of the most important opportunistic pathogens which does not typically cause disease but takes opportunity to cause infection when a person's immune system is weakened. This bacterium is associated with healthcare environment where it frequently infects people who already have variety of several medical problems (Podschun & Ullmann, 1998).

Klebsiella pneumoniae is a gram-negative, rod-shaped bacterium that cannot move independently, belongs to the Enterobacteriaceae family. This bacterium is encapsulated which means it has a thick protective outer layer that increases its survival and virulence (Paczosa & Mecsas, 2016). Even though it is commonly found in the human gut, it can also become pathogenic when it travels to other parts of the body. This organism causes a wide range of infections such as pneumonia, urinary tract infections and wound infections. Pneumonia, a respiratory infection, may often cause severe inflammation and tissue tissue damage which can lead to complications if it is not treated right away.

This bacterium plays a particularly significant role in hospital acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). Patients who require mechanical ventilation are at increased risk because the normal defense mechanisms of the respiratory tract are compromised. *Klebsiella pneumoniae* always wins in case of pathogenic success and it is due to its multiple virulence factors. Its polysaccharide capsule is one of its most important features as it helps the bacterium evade phagocytosis and resist destruction by the host immune system. Additionally, lipopolysaccharide (LPS) contributes to inflammation and septic shock, while siderophore molecule help the organism to bind iron from the host environment which is essential for its growth. By attaching firmly to respiratory epithelial cells, adhesin surface proteins facilitate the infection and colonization (Paczosa & Mecsas, 2016).

In recent years, the increasing emergence of multidrug-resistant (MDR) strains of *Klebsiella pneumoniae* has become a major global health challenge. The rise of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is even more alarming which significantly limits treatment options. The bacterium can produce enzymes such as extended-spectrum β-lactamases (ESBLs), which break down many commonly used antibiotics, including penicillins and cephalosporins. Furthermore, carbapenemase enzymes like KPC (Klebsiella pneumoniae carbapenemase) can inactivate carbapenem antibiotics which are often considered last-resort drugs (Nordmann et al., 2011). The wide misuse and overuse of antibiotics in both community and clinical settings have speed up the development and spread of these resistant strains, making treatment of illnesses more challenging and costly.

For effective management and identification of infections, accurate laboratory diagnosis is essential. Isolation and identification of *Klebsiella pneumoniae* from clinical samples such as sputum, play a crucial role in guiding appropriate antibiotic therapy. Conventional culture methods remain important for primary identification, however, they may require longer processing times. Molecular techniques, particularly Polymerase Chain Reaction (PCR), offer rapid, sensitive and specific confirmation of bacterial identity (Becker et al., 2004). In addition, antibiotic susceptibility testing (AST), using methods such as the Kirby-Bauer disk diffusion technique helps in determining the most effective antibiotics for the treatment and assists in monitoring resistance patterns.

The growing problem of antimicrobial resistance (AMR) in *Klebsiella pneumoniae* has made the treatment of respiratory tract infections highly challenging. Over the past years, this organism has developed resistance to many commonly used antibiotics reducing the effectiveness of standard therapies and increasing the risk of complications and mortality. Recognizing the seriousness of this threat, the World Health Organization (WHO) has identified carbapenem-resistant and extended-spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* as a critical priority pathogen (World Health Organization, 2017). This classification highlights the urgent need for new antibiotics and improved infection control strategies. Resistant strains of this bacterium produce enzymes like KPC (Klebsiella pneumonia carbapenemase), which are capable of breaking down β-lactam antibiotics and making many frontline treatments ineffective.

Accurate diagnosis is equally important in managing infections caused by *Klebsiella pneumoniae*. Sputum examination is one of the primary and most practical methods for identifying bacterial pathogens responsible for lower respiratory tract infections (LRTIs). Traditional culture techniques play a fundamental role in the initial isolation and identification of the organism. However, these methods may sometimes lack precision or require longer times. By identifying specific genetic markers unique to the bacteria, molecular techniques such as Polymerase Chain Reaction (PCR) provide greater sensitivity and specificity (Martin & Bachman, 2018). Combining culture based identification with molecular confirmation ensures more accurate diagnosis. In addition, determining the antibiotic susceptibility pattern of the isolated strains is essential for selecting appropriate therapy and reducing the misuse of antibiotics.

It is important that the patterns of drug resistance are continuously monitored, particularly in developing countries like Bangladesh, where irregular antibiotic use and lack of access to advanced diagnostic facilities play a significant role to the increase of multidrug-resistant (MDR) organisms. In Bangladesh, pneumonia and other lower respiratory tract infections are very common and they continue to be a major reason of hospitalization and mortality. Several studies conducted in the country have reported that *Klebsiella pneumoniae* is one of the main bacterial pathogens linked with hospital-acquired pneumonia and respiratory infections. According to a study conducted in a tertiary care hospital in Bangladesh reported that *Klebsiella pneumoniae* isolates showed high resistance to commonly used antibiotics such as cephalosporins and penicillins (Sonia et al., 2020). Similarly, multidrug-resistant *Klebsiella pneumoniae* strains with significant resistance genes were found in another investigation which indicated that antibiotic resistance is becoming more common in Bangladesh (Tanni et al., 2021). Since the prevalence and antibiotic resistance patterns of bacterial pathogens keep changing over time, it is necessary to keep it in continuous monitoring. Frequent investigation *on Klebsiella pneumoniae* isolation, identification and drug susceptibility patterns can help us by providing updated information that is useful for choosing effective antibiotics and improving infection control techniques.

Objective: The objective of this study was to isolate and identify *Klebsiella pneumoniae* from sputum samples of patients diagnosed with lower respiratory tract infections and to determine the antibiotic susceptibility pattern of the isolated strains.

Chapter 2: Methodology

2.1 Study Design:

This study was designed as an experimental laboratory based investigation to isolate, identify and analyze the antibiotic susceptibility pattern of *Klebsiella pneumoniae* obtained from clinical sputum samples. The research primarily focused on understanding the presence of this pathogen in respiratory infections and examining its resistance behaviour against some commonly used antibiotics. Standard microbiological culture techniques were first used to obtain bacterial isolates from sputum samples in a laboratory setting, followed by molecular confirmation using Polymerase Chain Reaction (PCR) that allowed accurate identification. It also aimed to determine how these bacterial isolates respond to different antimicrobial agents. For this purpose, antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method for assessing bacterial resistance patterns. This study provides an in-depth evaluation of *Klebsiella pneumoniae* from clinical sputum samples by combining traditional microbiological techniques with molecular confirmation and susceptibility testing.

2.2 Study Area and Duration:

The clinical sputum samples used in this research were collected from the National Institute of Diseases of the Chest and Hospital (NIDCH), Mahakhali, Dhaka. The entire study was completed over a period of several months. This duration included multiple stages of the research process, including clinical sample collection and transportation, isolation and culture of bacterial organisms, molecular confirmation by Polymerase Chain Reaction (PCR) and antibiotic susceptibility patterns evaluation. Each step was performed systematically to ensure reliable and accurate results throughout the study. The collected samples were then transferred to life science laboratories of BRAC University for further experiment.

2.3 Sample Collection and Transportation:

Sputum samples were collected from patients suspected of having lower respiratory tract infections at the hospital. The sputum specimens are generally thick and viscous in nature, so the collected samples required proper processing before microbiological analysis. The processed sputum

samples were first sub-cultured onto MacConkey agar medium which is commonly used for the isolation of gram-negative bacteria. After incubation, colonies (pink) suspected to be *Klebsiella* species were further sub-cultured on KPC (Klebsiella pneumoniae carbapenemase) selective medium for more specific detection and confirmation.

Following the initial culturing steps, the sample plates were transported to the life science laboratory of BRAC University for further analysis and were kept in a temperature controlled ice box during the transportation to preserve their viability and maintain sample integrity. This controlled condition helped prevent contamination and ensured that the bacterial isolates remained stable until further laboratory processing and analysis were done.

2.4 Incubation and Isolation of Bacterial Colonies:

After transportation to the research laboratory, the inoculated plates were incubated at 37°C for 24 hours. After incubation, distinct colonies were observed on the agar media plates, indicating successful growth of the isolates. Well isolated colonies were then selected for further microbiological and molecular analysis.

2.5 DNA Extraction (Boiling Method):

Genomic DNA was extracted from bacterial isolates by using the boiling method.

Procedure:

1. A loopful of bacterial colony was suspended in 400 µL TE buffer.
2. The suspension was vortexed for approximately 15 seconds to ensure proper mixing.
3. The mixture was centrifuged at 13,000 rpm for 10 minutes.
4. The supernatant was discarded carefully.
5. The pellet was resuspended in 400 µL TE buffer and vortexed again for 15 seconds.
6. The suspension was incubated in a water bath at 100°C for 10 minutes to lyse bacterial cells.

7. The mixture was kept at room temperature for 5–10 minutes for cooling.
8. The lysate was centrifuged again at 13,000 rpm for 10 minutes.
9. The supernatant (200 µL) containing genomic DNA was transferred into sterile microcentrifuge tubes.
10. Extracted DNA was stored at −20°C until PCR analysis.

2.6 Primer Information for PCR Identification:

Molecular identification of *Klebsiella pneumoniae* was performed by polymerase chain reaction (PCR) targeting a species-specific gene region. The targeted gene was 16S-23S rDNA. The primer sequences used in this study amplified a 130 bp fragment specific for *Klebsiella pneumoniae*.

Target Organism	Primer Name	Sequence (5'–3')	Product Size
Klebsiella pneumoniae	KP Pf-F	ATTTGAAGAGGTTGCAAACGAT	130 bp
Klebsiella pneumoniae	KP Pf-R	TTCACTCTGAAGTTTTCTTGTGTTC	

Table 1: *Primer details*

2.7 PCR Reaction Mixture Preparation:

PCR amplification was carried out in a final reaction volume of 13 µL per sample. The reaction mixture consisted of the following components:

Components	Volume per Reaction
Master Mix	6 µL
Nuclease-free water	3 µL

Forward primer	1 µL
Reverse primer	1 µL
Template DNA	2 µL

***Table 2**: PCR Mixture Preparation*

Initially, the master mix was prepared in a sterile 1.5 mL microcentrifuge tube by adding master mix, nuclease-free water, forward primer and reverse primer. A total of 11 µL of the prepared PCR mixture was then aliquoted into individual PCR tubes. Subsequently**,** 2 µL of extracted DNA was added to each labeled PCR tube to make the final reaction volume of 13 µL. The tubes were gently mixed and briefly centrifuged using a short spin to collect the contents at the bottom of the tube before placing them in the PCR machine or thermal cycler.

2.8 PCR Cycling Conditions:

PCR amplification was performed in a thermal cycler under the following conditions:

1. Initial denaturation at 94°C for 10 minutes
2. 35 cycles of-
    - Denaturation at 94°C for 30 seconds
    - Annealing at 57°C for 20 seconds
    - Extension at 72°C for 20 seconds
3. Final extension at 72°C for 10 minutes

After completion of amplification, the PCR products were stored at 4°C until further analysis.

2.9 Agarose Gel Electrophoresis:

After all the preparations of PCR, the amplified PCR products were analyzed by agarose gel electrophoresis to confirm the presence of the expected 130bp amplicon.

Gel Preparation

- Agarose powder: 1.7 g
- Distilled water: 98 mL
- 50× TAE buffer: 2 mL

The mixture was heated until completely dissolved and cooled for 10 minutes. Later, 4 µL of Ethidium Bromide (EtBr) was added to the mixture and poured into a casting tray with combs. After solidification, the gel was placed in an electrophoresis chamber filled with running buffer. Then, PCR products and DNA ladder were loaded into wells. Electrophoresis was performed at appropriate voltage until separation was achieved. After the gel run, bands were visualized under UV transillumination and the presence of a distinct band at approximately 130bp was considered positive for *Klebsiella pneumoniae*.

2.10 DNA Ladder Used:

A Quick-Load 100 bp DNA Ladder was used as a molecular size marker. The ladder contained fragments ranging from 100 bp to 1517 bp, allowing accurate identification of the expected 130bp PCR product.

2.11 Controls Used in PCR:

To ensure accuracy and reliability of PCR results, the following controls were used:

- Positive control (known *Klebsiella pneumoniae* DNA)
- Negative control (no template DNA)

2.12 Antibiotic Susceptibility Testing (AST):

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines, 2020.

Preparation of Mueller-Hinton plate

Mueller-Hinton Agar (MHA) was prepared using:

- Distilled water: 750 mL
- Agar powder: 28.5 g

30 plates were prepared for testing because two plates were prepared per sample.

Preparation of Normal Saline (0.9% NaCl)

Sterile normal saline (0.9% NaCl) was prepared for the preparation of bacterial suspension. Each test tube contained 5 mL of saline solution. A total of 15 test tubes were prepared; therefore, the total required volume was calculated as:

$$15 \times 5 \text{ mL} = 75 \text{ mL}$$

To prepare 75 mL of 0.9% saline solution, 0.675g of sodium chloride (NaCl) was required and weighed and dissolved in distilled water to make a final volume of 75 mL. The solution was mixed thoroughly until completely dissolved and then dispensed aseptically into 15 sterile test tubes (5 mL per tube). The prepared saline was sterilized by autoclaving at 121°C and allowed to cool before use.

Preparation of Bacterial Suspension

Fresh bacterial colonies were suspended in sterile normal saline and gently vortexed to ensure uniform mixing and to obtain a homogeneous bacterial suspension. The turbidity of the suspension was visually adjusted to 0.5 McFarland standard to achieve an even distribution of bacterial cells. Then, the suspension was uniformly spread on Mueller-Hinton agar plates using sterile cotton swabs.

8 antibiotics were tested against the bacterial isolates.

Serial No	Antibiotic Name	Disc Code	Disc Potency	Sensitive (S) ≥ mm	Intermediate(I) mm	Resistant(R) ≤ mm
1	Kanamycin	K	30 µg	≥18	14–17	≤13
2	Tetracycline	TE	30 µg	≥15	12–14	≤11
3	Cefepime	FEP	30 µg	≥25	19–24	≤18
4	Imipenem	IPM	10 µg	≥23	20–22	≤19
5	Cefixime	CFM	5 µg	≥19	16-18	≤15
6	Meropenem	MEM	10 µg	≥23	20–22	≤19
7	Amoxicillin-clavulanic acid	AMC	30 µg	≥18	14–17	≤13
8	Ceftriaxone	CRO	30 µg	≥23	20–22	≤19

***Table 3**: List of Antibiotics and Interpretation criteria used in the AST test (CLSI, 2020)*

2.13 Placement of Antibiotic Discs:

For each bacterial isolate:

- Two Mueller-Hinton agar plates were used.
- Four antibiotic discs were placed per plate.
- Plates were incubated at 37°C for 18–24 hours.

After incubation, zones of inhibition were measured in millimeters following the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2020.

Results were interpreted as:

- Sensitive (S)
- Intermediate (I)

- Resistant (R)

2.14 Data Collection and Analysis:

All experimental observations including PCR band presence, gel electrophoresis results and antibiotic susceptibility zones were recorded systematically. Zone diameters were measured using a ruler in millimeters and compared with CLSI interpretative charts to determine susceptibility patterns. Data were organized into tables and analyzed descriptively.

2.15 Biosafety and Ethical Considerations:

Standard microbiological safety procedures were followed throughout the study. Personal protective equipment (PPE) including gloves and lab coats were used during sample handling and experimentation. Clinical samples were handled confidentially and no patient identifying information was recorded.

Chapter 3: Result

3.1 Molecular Detection of *Klebsiella pneumoniae:*

Polymerase Chain Reaction (PCR) was carried out to confirm the presence of *Klebsiella pneumoniae* using species-specific primers targeting a 130 bp gene fragment. Due to laboratory workflow and sample processing limitations, the PCR amplification and subsequent agarose gel electrophoresis were performed in three separate batches.

The amplified PCR products were analyzed through agarose gel electrophoresis and visualized under UV transillumination. A 100 bp DNA ladder was used as a molecular size marker to estimate the size of the amplified fragments. The appearance of a clear and distinct band at approximately 130 bp was considered a positive result- confirming the presence of *Klebsiella pneumoniae* in the tested isolates.

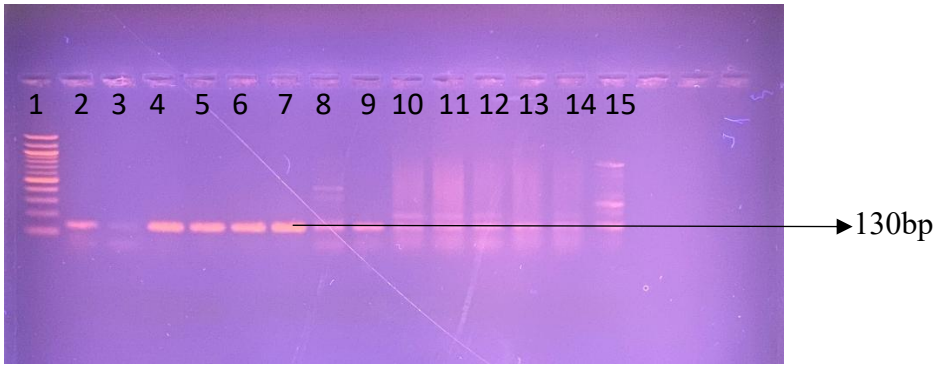

Figure 3.1.1: *Agarose gel electrophoresis of PCR products of Klebsiella pneumonia isolates. Here, well 1 is 100 bp DNA ladder, and well (4-7) showed distinct bands at 130bp. Well 2 and 3 contained positive and negative control respectively.*

These samples exhibited well defined bands corresponding to the expected 130 bp size, indicating successful detection of the target organism. However, some isolates did not show any visible amplification band which suggests either the absence of the target gene or sample DNA contamination. Overall, the PCR results supported the identification of *Klebsiella pneumoniae* in a significant number of isolates, demonstrating the reliability of molecular techniques in confirming bacterial identity alongside conventional microbiological methods.

3.2 Zone of Inhibition (mm) of Antibiotics Against *Klebsiella pneumoniae* Isolates:

Antibiotic susceptibility testing of the confirmed *Klebsiella pneumoniae* isolates was carried out using the Kirby-Bauer disk diffusion method on Mueller- Hinton agar. The zone of inhibition was measured in millimeters (mm) and interpreted according to Clinical and Laboratory Standards Institute guidelines, 2020.

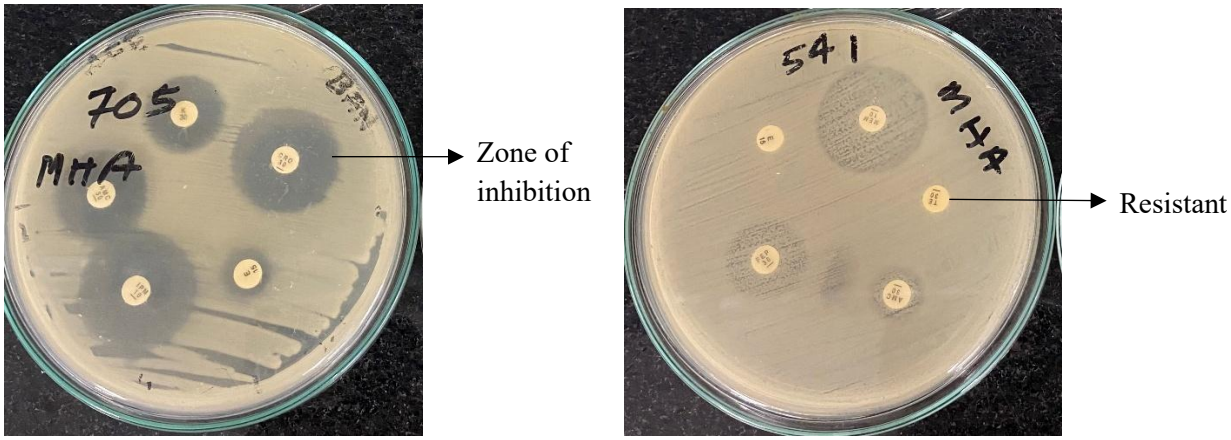

Figure 3.2.1: *Antibiotic Susceptibility Test plates showing zones of inhibition and no zone of inhibition (resistance) of Klebsiella pneumoniae isolates*

3.3 Antibiotic Resistance Pattern:

The antibiotic susceptibility testing results revealed a varied resistance pattern among the *Klebsiella pneumoniae* isolates. Overall, the isolates exhibited a high level of resistance to several commonly used antibiotics, while showing better sensitivity toward carbapenem class.

Percentage= (no. of category/ total valid samples) *100

Disc code	Antibiotic Name	Sensitive (%)	Intermediate (%)	Resistant (%)
K	Kanamycin	7.14%	35%	57%
TE	Tetracycline	21.4%	7.14%	64.28%
FEP	Cefepime	30.76%	15.38%	53.84%
IPM	Imipenem	42.85%	7.14%	42.85%
CFM	Cefixime	0%	0%	100%
MEM	Meropenem	16.67%	25%	58.34%
AMC	Amoxicillin clavulanic acid	35.71%	28.57%	35.71%
CRO	Ceftriaxone	21.42%	7.14%	71.42%

Table 4*: Percentage Distribution of Antibiotic Resistance pattern of 15 confirmed Klebsiella pneumoniae isolates*

A high level of resistance was observed against several widely used antimicrobial agents among the tested antibiotics. The highest resistance was observed against Cefixime (CFM) which was 100%. A high resistance rate was also observed for Ceftriaxone (CRO- 71.42%) and Tetracycline (TE- 64.28%). Moderate resistance levels were detected for Cefepime (FEP- 53.84%) and Meropenem (MEM- 58.34%), indicating reduced susceptibility among several isolates.

In contrast, Imipenem (IPM) showed considerably higher susceptibility with 42.85% of the isolates being sensitive, while the same percentage also showed resistance (42.85%). The action of amoxicillin-clavulanic acid (AMC) was moderate with 35.71% of isolates showing sensitivity and an equivalent percentage showing resistance. Furthermore, Cefepime (FEP) showed moderate susceptibility with 30.76% sensitive isolates indicating that some strains remain responsive to cephalosporins. These findings indicate that resistance to several β-lactam antibiotics (such as penicillins, cephalosporins, carbapenems) is common among the isolates. Carbapenem antibiotics (IPM and MEM) continue to be the most reliable and effective treatment options against the *Klebsiella pneumoniae* isolates.

Moreover, several isolates showed resistance to more than three different classes of antibiotics, indicating the presence of multidrug-resistant (MDR) strains which is consistent with global reports on increasing MDR *Klebsiella pneumoniae* infections (World Health Organization, 2022).

MDR organisms play a great role in restricting treatment options and are significantly linked to higher rates of morbidity and mortality.

Disc Code	Antibiotic	Antibiotic class
K	Kanamycin	Aminoglycoside
TE	Tetracycline	Tetracycline
FEP	Cefepime	Cephalosporin
CFM	Cefixime	
CRO	Ceftriaxone	
IPM	Imipenem	Carbapenem
MEM	Meropenem	
AMC	Amoxicillin-Clavulanic Acid	Penicillin/ β-lactam

Table 5: *Classification of Antibiotics Used in the Study*

Based on the resistance patterns observed in the sputum sample isolates, 6 out of 15 isolates exhibited multidrug resistance, showing resistance to at least three different classes of antibiotics. The presence of MDR strains emphasizes how difficult it is becoming to cure infections or diseases caused by this organism (*Klebsiella pneumoniae)*.

Chapter 4: Discussion

The study highlighted the antimicrobial resistance profile and molecular detection of selected *Klebsiella pneumoniae* isolates from clinical sputum samples providing us important insights into the growing concern of multidrug resistance in clinical settings. The findings demonstrated a clear observation of reduced susceptibility to commonly used antibiotics among the isolates, suggesting limited effectiveness of these drugs in treating infections caused by *Klebsiella* isolates.

A key observation of the study was the high level of resistance exhibited by the isolates across multiple classes of antibiotics which reflected the growing challenge of antimicrobial resistance. Complete resistance was observed against Cefixime (100%), indicating that this antibiotic was entirely ineffective against the tested isolates. A high level of resistance was also observed for Ceftriaxone (71.42%). Similar observations have been observed in a study conducted in tertiary care hospitals in Bangladesh which reported an even higher Ceftriaxone resistance rate of 90.67% among clinical *Klebsiella pneumoniae* isolates (Sonia et al., 2020). Although the resistance percentages vary between studies, both findings indicate a concerning level of resistance to third-generation cephalosporins in Bangladesh. Such variations in resistance rates may occur due to differences in study period, sample size, hospital environment and antibiotic usage patterns. Moreover, cefepime and meropenem showed considerable resistance among the isolates. These findings are similar to previous studies which showed that *Klebsiella pneumoniae* produces ESBL enzyme, which is resistant to many β-lactam and cephalosporin that can break down these drugs and make them ineffective or useless against infection (Podschun & Ullmann, 1998).

In contrast, carbapenem antibiotics demonstrated comparatively better activity against the isolates tested in this study. Among the tested drugs, Imipenem exhibited the lowest resistance rate and the highest sensitivity of about 42.85%, indicating that carbapenems may still remain relatively effective treatment options for infections caused by *Klebsiella pneumoniae*. Similar observations have been reported in international studies, where carbapenems are frequently considered reliable antibiotics for severe infections caused by resistant gram-negative bacteria (Nordmann et al., 2011). However, the detection of resistance to carbapenems among several isolates suggests the potential emergence of carbapenem resistant strains, which is a growing global concern. Amoxicillin-clavulanic acid also showed a high sensitivity of 35.71%, suggesting that carbapenems and β-lactam combinations remain comparatively effective treatment options. The

amoxicillin is an antibiotic and clavulanic acid helps by blocking the bacteria's resistance enzyme (ESBL). So together, they can kill or stop the bacteria.

The study also revealed evidence of multidrug resistance (MDR) among the isolates as many samples exhibited resistance to three or more classes of antibiotics. This pattern is particularly concerning because MDR organisms significantly limit treatment options and they are associated with increased morbidity, mortality and healthcare costs. Out of the 15 confirmed *Klebsiella pneumoniae* isolates tested, 6 isolates (about 40%) showed resistance to multiple classes of antibiotics. A comparable prevalence of MDR *Klebsiella pneumoniae* has also been reported in Bangladesh, where around 41% of clinical isolates were identified as multidrug resistant (Kawser et al., 2024). The presence of MDR strains emphasizes the increasing challenge of antibiotic resistance and highlights the importance of continuous monitoring, rational antibiotic use and effective infection control strategies.

Moreover, the study highlighted the importance of strict procedural control and optimization in microbiological assays. The molecular analysis using PCR further supported the identification of selected isolates. The presence of distinct bands at the expected size (approximately 130 bp) in 30 multiple wells across three separate gel runs confirms the successful amplification of the target gene. Conducting PCR in multiple batches ensured specificity and minimized the chances of experimental error, thereby strengthening the reliability of the results. Another important factor in this study was the variation observed in the isolate's response to different antibiotics. Certain isolates showed sensitivity while others showed total resistance, even within the same class of antibiotics. This variation suggested the presence of diverse resistance mechanisms within the bacterial population such as enzymatic degradation of antibiotics etc. Furthermore, the detection of resistance to cephalosporins, such as ceftriaxone and cefepime, indicated the likely presence of extended-spectrum β-lactamase (ESBL) producing strains. ESBL production enables bacteria to hydrolyze or break down a wide range of β-lactam antibiotics, meaning making them ineffective. This not only makes the treatment complicated but also increases the possibility of resistant strains spreading quickly in healthcare environments. The resistance pattern found in this study aligns with global trends where ESBL producing *Klebsiella pneumoniae* has become increasingly prevalent (Paterson & Bonomo, 2005). In addition, certain outcomes may have been affected by environmental and procedural factors. Variations in inoculum density, incubation conditions etc.

can all affect zone sizes in disk diffusion tests. Although standard procedures were followed, minor inconsistencies are inevitable in laboratory based studies. Therefore, antimicrobial susceptibility testing should be strongly encouraged to be done routinely in clinical settings to direct an appropriate antibiotic therapy. In summary, this study demonstrated a concerning level of antibiotic resistance among *Klebsiella pneumoniae* isolates with a clear indication of multidrug resistance and reduced effectiveness of commonly used antibiotics, while certain antibiotics such as carbapenems showed relatively better activity. The molecular confirmation of isolates and resistance patterns observed across samples strengthen the validity of the findings.

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