Study on Bacteriological Profile and Antimicrobial Susceptibility Pattern of *Klebsiella pneumoniae* and *Escherichia coli* From Blood of Suspect Septicemia Patient

By

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A thesis submitted to the in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Bachelor of Science in Microbiology
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Declaration

It is hereby declared that

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

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Approval

The thesis/project titled Study on "Study on Bacteriological Profile and Antimicrobial Susceptibility Pattern of Klebsiella pneumoniae and Escherichia coli From Blood of Suspect Septicemia Patient" submitted by

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of Spring, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of bachelor of Science in Microbiology on 15th May,2025.

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Ethics Statement

This study was approved by the departmental review committee of Brac University, Dhaka, Bangladesh. All the participants were provided written and informed consent to participate in the study in the study. To ensure participants' confidentiality and privacy, their personal identities were strictly and carefully anonymized in all records and reports. This research was completed in accordance with BRAC University's ethical guidance. All the protocols were carefully followed to maintain standards of ethical research practices.

Abstract

Septicemia, a life-threatening bloodstream infection, continues to pose significant public health challenges, particularly in low- and middle-income countries such as Bangladesh where multidrug-resistant (MDR) pathogens are prevalent. This study aimed to identify the bacteriological profile and evaluate the antimicrobial susceptibility patterns of *Klebsiella pneumoniae* and *Escherichia coli* in septicemia-suspected patients. Blood samples from 20 patients were collected from different hospitals in Dhaka and analyzed using selective culture methods, DNA extraction, PCR, and antibiotic susceptibility testing via the Kirby-Bauer disc diffusion method. Out of 20 samples, 9 (45%) showed positive bacterial growth. *Klebsiella pneumoniae* was identified in 5 samples (25%) and *Escherichia coli* in 4 (20%). A significant age-based prevalence was observed, with all infections occurring in individuals aged 30 and above, particularly those aged 40–69. Gender-based analysis showed a higher infection rate among males (66.7%). Antimicrobial susceptibility testing revealed high resistance to commonly used antibiotics such as ampicillin, erythromycin, cefixime, and chloramphenicol. In contrast, imipenem, meropenem, and ciprofloxacin retained high efficacy against both pathogens. Notably, MDR and extended drug-resistant (XDR) patterns were detected in both bacterial species. From the result observation of *Klebsiella pneumoniae* 25% of the drugs exhibited multidrug resistance (MDR) and for *Escherichia coli* 21.43% of the drugs exhibited multidrug resistance (MDR). It is clear that the urgent need for enhanced diagnostic capabilities, age-targeted infection control, and antibiotic stewardship in Bangladesh. Findings contribute valuable data to local antimicrobial resistance surveillance and highlight the need for evidence-based treatment protocols to combat septicemia and its associated morbidity and mortality.

Keywords: Septicemia, bloodstream infection, *Klebsiella pneumoniae*, *Escherichia coli*, antimicrobial resistance, multidrug resistance (MDR), extended drug resistance (XDR), antibiotic susceptibility, Bangladesh, PCR diagnostics.

Dedication

“To my beloved family”

I am also grateful to my friend Musharrat Quazi for her cooperation and assistance in conducting the project seamlessly. Her support was crucial for the smooth progression of my work.

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I would like to start by expressing my deepest gratitude to my Almighty Allah for allowing me to pursue and complete this research and to my parents and sibling for supporting me from the very beginning till the end. I am grateful to the Chairperson of the Department of MNS, Md. Firoze H. Haque Sir and to all the teachers of BRAC University for always supporting me. Special thanks to my supervisor Dr Fahim Kabir Monjurul Haque, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University whose guidance, insightful feedback, and endless support was instrumental when it came to the success of this research. The research would never have reached its potential if she had not given dedication and constructive criticism at times.

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List of Acronyms

AST	Antimicrobial Susceptibility Testing
MDR	Multidrug Resistant
MHA	Mueller-Hinton Agar
NA	Nutrient Agar
TE	Tris-EDTA Buffer
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
CFU	Colony-Forming Unit
ESBL	Extended-Spectrum Beta-Lactamase
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*

MIC	Minimum Inhibitory Concentration
CLSI	Clinical and Laboratory Standards Institute

Chapter 1

Introduction

1.1 Background

Septicemia, or bloodstream infection (BSI), is a life-threatening condition with the presence of pathogenic microbes or their toxins within the circulation, eliciting systemic inflammatory responses and multi-organ dysfunction (Seifert, 2009). It remains a worldwide cause of morbidity and mortality, including among low- and middle-income nations like Bangladesh, where diagnostic facilities are scarce and antimicrobials are abused, exacerbating the condition (Bhuiyan et al., 2017). Among the bacterial causes of septicemia, both Gram-negative and Gram-positive organisms are significant; however, of recent trends is the multidrug-resistant Gram-negative organisms, which pose a daunting challenge to clinical management (Kumarasamy et al., 2010).

Gram-negative organisms such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are leading causes of bloodstream infections both in hospital and community settings (Diekema et al., 2019). Their natural and acquired mechanisms of resistance—like the production of extended-spectrum beta-lactamases (ESBLs), carbapenemases, and efflux pumps—have rendered most of the frontline antibiotics ineffective (Paterson & Bonomo, 2022). The lipopolysaccharide-enriched outer membrane structure of Gram-negative bacteria not only serves as a physical barrier but also assists them in resisting antimicrobial agents and host immune systems (Nikaido, 2003). This structural protection, in addition to the rampant horizontal transfer of resistance genes, emphasizes their stability and endurance in healthcare environments (Livermore, 2002).

In Bangladesh, septicemia caused by multidrug-resistant pathogens is on the rise, especially in intensive care units (ICUs) and neonatal intensive care units (NICUs) (Rahman et al., 2020). Studies have reported high rates of bloodstream infections caused by *K. pneumoniae, E. coli* and *Acinetobacter spp.* with regular resistance to third-generation cephalosporins, aminoglycosides, and even carbapenems (Shamsuzzaman et al., 2017). Lack of infection control measures and unselective use of antibiotics coupled with poor surveillance cause the dissemination of these resistant isolates (Islam et al., 2019). Bloodstream infection in neonates and immunocompromised individuals is particularly important due to increased mortality and lack of therapeutic options (Saha et al., 2018). Timely and accurate identification of the causative microbes and their antimicrobial sensitivity pattern is vital for the effective clinical treatment of septicemia. Conventional culture-based methods like blood culture, Gram stain, and biochemical analysis are yet to be the cornerstone of diagnosis in most of the clinical laboratories of Bangladesh. These methods are time-consuming, labor-intensive, and require 48–72 hours or even more time to yield ultimate results (Forbes et al., 2016). The late identification of the pathogen and reporting often lead to empirical broad-spectrum antibiotic treatment, further contributing to antimicrobial resistance (Rajkumari & Roy, 2023). Septicemia, if not promptly diagnosed and adequately treated, can lead to septic shock, disseminated intravascular coagulation, and multiple organ failure, highlighting the need for effective diagnostic strategies and antimicrobial stewardship (Singer et al., 2016). The increasing prevalence of multidrug-resistant organisms among bloodstream infections highlights the need to monitor local pathogen epidemiology and resistance trends to guide empirical treatment and infection control guidelines (Saxena et al., 2023).

1.2 Age as a Risk Factor for Septicemia

Although septicaemia can strike anyone at any age, as people age, their risk and severity of the illness rise dramatically. Notably, due to physiological, medicinal, and immunological variations, the demographic group most at risk of septicaemia is those aged 35 and older. This underlying tendency gradually rises with age, and according to the patient data gathered for this investigation, every occurrence of septicaemia found involved individuals 40 years of age or older. The condition known as immunosenescence, or a reduction in the body's immunological efficacy, is brought on by hormonal changes during the ageing process. As a result, older folks are less equipped to combat diseases as effectively as feasible. Additionally, noncommunicable disorders lower immunity and promote septicaemia. These include diabetes, cardiovascular disease, chronic renal disease, and many other conditions that disproportionately impact the elderly due to their advanced age (Clegg et al. 2013). Additionally, the elderly population requires invasive medical procedures such as central line insertions or catheterisation, where bloodstream infections might be caused by nosocomial microorganisms.

This patient data of this work reveals a clear pattern: One noteworthy aspect of a cross-sectional investigation is that every verified case of *Staphylococcus aureus* and *Klebsiella pneumonia* was discovered in people older than 40. This finding is consistent with global research showing that one of the main variables influencing severe septicaemia is advanced age. The risk of bloodstream infection in this group is increased by a number of predisposing variables, such as elderly patients receiving immunosuppressive medication, frequent hospitalization, and multidrug use (Weiner-Lastinger et al., 2020).

Age-specific preventative measures are therefore quite desirable. Elderly septicaemia can be effectively prevented by early infection surveillance, adherence to general precautions in the health system, and cohort-specific appropriate antibiotic use methods. It is essential to improve the clinical index and reduce the frequency of septicaemia in the elderly in order to target these characteristics as risk factors.

1.3 Objectives

Objectives:

1. To Determine the Typical Bacterial Pathogens in Cases of Septicaemia:

Finding common bacterial pathogens which are present in Bangladeshi.

2. To Evaluate Antimicrobial Susceptibility Patterns: This is done to determine how susceptible the microorganisms are to the widely used antibiotics, with an emphasis on MDR.

3. To Examine Trends in Septicaemia Associated with Age:

to look at the connection between septicaemia and patient age, specifically with regard to older persons over 36.

By achieving these goals, the nation's epidemiology and antibiotic resistance in septicaemia will be better understood, and patient treatment and public health will be improved overall.

Chapter 2

Materials and Methods:

2.1 Workflow

Sample Collection

Through sterilized syringe and blood collecting tubes sample was collected from the patient

⬇

Serially sample spread on 6 different media

⬇

Sub cultured on Nutrient Agar (NA)

⬇

DNA Extraction by Boiling Method

Extraction of bacterial DNA

⬇

Polymerase Chain Reaction (PCR)

⬇

Agarose Gel Electrophoresis

Detection of PCR products

⬇

Antibiotic Susceptibility Testing

2.2 Study Design

Investigating the bacteriological profile and patterns of antibiotic resistance in patients with a suspicion of septicaemia is the goal of the current investigation. Two sites in Dhaka, Bangladesh—the National Heart Institute and Asha Private Clinic—were used to get blood samples. In order to comply with ethical standards and procedures, ethical approval was acquired from the relevant institutions before to conducting this research project. In the context of a suspected bloodstream infection, septicaemia suspected is a clinical disease marked by systemic signs and symptoms. It usually exhibits the following key characteristics in a classical presentation:

Chills and Fever: Fever, which often manifests as chills or rigours in the context of the systemic inflammatory response to an infection, can be chronic or recurrent.

Changes in Mental Status: The systemic consequences of an illness or reduced brain perfusion might cause confusion, sluggishness, or disorientation. The Third International Consensus Definitions for Sepsis and Septic Shock's Sepsis-3 definition states that these symptoms are typical of sepsis, which supports a stronger focus on the host's dysregulated response to infection, which results in potentially fatal organ failure. These clinical indicators are frequently accompanied by laboratory results that further support the diagnosis of probable septicaemia, such as high levels of procalcitonin, C-reactive protein, and white blood cell count. To identify the infectious agent and establish the presence of pathogens in the blood, blood culture is necessary.

Sepsis-infection is defined by the signs of a serious infection as something that endangers life or function. Suspected septicaemia must be identified and treated promptly since failure to do so may worsen the condition and result in septic shock or severe sepsis, which would primarily increase the risk of death (Rhee et al., 2017). In the study described here, the sample collection techniques are predicated on rigorous protocol inclusion for accurately detecting bacteria among patients falling under the category of probable septicaemia.

2.2.1: Collection of Samples:

The technique of collecting specimens was designed to be accurate, standardised, and aseptic. The following methods were used to obtain blood samples from individuals who were suspected of having septicaemia:

Aseptic Technique: All blood samples were obtained aseptically to minimise contamination. Sterilisation of the syringes, needles, and collecting tubes was one of the universal precautions used while touching the patient. Before the samples were collected, the venipuncture site was cleaned with an alcohol-based antiseptic.

Volume of the Sample: Three millilitres of blood were drawn from each subject on each occasion. Aliquoting the samples into sterile, heparinised tubes was done right away. In order to keep the blood samples liquid during the ensuing culturing and processing, heparin was used to reduce coagulation.

Transport and Storage: After the blood samples were collected, they were identified based on the patient's specific characteristics, including age, gender, and whether they were inpatient or outpatient. The samples were collected, properly labelled, and collected samples were transferred to Brac University's lab for maintaining cold chain at 4C.

2.2.2: Preparation of Sample:

As soon as the blood samples arrived at the microbiology lab, they were treated to guarantee maximum cleanliness. To ensure process sterility, all of the aforementioned procedures were carried out in a laminar flow biosafety cabinet. 70% ethanol was used to disinfect the outside of the test tubes containing the heparinized blood samples in order to lessen the impact of outside factors on the samples. Following sample collection, labels were placed on each one and verified for correct identification using patient data. To identify bacteria and their sensitivity to antibiotics, the prepared samples were then aseptically injected using the proper culture media. They used this methodical methodology to guarantee the tests' dependability, which was extremely important for their experiment.

2.2.3: Isolation and Identification of bacteria:

100 µL of each blood sample was aseptically transferred and dispersed using the spread plate method across six distinct selective and differential media designed for recognising certain organisms in order to isolate and identify bacterial infections. Hichrome KPC agar was utilised for *Klebsiella pneumoniae* (KP), allowing for distinction by unique colony colouration. HiChrome UTI agar was used to isolate *Escherichia coli*, enabling precise identification using chromogenic substrate reactions.

SS (*Salmonella-Shigella*) agar was utilized to identify *Salmonella* species, highlighting enteric pathogens through colony morphology and hydrogen sulfide production. MacConkey agar, a versatile selective and differential medium, was also used to isolate Gram-negative bacteria and differentiate lactose fermenters from non-lactose fermenters. All plates were incubated under optimal conditions specific to the growth requirements of the respective organisms, ensuring reliable isolation for downstream analyses.

Profiling of Pathogens from Blood Cultures:

Based on their unique colony shape on selective medium, two target species were successfully identified and verified. On both HiChrome KPC agar and HiChrome UTI agar, *Klebsiella pneumoniae* (KP) was distinguished by its distinctive blue colonies. *Escherichia coli's* purple colonies on the same medium, which demonstrated its chromogenic response, served as confirmation. However, because there was no discernible growth on SS agar or Leeds *Acinetobacter Baumannii* agar, respectively, the other two target organisms—*Salmonella spp*. and *Acinetobacter baumannii*—could not be identified. These results highlight the specificity of the isolation techniques and the absence of the latter pathogens in the analyzed samples.

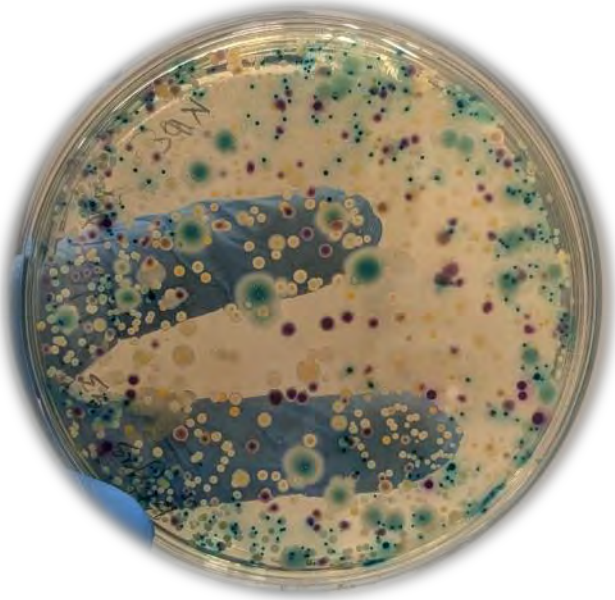

Figure 1: *Klebsiella Pneuomoniae's* growth of blue color on HiCrome KPC agar.

Figure 2: *Escherichia coli's* growth of purple color on HiCrome UTI agar.

2.2.4: DNA Extraction:

As the basis solution for cell lysis, 1X Tris-EDTA buffer was prepared before the DNA extraction process started. First, an Eppendorf tube was filled with 400 µL of 1X TE buffer. The buffer-containing tube was then infected with a loopful of the bacterial culture. After that, the sample was vortexed to fully combine the TE buffer and the bacteria. To pellet the bacterial cells, the sample was centrifuged for 10 minutes at 13,000 rpm and 25°C after initial mixing. Following centrifugation, 400 µL of 1X TE buffer was added to the Eppendorf tube along with the supernatant. The bacterial pellet was then resuspended by vortexing the sample one more. In order to rupture the bacterial cells and liberate their genomic DNA, the samples were to undergo a heat treatment. For ten minutes, the tubes were kept at 100°C in a heat block. The bacterial cell wall and membrane will be properly lysed by this heat treatment, releasing the DNA into the solution. Following this heat block process, the samples were allowed to cool for five minutes at room temperature. To remove the recovered DNA from any leftover debris or biological components, the cooled samples were centrifuged for 10 minutes at 13,000 rpm. 200 µL of the DNA supernatant was transferred to a new Eppendorf tube after these centrifugation procedures. Extreme caution was used to avoid disturbing the pellet at the tube's bottom during the transfer. The isolated DNA was now present in this supernatant, making it suitable for further examination. For use in PCR or sequencing, this technique guaranteed the effective extraction of high-quality DNA from the bacterial cells.

2.2.5: PCR (Polymerase Chain Reaction)

It is a method used in laboratories to quickly create millions to billions of copies of a certain DNA sequence. This process readily and rapidly creates a large number of copies, which is helpful for medical diagnostics, forensic analysis, and molecular biology testing (Britannica, T. Editors of Encyclopaedia 2023). rounds of replication utilising a PCR equipment (The Applied Biosystems 2720 Thermal Cycler) in a matter of hours. Each primer was added to a 25 µL PCR mixture, which also contained 4 µL of template DNA, 12 µL of PCR master mix (Emerald Amp), 2 µL of forward primer, 2 µL of reverse primer, and 5 µL of nuclease-free water. To guarantee proper mixing, the liquid was vortexed for three to five seconds. After that, PCR was performed while maintaining the PCR parameters for every target gene. After the PCR was finished, all of the PCR results were to be segregated at -20°C for further analysis.

Targeted Genes and Primers: Escherichia coli and Klebsiella pneumoniae virulence-specific genes were selected for the current study in order to screen the samples for bloodborne pathogens. Targeting the particular genes linked to these bacteria' pathogenicity became crucial because of their strong resemblance to septicaemia and bloodstream infections. For example, the pathogenic strains of Escherichia coli are responsible for severe sepsis, and they are mostly recognized to cause illnesses including urinary tract infections and diarrhoea. In order to identify the bacterial cause of septicaemia, this study aimed to identify blood samples that tested positive for pathogenic strains of Klebsiella pneumoniae, and E. coli .

Name of bacteria	Targeted gene	Sequence	Product size	PCR conditions	Agarose gel	Reference
Klebsiella pneumoniae	16S–23S rRNA internal transcribed spacer (ITS) region	5′-ATTTGAAGAGGTTGCAAACGAT-3′	130 bp	The cycling conditions were 10 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 20 s at 57 °C and 20 s at 72 °C, followed by a 10 min hold at 72 °C.	2%	(Ranjbar et al.,2012)
		5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′				
Escherichia coli	*Gnd* gene. encodes 6-phosphogluconate	5′-GACCTCGGTTTAGT	585 bp	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for	1.5 %	(Hossain et al.,

Table 1: List primers and conditions for this study

2.2.6: Agarose Gel Electrophoresis:

DNA fragments with sizes between 100 bp and 25 kp. The agarose separates the biomolecules based on size. An electric field is used in a gel matrix to force charged molecules through the substance. There are several methods for determining the size of DNA, including agarose gel electrophoresis. In this, DNA travels through a heavily cross-linked agarose substrate due to an electric current. Because the DNA phosphates in the solution have a negative charge, the molecule will shift to the positive. pole that is red. Three factors affect how quickly DNA moves across a gel, providing information about the amount of the DNA and verifying its existence as a specific DNA ladder. The necessary agarose powder (usually 1.5–2%) is boiled with 2000 µL Tris-acetate-EDTA Buffer and 98% distilled water to create a gel for the tests. Then, four microlitres of ethidium bromide and

The intercalating agent is utilised as a fluorescent tag. Then, using autoclaved tips, the previously completed PCR products were deposited onto an Agarose gel. Tris-acetate-EDTA, or TAE, was utilised in this instance for jogging. The migration of DNA to the positive electrode is aided by a buffer. Agarose gel electrophoresis is always used. Five microlitres of Biolab's 100 bp ladder and 100 volts of electricity were used. The DNA profession can be tracked through the gel since the DNAs are coloured with MIDORI Green. A material that primarily binds is used to dye the DNA after it has passed through the gel. DNA molecules can either glow a certain colour when viewed under ultraviolet light or reflect a particular colour when exposed to visible light. Therefore, the band sizes are then calculated and recorded using the DNA ladder.

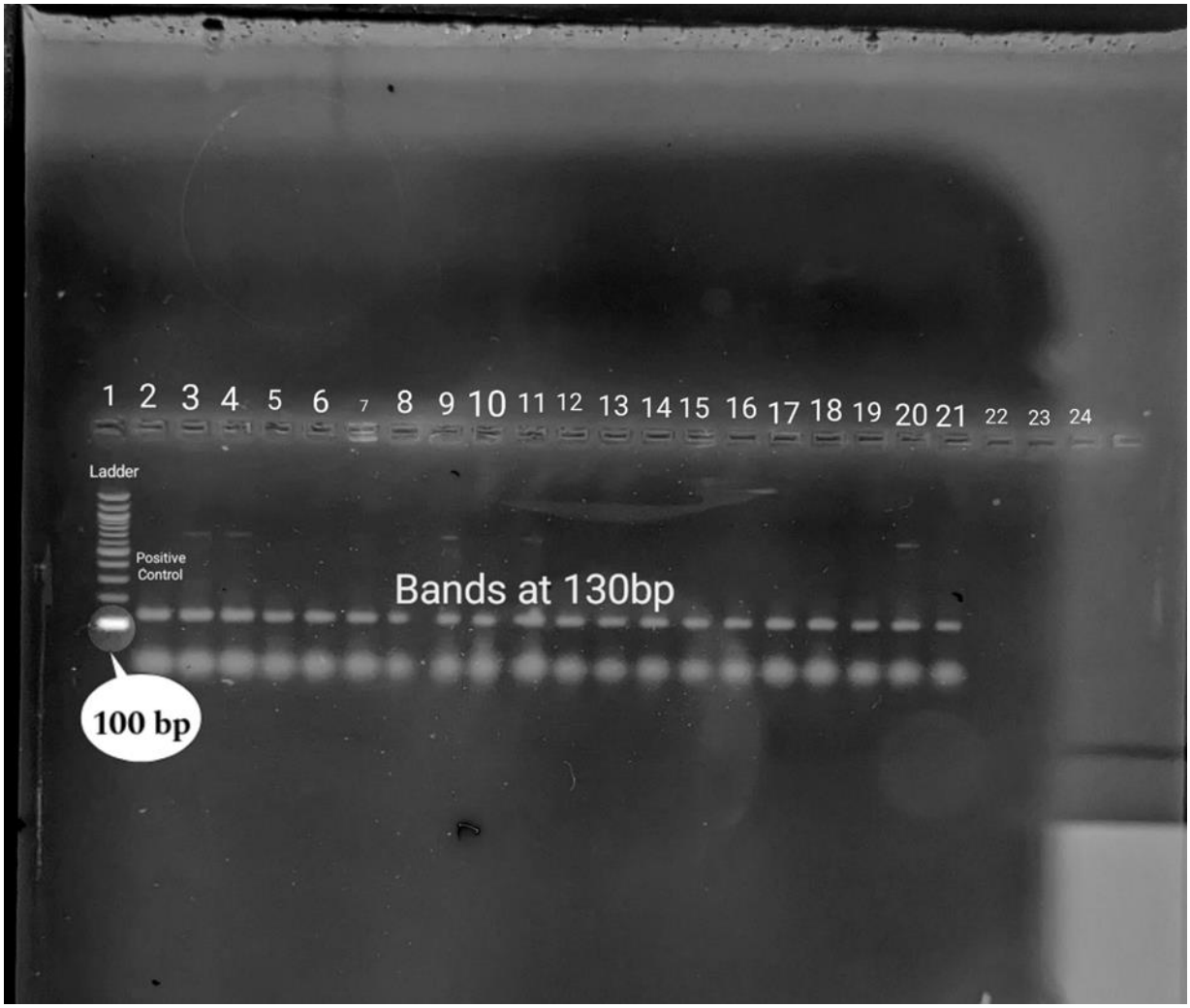

Figure 3: *Klebsiella pneumoniae* after the agarose gel electrophoresis showing band from the 3rd well to the 21st well.

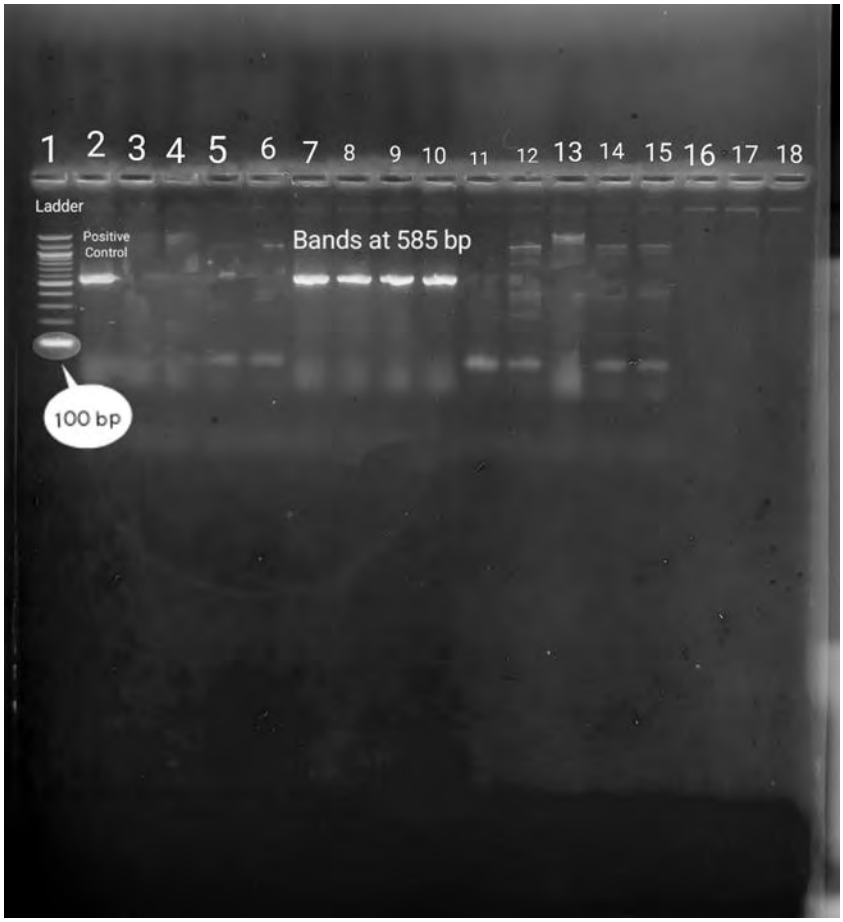

Figure 4*: E. coli* after agarose gel electrophoresis showing bands from the 7th well to the 9th well.

2.2.7: Antibiotic susceptibility testing:

The Kirby-Bauer disc diffusion method, which is faster and more useful than the broth dilution method, was used to assess antimicrobial susceptibility profiles. This technique is widely used to assess anaerobes' and aerobes' sensitivity to and resistance to a variety of antibiotic classes.

The sensitivity of isolated isolates of *Escherichia coli, Klebsiella pneumoniae* was tested using standard antibiotic discs on Mueller-Hinton agar plates. To ascertain the corresponding resistance or sensitivity pattern, each strain was exposed to a variety of antibiotics. Therefore, the effectiveness of the antibiotics in preventing bacterial growth was assessed by measuring the inhibitory zones surrounding each antibiotic disc.

By clarifying the pathogens' susceptibility pattern, this method helped to guide appropriate therapy alternatives. Clinicians and public health officials need to know the results of these tests in order to address the problems caused by antibiotic resistance and guarantee efficient treatment methods.

2.2 Antibiotic test on Muller Hinton Agar (MHA):

The disc diffusion method's antibiotic susceptibility tests are the primary usage for this.

For example, the disc diffusion method of Kirby-Bauer. Its nonselective and non-differential character is the main reason why the Clinical and Laboratory Standards Institute has suggested it as the best medium for AST (Vasylevskyi S. et al., 2018). Because starch is present, the bacteria's toxins are absorbed by it, preventing antibiotics from working negatively. Additionally, it exhibits strong batch-to-batch repeatability. It promotes improved antibiotic diffusion since it is a loose agar. Thus, MHA has been applied. For the AST in order for the investigation to yield accurate findings.

2.3 Selected Antibiotic Disks:

Antibiotics are a family of medications that have a variety of effects on various aspects of bacteria. These actions allow bacteria to develop an additional mechanism of resistance to antibiotics. Twelve to sixteen medications were utilized in this investigation to determine general features of the antimicrobial susceptibility of *Klebsiella pneumoniae*, and *Escherichia coli*. Because these antibiotics come from a variety of classes and have distinct modes of action, it is possible to take the isolates' resistance levels into account.

Below is a table containing the information gathered from the data from the Excel spreadsheet:

Antibiotic Group	Antibiotic Name	Disc Code	Disc Potency (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Carbapenems	Meropenem	MEM10	10	≥23	20-22	≤19
Beta-lactams	Amoxicillin	AMC30	30	≥18	14-17	≤13
	Cefixime	CFM5	5	≥19	15-18	≤14
	Ceftazidime	CAZ30	30	≥18	15-17	≤14
	Cefepime	CPM30	30	≥18	15-17	≤14
	Ampicillin	AMP10	10	≥17	14-16	≤13
	Ampicillin (High Dose)	AMP25	25	≥17	14-16	≤13
Aminoglycosides	Amikacin	AK30	30	≥17	15-16	≤14
	Gentamicin	CN10	10	≥15	13-14	≤12
	Kanamycin	K30	30	≥18	14-17	≤13
Macrolides	Erythromycin	E15	15	≥23	14-22	≤13

Table 2: Concentrations and diffusion zones of the antibiotics. (Kirby-Bauer)

2.4 Muller Hinton Agar Plates:

Prior to inoculating bacterial pathogens on Mueller-Hinton Agar (MHA) plates for isolation, a bacterial suspension according to the McFarland Standard had to be prepared. First, a test tube was autoclaved with 5–6 mL of regular saline. Three to five bacterial colonies from a freshly streaked NA were aseptically collected using an inoculating loop and then submerged in saline. After that, the test tube was aggressively vortexed until the suspended material's cloudiness reached the level specified by the 0.5 McFarland Standard, which is around 1.5 x 10^8 CFU/mL of bacteria. To ensure a consistent and standardized bacterial density suitable for testing for antibiotic susceptibility, this standard is necessary. A new autoclaved cotton swab was inserted into the bacterial suspension after the necessary turbidity was reached. This allowed the bacteria to multiply and aid in colony counts. The swab was then rolled against the test tube's inner surface to remove any remaining suspension. To cover the whole petri plate, the swab made from the bacterial sample was streaked across the surface of a sterile Mueller Hinton Agar (MHA) plate. To create a uniform lawn of bacterial growth, the cotton swab was rotated several times, with the plate rotating at each angle.

After the grass was finished, the antibiotic discs were moved to the MHA plate's surface using sterile forceps. The forceps used to position the antibiotic discs were cleaned with 70% ethanol and then flamed before each antibiotic disc was picked in order to prevent bacterial specimen contamination. To ensure that the antibiotic discs made excellent contact with the bacterial lawn, they were equally gently dropped onto the agar. After the plates were placed, they were incubated at 37 degrees Celsius, which is the temperature at which the bacteria grow and prevent the inhibitory zone from forming. The exact and consistent technique of inoculating the bacterial isolates produced a standard bacterial density and reasonably accurate findings for the antimicrobial susceptibility of the bacteria present because of the combination of these characteristics.

2.5 Zone of Inhibition:

Bacterial growth is visible around each disc when the MHA plates are removed after a day of incubation. There should be "no growth" surrounding the tested organism if the antibiotic is working. Similar to that, there will be antibiotics that are less effective in such situation; a zone of inhibition will be evident. A scale is used to quantify the zone of inhibitions in these situations, which serves to provide a clear picture of the appropriate development constraint zones surrounding the discs. (Institute for Clinical and Laboratory Standards, 1997, 1999).

The findings are then classified as sensitive, moderate, or resistant using a standard table created by Oxoid Limited (England). It is advised by the National Antimicrobial Resistance.

Chapter 3

Result 3.1:

Isolation of *Klebsiella pneumoniae* and *Escherichia coli* from blood samples of suspected septicemia patients 20 blood samples were examined in this study in order to determine the bacterial pathogens linked to septicaemia. Of them, 9 samples (45%) had positive bacterial growth tests, meaning that harmful organisms were present. Of the 20 samples that tested positive: The most common pathogen found was *Klebsiella pneumoniae* (*KP),* which was found to be present in 5 samples (25%). *Escherichia coli (E. coli),* a known Gram-negative bacterium linked to instances of severe septicaemia, was found to be present in four samples (20%). These results highlight that, among the examined samples, *Klebsiella pneumoniae* was the most common cause of bloodstream infections, followed by *Escherichia coli*. The lack of bacterial growth in the remaining 11 samples (55%) under the testing circumstances suggests that these specific pathogens or possible non-bacterial sources of infection are not present.

3.2: Positive result based on Gender:

The distribution of bacterial infections by gender was established by further analyzing the 9 positive blood samples. Positive examples include:

Six of the samples (30%) were from male patients, suggesting that bloodstream infections are far more common in men. Three samples (15%) came from female patients, indicating that females were less likely to have infections.

The study's gender distribution indicates that male patients were more likely to have septicemia. Factors including underlying medical disorders, behavioral and biological differences that impact vulnerability to bacterial infections, or variances in exposure to healthcare environments might all have an impact on this gender-based gap. Additional research on these variables may provide important light on the pattern that has been noticed.

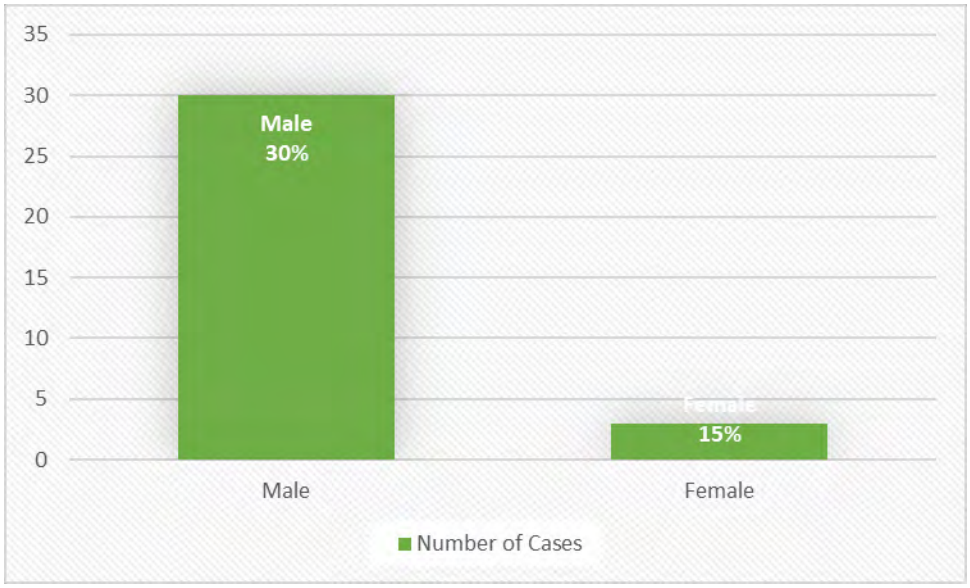

Figure 5: A graph showing the percentage of infected males and females in the total sample.

3.3 Positive Results Based on Age

The age-based distribution of positive cases reveals that infections were found in people ranging in age from 26 to 94. To facilitate comprehension, the data was categorized by age:

Age	Cases	Percentage
Ages 30-39 years	1	4%
Ages 40-49 years	4	20%
Ages 60-69 years	4	20%

Table 3: Results based on Age

According to the findings, the 30-39 age group 4% has lowest prevalence, with the 40-49 age group is having at 20% of all illness. The age range of 60 to 69 has the same percentage like the previous one 20%. These results demonstrate that bloodstream infections are more prevalent in middle-aged and older people in our research.

3.4: Antibiotic Susceptibility Profiling of Isolated Pathogens

Antibiotic Susceptibility Analysis of *Klebsiella pneumoniae*:

The majority of the positive *Klebsiella pneumoniae* isolates exhibited relatively good antibiotic sensitivity, with the exception of Ampicillin, Erythromycin, and Colistin, which showed notable resistance. The efficiency of these 12 antibiotics against the isolates is displayed in the data table below. With 96% to 100% of the isolates exhibiting susceptibility, the data clearly shows that imipenem (IPM10), meropenem (MEM10), and ciprofloxacin (CIP5) shown high susceptibility. Conversely, 88% to 92% of the isolates exhibited high levels of resistance to ceftazidime (CAZ30), cefixime (CFM5), and chloramphenicol (CPM30). 60% of isolates showed moderate resistance to ceftriaxone (CTR30), whereas 20% showed intermediate resistance.

With 8% of isolates exhibiting intermediate susceptibility, kanamycin (AK30) and tetracycline (TE30) showed the greatest percentages of intermediate resistance. In contrast, the intermediate resistance of Piperacillin-Tazobactam (PIT100/10) was 12%, which is less than that of other antibiotics. The lack of intermediate imipenem (IPM10) resistance, which shows 100% susceptibility and emphasizes its efficacy against the studied isolates, is one important discovery. Erythromycin (AZM15/30), on the other hand, demonstrated a notable resistance pattern (20%) with just 64% susceptibility, highlighting the antibiotic's diminished efficacy.

These findings show that there is considerable resistance in several types of antibiotics, including beta-lactams and erythromycin, even if many isolates are still susceptible to popular antibiotics like imipenem, meropenem, and ciprofloxacin. This emphasizes the necessity of ongoing monitoring and antibiotic stewardship in order to stop the emergence of new resistance.

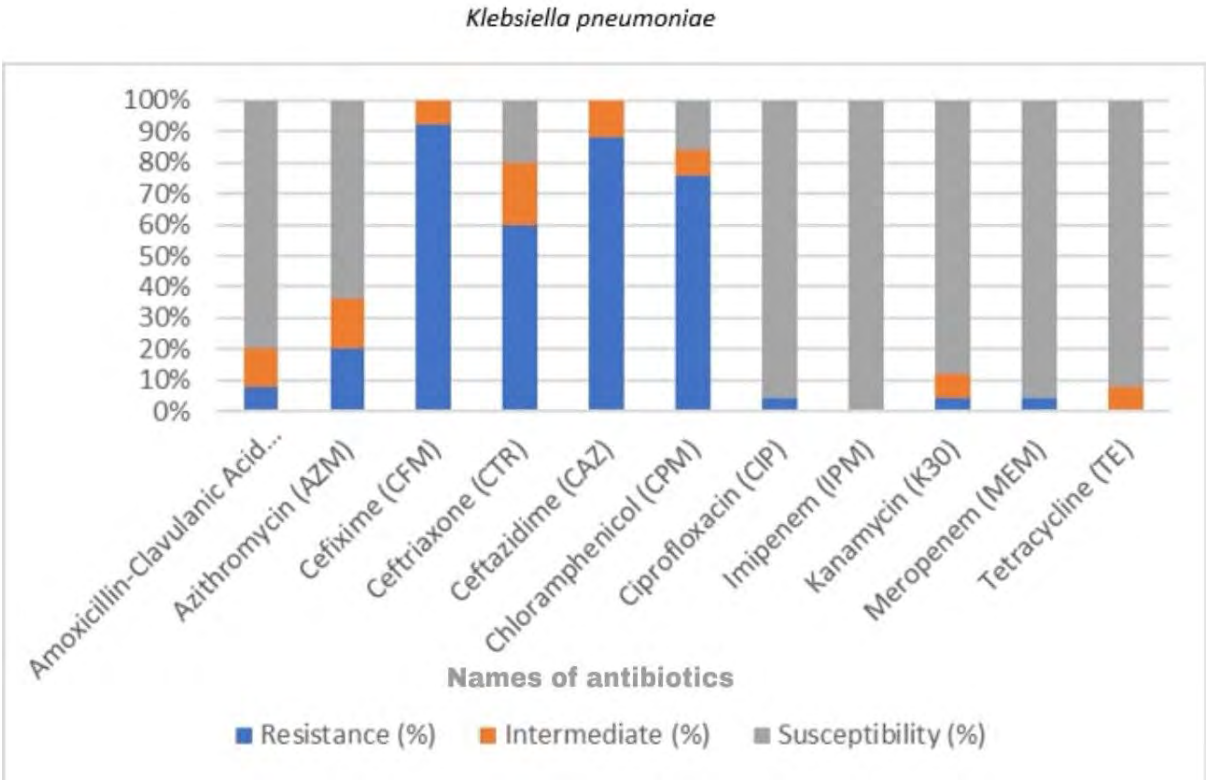

Figure 6: A graph showing the resistance, intermediate and susceptible results of *Klebsiella pneumoniae*

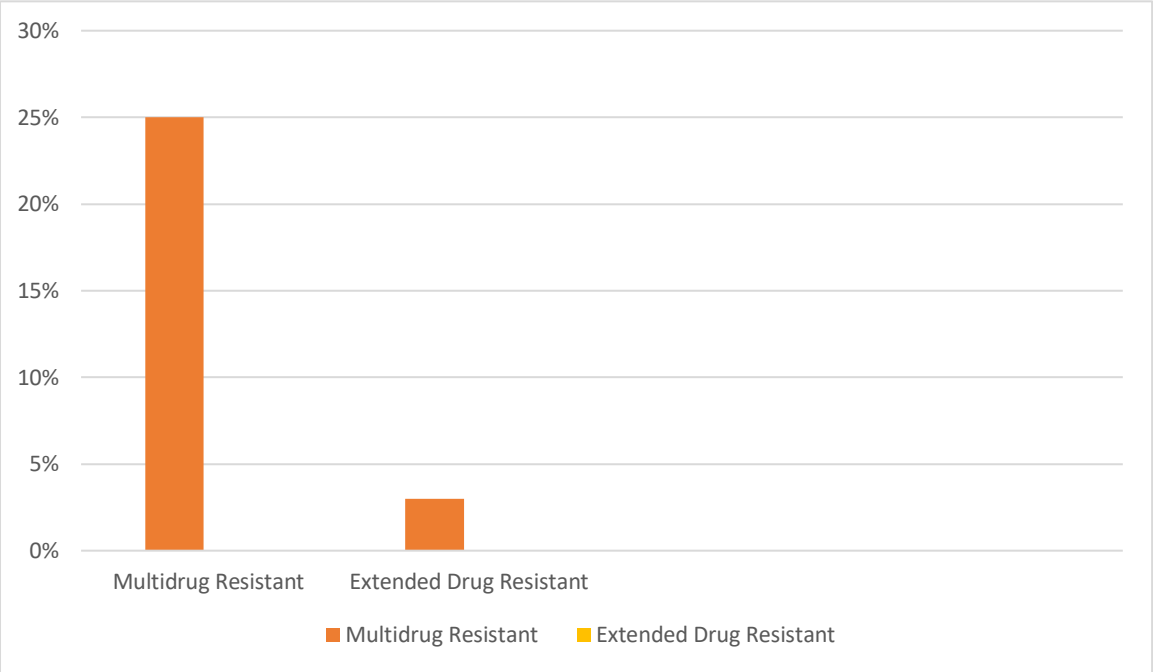

Figure 7: A graph showing the MDR and XDR isolate percentage of *Klebsiella pneumoniae*

Antibiotic Susceptibility Analysis of *E. coli*:

Ampicillin, imipenem, ciprofloxacin, tetracycline, meropenem, and kanamycin (AK) are among the key antibiotics that are 100% susceptible to the isolate, according to the study of the 14 medicines tested against it. This makes them excellent therapy alternatives. However, 100% resistance to Erythromycin, Cefixime, and Chloramphenicol (CPM) was found, indicating that these antibiotics are ineffective against the isolate. As stand-alone therapies, antibiotics such ceftriaxone, gentamicin, and chloramphenicol (CL) showed moderate susceptibility, indicating limited effectiveness. These results showed that 21.43% of the drugs exhibited multidrug resistance (MDR), whereas 71.43% were categorized as susceptible. One antibiotic (chloramphenicol [CPM]) showed total resistance and was classified as extended drug-resistant (XDR). These findings highlight how crucial it is to prioritize highly effective antibiotics while avoiding highly resistant ones in order to get the best possible treatment results.

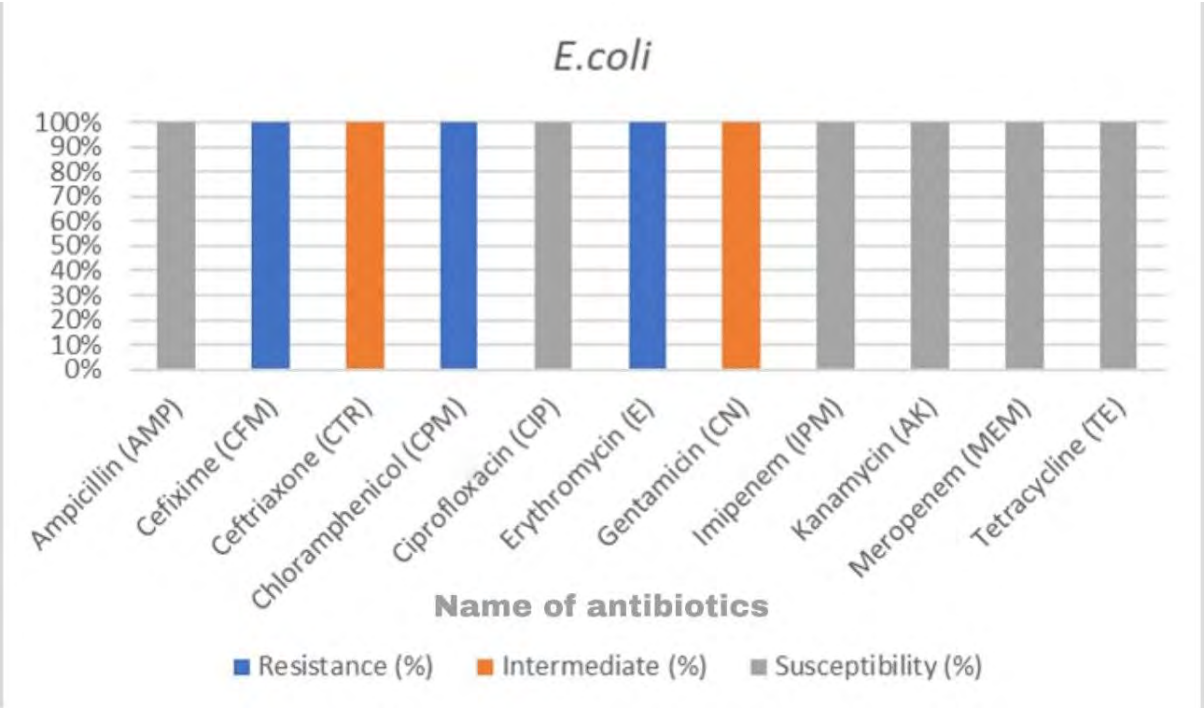

Figure 8: A graph showing the resistance, intermediate and susceptible results of *E. coli.*

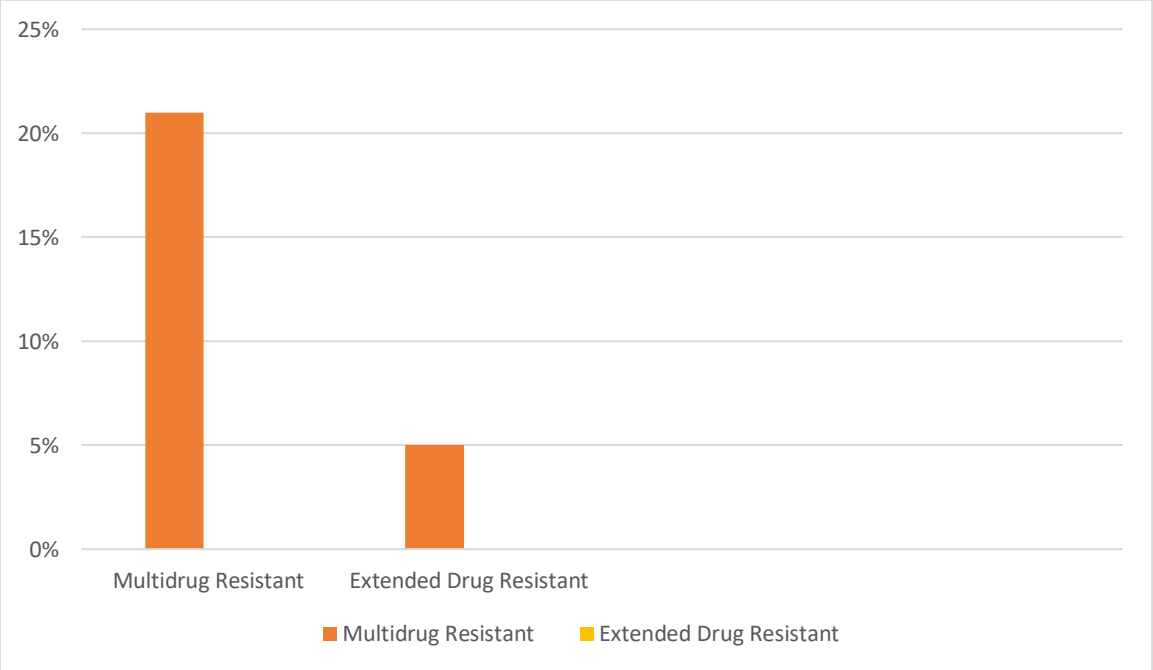

Figure 9: A graph showing the MDR and XDR percentage of the isolates of *E. Coli.*

Chapter 4

Discussion:

The present study highlights the ongoing threat posed by bloodstream infections (BSIs), especially those caused by multidrug-resistant (MDR) organisms such as *Klebsiella pneumoniae* and *Escherichia coli*. Among the 20 suspected septicemia cases analyzed, 45% yielded positive cultures, with *K. pneumoniae* (25%) being the most prevalent pathogen, followed by *E. coli* (21.43%). This bacteriological profile aligns with global data indicating these organisms as leading causes of BSIs, particularly in hospital and ICU settings (Diekema et al., 2019; WHO, 2023).

In global terms, the prominence of *K. pneumoniae* mirrors findings from other low- and middle-income countries (LMICs), such as India and Egypt, where carbapenem-resistant strains have become endemic, often exceeding resistance rates of 50% (Khalifa et al., 2021). These trends pose a significant clinical challenge due to limited therapeutic options. Similarly, high-income countries have reported increasing resistance in *E. coli*, especially against fluoroquinolones and third-generation cephalosporins, but to a lesser extent due to more robust antimicrobial stewardship and diagnostics (Antimicrobial Resistance Collaborators, 2022).

In Bangladesh, the findings of this study are consistent with local surveillance reports showing a growing burden of MDR organisms in blood cultures, particularly in ICU settings (Rahman et al., 2020; Shamsuzzaman et al., 2017). The prevalence of *K. pneumoniae* and *E. coli* in this study supports earlier reports from tertiary hospitals where these pathogens displayed significant resistance to β-lactams and macrolides (Bhuiyan et al., 2017). Notably, in this study, resistance to ceftazidime, cefixime, and erythromycin was high, while imipenem and ciprofloxacin showed high efficacy, underscoring the continued—albeit narrowing—utility of these antibiotics.

The age-wise distribution of positive cases indicated a clear predominance in middle-aged and older adults (40–69 years), with no positive cases below age 30. This finding is consistent with the concept of immunosenescence and increased comorbidities in older adults, both of which elevate the risk of BSIs (Clegg et al., 2013; Simon et al., 2015). The gender-wise distribution also revealed a marked male predominance (66.7%), consistent with international trends attributing this to a complex interplay of behavioral, genetic, and immunological factors (Weiner-Lastinger et al., 2020; Rudd et al., 2020).

From an antimicrobial susceptibility standpoint, both *K. pneumoniae* and *E. coli* demonstrated patterns typical of MDR pathogens in LMICs. The *E. coli* isolates were 100% resistant to cefixime, erythromycin, and chloramphenicol, mirroring studies in Bangladesh and neighboring countries that report high resistance to these agents (Rahman et al., 2014). While susceptibility to imipenem and ciprofloxacin remained high, emerging intermediate resistance to tetracyclines and aminoglycosides in *K. pneumoniae* raises concern over the gradual erosion of available treatment options.

Compared to national averages, this study's data reflect a slightly lower positivity rate (45%) than larger Bangladeshi studies, which often report culture positivity rates between 50–60% (Rahman et al., 2014). However, the pattern of resistance closely mirrors national surveillance data, with carbapenem and fluoroquinolone resistance steadily increasing (Islam et al., 2019).

Study Limitations:

The study is limited by its small sample size (n=20), which restricts generalizability. Furthermore, the study excluded non-bacterial pathogens and did not assess clinical comorbidities, which could have provided deeper insights into the etiology and outcomes of septicemia. Additionally, while standard culture and susceptibility techniques were used, the

absence of genotypic resistance profiling limits understanding of underlying resistance mechanisms.

Chapter 5:

Conclusion:

This study provides important insight into the bacteriological etiology and antimicrobial susceptibility patterns of bloodstream infections among septicemia-suspected patients in Bangladesh. The findings confirm that Klebsiella pneumoniae and Escherichia coli remain the predominant pathogens, particularly affecting middle-aged and elderly individuals. These pathogens showed substantial resistance to several commonly used antibiotics, including third-generation cephalosporins and macrolides, while carbapenems and fluoroquinolones retained relatively high efficacy.

Comparatively, the resistance patterns observed in this study reflect global and national trends, particularly in LMICs where inadequate diagnostics and indiscriminate antibiotic use continue to fuel the rise of MDR organisms. The detection of high MDR rates underscores the urgency of strengthening antimicrobial stewardship, implementing robust infection control policies, and expanding access to molecular diagnostics.

In conclusion, this study highlights the critical need for improved diagnostic capabilities, larger-scale epidemiological surveillance, and evidence-based antibiotic prescribing in Bangladesh. Future studies should incorporate larger sample sizes, genomic resistance profiling, and patient outcome data to inform clinical guidelines and public health strategies more effectively.

Chapter 6

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