Study on Bacteriological Profile and Antimicrobial Susceptibility Pattern in Septicemia Suspected Patients.

By

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A thesis submitted to the Department of Mathematics & Natural Sciences in partial fulfillment of the requirements for the degree of

Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences,

Brac University

Declaration

It is at this moment declared that.

1. The thesis submitted titled "**Study on Bacteriological Profile and Antimicrobial susceptibility pattern in septicemia suspected patients in Bangladesh.**" is our original work while completing our degree at BRAC University.

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

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

4. We have acknowledged all main sources of help.

Student's Full name & signature:

_____

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Approval

The thesis titled "**Study on Bacteriological Profile and Antimicrobial susceptibility pattern in septicemia suspected patients in Bangladesh.**" submitted by
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In Summer, 2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology in Spring, 2024.
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Ethics Statement:

This study will fully conform to the highest ethical consideration for research: The blood samples from patients were collected from the National Heart Institute and the Asha Private Clinic, strictly adhering to ethical guidelines. Ethical clearance was sought and attained from both institutes before commencing this study. Therefore, the institutional review boards studied and approved the protocols to ensure they adhered to and put into practice the requirements necessary in the National Guidelines on Health Research and the International Guidelines of Ethical Considerations on Research.

This study was further reviewed and approved by the Ethics and Research Review Committee of the Faculty of Mathematics and Natural Sciences, MNS, BRAC University, Dhaka, Bangladesh, under the supervision of Dr. Fahim Kabir Monjurul Haque. All dimensions of the research were done following the guidelines on ethics set by BRAC University.

Informed consent was taken from all subjects before the collection of samples. All subjects clearly explained the aims of the research, procedures involved, possible risks, and benefits. Data confidentiality and patient anonymity were maintained, and personal identifiers were removed during analysis.

This solid ethical framework underpins the integrity and validity of the study, reinforcing established norms for research ethics.

Acknowledgment

I would like to start by expressing my deepest gratitude to Almighty Allah for allowing me to pursue and complete this research and to my parents and siblings for supporting me from the very beginning to 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 were instrumental in 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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Abstract

Septicemia, a critical bloodstream infection, poses significant health challenges, particularly with the increasing prevalence of multidrug-resistant (MDR) pathogens. This study aimed to investigate bacterial isolates' bacteriological profile and antimicrobial susceptibility patterns from septicemia-suspected patients in Bangladesh. Fifty-six blood samples were analyzed, of which 33 (58.9%) tested positive for bacterial growth. The predominant pathogens identified were *Klebsiella pneumoniae* (75.8%), *Staphylococcus aureus* (12.1%), and *Escherichia coli* (12.1%). Most positive cases were observed in males (90.9%), with a significant age distribution among patients above 40. The eminence of antimicrobial susceptibility testing by Kirby Bauer disk diffusion method in sterile Muller Hinton Agar plates was done. The isolates efficacy and resistance profile was as follows: The highest resistance, 70 %, was seen against beta-lactams and fluoroquinolones, and carbapenems had better results than the other two drugs. These results also highlight the increasing threat from multi-drug resistance pathogens such as *Klebsiella pneumoniae* to warrant optimized and personalized antimicrobial stewardship. Thus, the present study underlines the essence of performing periodic scans of septicemia pathogens and resistance patterns, as the obtained data might be beneficial when developing guidelines and creating corresponding approaches in empiric treatment and public health. Large-scale population-based studies and the utilization of improved molecular approaches are encouraged for further research to build up the research findings and to eradicate the increasing menace of septicemia in Bangladesh.

Keywords: Sepsis, Multiple drug resistance, Klebsiella pneumonia, Antimicrobial resistance.

List of Tables:

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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*
S. aureus	*Staphylococcus aureus*
MIC	Minimum Inhibitory Concentration
CLSI	Clinical and Laboratory Standards Institute

Chapter 1

1. Introduction

1.1 Background

Septicemia, also commonly referred to as blood infection, is among the serious modern medical conditions at the core of the pathogens throughout the bloodstream. If the condition is not curtailed, it is likely to aggravate critical sepsis or septic shock with multi-organ dysfunction, which exposes one to high morbidity rates (Singer Mervyn et al., 2016). Blood ream infections have conventionally taken a heavy toll in terms of morbidity and mortality and continue unabatedly to expand with the emergence of multidrug-resistant pathogens. Septicemia remains one of the significant global health concerns, with an estimated 49 million cases and 11 million related deaths annually. This fundamental problem is much higher in low- and middle-income countries than in high-income countries due to inadequate infrastructure in health care provided, time taken for diagnosing, and suboptimal infection control practices. The cases are also on the rise in high-income countries, where most bloodstream infections in facilities develop from resistant organisms, such as *Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli* (CDC, 2023). In Bangladesh, the incidence of septicemia has increased with delays in prescription, poor infection control practices, and irrational use of antibiotics.

These factors encourage the growth of MDR organisms such *as Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli* commonly implicated in septicemia. These mechanisms of AMR include beta-lactamases and efflux pumps that contribute to poor treatment

outcomes by increasing the risk of prolonged hospitalization and adverse clinical outcomes. This is further complicated by mechanisms of AMR, including beta-lactamases and efflux pumps impeding treatment efficacy, prolonging hospitalization, and leading to worse clinical outcomes (Philippon et al., 1986; Khalifa et al., 2021). Anti-microbial resistance further complicates the management of septicemia worldwide. The WHO recently reported that carbapenem resistance rates are above 50% in some countries for *Klebsiella pneumoniae.* Similarly, methicillin-resistant *Staphylococcus aureus* remains one of the leading causes of severe sepsis worldwide, especially in medical settings; WHO, 2023. Recently, the WHO estimated that through direct attribution, AMR accounted for 1.27 million deaths in 2019, stressing how vital such interventions are needed to contain the challenge; WHO, 2023. Overuse and misuse of antibiotics complicate septicemia by increasing the speed towards resistance development. Recent studies indicate that bloodstream infections by carbapenem-resistant *Enterobacteriaceae* can have mortality rates higher than 40% (CDC, 2023). In Bangladesh, however, resistance rates to cephalosporins and carbapenems among TSBF isolates were reportedly relatively high; hence, empirical treatment guidelines are challenging to decide upon (Rahman et al., 2014). Couple with this problem is that no routine antimicrobial stewardship programs are instituted without studies based on local microbial resistance and therapy trends. This recent study determines the bacteriological profile and resistance rate of bacteria involved in bloodstream infections in Bangladesh to address this global and local challenge. The findings from this study will contribute significantly to informed clinical practice, antimicrobial stewardship program implementation, and public health strategies against the growing threat.

1.2 Bacteriological Profile and Antimicrobial Resistance in Septicemia

Septicemia is a multifaceted condition defined by the presence of pathogens or toxins in the bloodstream, giving rise to inflammatory responses throughout the body. Septicemia is a multifaceted condition defined by pathogens or toxins in the bloodstream, giving rise to inflammatory responses throughout the body. Many bacteria, including aerobic and anaerobic Gram-positive and Gram-negative fungi and viruses, may cause septicemia. Among the Gram-negative bacteria, the most common are *E. coli, K.pneumoniae & P.aeruginosa*; for Gram-positive bacteria, the most commonly found bacteria are *S. aureus, S.pneumoniae & Enterococcus spp*. Pathogenicity factors differ in biofilm production, toxins, and immune system modulation factors. *Klebsiella pneumoniae and Escherichia coli* are Bangladesh's most common pathogens causing septicemia, more frequently among hospitalized patients. These organisms present a significant public health problem as their multi-drug resistant strains rise. These are generally associated with fewer options for treatment and poorer clinical outcomes than patients with infections due to susceptible strains (Rahman et al., 2014). These include molecular factors such as overexpression of Extended-spectrum beta-lactamases , carbapenemases, and efflux pumps, which play a vital role in the persistence and dissemination of MDR organisms (Philippon et al. 1986; Khalifa et al.2021). Of concern, due to anti-microbial resistance, septicemia has reached a dangerously significant level in the modern world. Misuse and non-adherence to proper prescription of antibiotics have promoted resistance to the strains, hence increasing mortality and morbidity not just in the health facilities in Low- and middle-income countries, including Bangladesh. Previous work has also revealed that patients resist other important classes of antibiotics, including carbapenems, cephalosporins, and fluoroquinolones used in the treatment of bloodstream infections (Jensen et

al., 2022). According to the World Health Organization, antimicrobial resistance is considered one of the top ten threats to global public health; this calls for enhanced Monitoring and Antimicrobial Use Control Programs (WHO, 2023).

Therefore, the current research aims to identify the bacteriological profile and pattern of antimicrobial resistance of septicemia in Bangladeshi patients. The goals are to offer an understanding of resistance profiles so that clinicians can fine-tune empirical therapy and to help public health countries specifically manage the effects of AMR on septicemia care.

1.3 Age as a Risk Factor for Septicemia

Septicemia can affect anyone regardless of age; nevertheless, both the risk and severity of this condition increase significantly with age. Notably, the population group that is most at risk of septicemia is the one that is 35 years and above because of physiological, medical, and immunology differences. This underlying propensity increases progressively with age, and from the patient data collected in this study, all the cases of septicemia identified were in patients who were 40 years and above.

Hormonal changes in the aging process lead to the phenomenon called immunosenescence, which is a decline in the immunological efficiency of the organism. This makes older adults less capable of fighting infections in the most effective way possible. Furthermore, noncommunicable diseases, including diabetes, cardiovascular diseases, chronic kidney disease, and many other diseases that affect the elderly majority due to their advanced age, reduce immunity and foster septicemia (Clegg et al.2013). Besides, the older population needs invasive medical procedures like

catheterization or central line insertions, in which the infection from nosocomial pathogens may result in bloodstream infections.

The patient data from this study highlights a stark pattern: A striking feature in a cross-sectional study is that all the confirmed *Klebsiella pneumonia* and *Staphylococcus aureus* cases were found in individuals aged more than 40 years. This result coheres with international investigations presenting older age as one of the leading factors affecting severe septicemia. Some of the predisposing factors include more frequent hospitalization, the consumption of multiple drugs, and immunosuppressive treatment for older patients, which increases the risk of bloodstream Infection in this population (Weiner-Lastinger et al., 2020).

Therefore, age-specific prevention interventions are highly desirable. Early infection surveillance, adherence to universal precaution measures in health sectors, and cohort-specific appropriate antimicrobial use measures can effectively reduce the probability of septicemia among elders. To target these aspects as risk factors, it is crucial to enhance the clinical index and minimize the incidence of septicemia in elderly people.

Study Limitations

Though the present study provides essential information on septicemia's bacteriological profile and antimicrobial resistance pattern, it has some limitations. First, the sample consisted of 56 blood samples, which, though adequate for achieving preliminary observations, could not represent a broader population, especially concerning the different healthcare settings across Bangladesh. This study focused on bacterial pathogens only and did not include other causative agents of septicemia, such as fungi and viruses, which will add a broader dimension to the findings. Thirdly, the

demographic distribution in the positive cases was incredibly imbalanced between genders, with the male population constituting 90.9% of the positive samples. This disparity can limit the generalization of findings to female populations.

Moreover, no review of the clinical history and comorbid conditions of the patients was considered, factors that are highly relevant and play a vital role in the development of susceptibility to bloodstream infection and its treatment outcome. Finally, reliance on conventional culture may exclude fastidious or non-culturable organisms, while the absence of molecular diagnostics provides limited detection for specific resistance genes or virulence factors. Future studies considering larger and more heterogeneous sample sizes, state-of-the-art diagnostic facilities, and all relevant patient data would offer a more valid understanding of septicemia.

1.4 Objectives

Objectives:

1. To Identify the Common Bacterial Pathogens in Septicemia Cases:

   This study aims to identify the common bacterial species involved in septicemia-suspected patients in Bangladesh.

2. To Assess Antimicrobial Susceptibility Patterns:

   This is done to estimate the pathogens' susceptibility to the commonly used antibiotics, with a particular focus on MDR.

3. To Analyze Age-Related Trends in Septicemia:

   To investigate the relationship between patient age and septicemia with particular reference to older adults who are over 36 years of age.

These objectives will fill the knowledge gap about the country's epidemiology and antimicrobial resistance in septicemia and promote improvement in patient care and overall public health.

Chapter 2

Materials and Methods:

Study Design

The present study has been undertaken to investigate the bacteriological profile and antimicrobial resistance patterns in patients where suspicion of septicemia had been cast. Blood samples were collected from two locations in Dhaka, Bangladesh: National Heart Institute and Asha Private Clinic. Before undertaking this research work, ethical clearance was obtained from the concerned institutions, thus meeting ethical requirements and protocols. Septicemia suspected refers to a clinical condition characterized by systemic signs and symptoms in the setting of presumed bloodstream infection. It typically presents classically with the following cardinal features:

Fever and Chills: Fever may be persistent or recur, classically presenting with chills or rigors in the setting of the systemic inflammatory response to an infection.

Hypotension: There is a drop in blood pressure; this could be a sign of septic shock, which is a critical complication of septicemia.

Mental Status Changes: Confusion, lethargy, or disorientation may result from the systemic effects of infection or decreased brain perfusion. According to the Sepsis-3 definition by the Third International Consensus Definitions for Sepsis and Septic Shock, these symptoms are consistent with sepsis, which encourages a greater emphasis on the dysregulated host response to infection,

which leads to life-threatening organ dysfunction. Laboratory findings that commonly accompany these clinical indices include an elevated white blood cell count, C-reactive protein, and levels of procalcitonin, all of which further support the diagnosis of suspected septicemia. Blood culture is essential for confirming pathogens in the blood and specifying the infectious agent. The manifestations of significant infection define sepsis-infection as something that threatens life or function. Early recognition and appropriate treatment of suspected septicemia are essential, as delay in appropriate treatment may lead to deterioration of the disease into severe sepsis or septic shock, mainly raising the risk of mortality (Rhee et al., 2017). The sample collection methods are based on strict protocol inclusion for correctly identifying pathogens among patients falling under the category of suspected septicemia in the study presented here.

Exclusion of contaminated samples: The samples suspected to be contaminated at collection, handling, or processing levels were excluded from the study immediately. Contamination was assessed by deviation from sterility or atypical growth that did not correspond to the clinical presentation. This strict exclusion ring-fenced the results to their reliability and accuracy, thereby preserving the integrity of the findings.

2.1: Collection of Samples:

The specimen collection process was planned for accuracy, asepsis, and standardization. Blood samples were collected from patients suspected of septicemia using the following procedures:

Aseptic Technique:

To reduce any form of contamination, all blood samples were taken aseptically. Universal precautionary measures were taken in handling the patient, including sterilization of the syringes, needles, and collection tubes. The choice of site for the venipuncture was washed with an alcohol-based antiseptic before the samples were taken.

Sample Volume

On each occasion, 3 milliliters of blood were collected from each patient. The samples were promptly aliquoted into sterile heparinized tubes. Heparin was applied to minimize the coagulation of the blood samples and maintain their liquidity in the subsequent culturing and processing.

Transport and Storage:

Following the collection, the blood samples were identified according to the patient's unique features, such as age, gender, and status: inpatient or outpatient. The samples were taken, appropriately labeled, and transported immediately to the microbiology laboratory in conditions that would not compromise their quality.

2.2: Preparation of Sample:

The blood samples were handled on reaching the microbiology laboratory to ensure they were as clean as possible. All the manipulations described above were performed in a laminar flow biosafety cabinet to maintain the sterility of the process. To reduce external influences on the samples, the external part of the test tubes with heparinized samples of blood was disinfected using 70% ethanol. After collecting each sample, labels were applied to them and checked again with

patient information for proper identification. Subsequently, the prepared samples were inoculated aseptically using an appropriate culture media to identify bacteria and their antimicrobial susceptibility. They applied this systematic approach to ensure the reliability of the experiments that were so crucial for their experiment.

2.3: Isolation and Identification:

Spreading on Selective and Differential Media

To isolate and identify bacterial pathogens, 100 µL of each blood sample was aseptically transferred and spread using the spread plate method across six different selective and differential media tailored for identifying specific organisms. For *Klebsiella pneumoniae* (KP), HiCrome KPC agar was used, enabling differentiation through distinct colony coloration. *Escherichia coli* was isolated on HiCrome UTI agar, which allows clear identification based on chromogenic substrate reactions. *Staphylococcus aureus* (Staph A) was cultured on Mannitol Salt Agar (MSA), a medium selective for staphylococci that exhibits mannitol fermentation as a color change. SS (Salmonella-Shigella) agar was utilized to identify Salmonella species, highlighting enteric pathogens through colony morphology and hydrogen sulfide production. Leeds Acinetobacter Baumannii agar was employed for the selective isolation of *Acinetobacter baumannii*. 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.

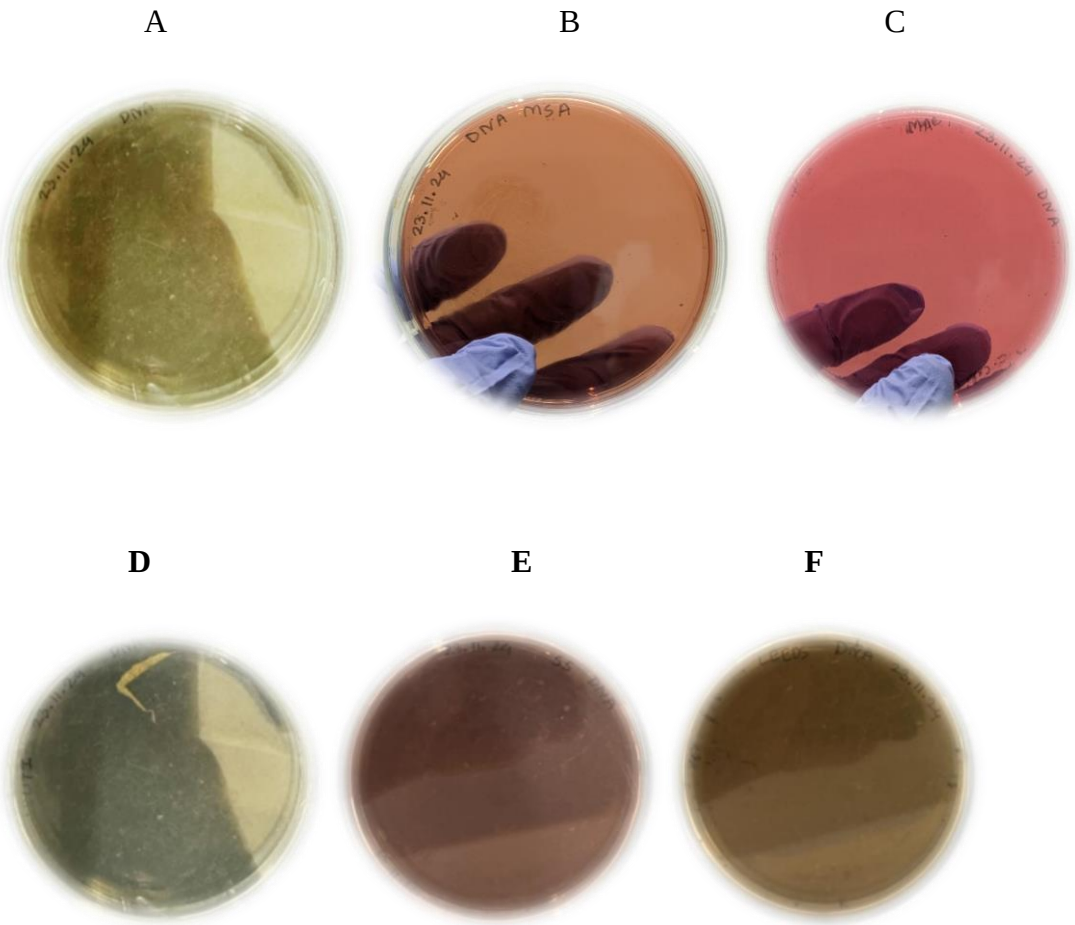

Figure 1: Six different media A- *HiCrome UTI Agar*, B-*Salmonella Shigella Agar*, C-*Leeds Acinetobacter Agar*, D-Mannitol Salt Agar , E-MacConkey agar and F-*HiCrome Klebsiella pneumoniae carbapenemase agar*

Profiling of Pathogens from Blood Cultures

Three target organisms were successfully isolated and confirmed based on their distinctive colony morphology on selective media. *Klebsiella pneumoniae* (KP) was identified by its characteristic blue-colored colonies on both HiCrome KPC agar and HiCrome UTI agar. *Escherichia coli* was confirmed through its purple-colored colonies on the same media, indicating its chromogenic reaction. *Staphylococcus aureus (Staph A)* was distinctly identified by its yellow-colored colonies on Mannitol Salt Agar (MSA), demonstrating mannitol fermentation. However, the remaining two target organisms, *Salmonella spp.*, and *Acinetobacter baumannii*, could not be isolated, as there was no observable growth on SS agar and Leeds Acinetobacter Baumannii agar, respectively. These results highlight the specificity of the isolation techniques and the absence of the latter pathogens in the analyzed samples.

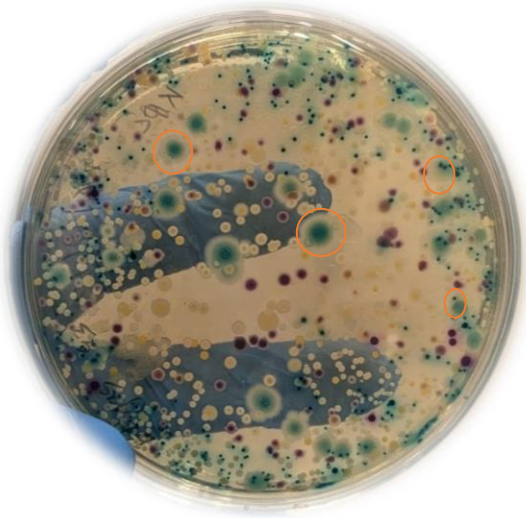

Figure 2: *Klebsiella Pneuomoniae's* growth of blue color (red circled) on HiCrome KPC agar

Figure 3: *Escherichia coli*'s growth of purple colony (red circled) on HiCrome UTI agar.

Figure 4: *Staphylococcus aureus's* growth of yellow colony (red circled) on MSA.

Further Confirmation of *Escherichia coli* and *Klebsiella pneumoniae*

To further confirm the identity of *Escherichia coli* and *Klebsiella pneumoniae*, the isolates were subcultured on HiCrome UTI agar and Eosin Methylene Blue (EMB) agar. On HiCrome UTI agar, *E. coli* retained its characteristic purple-colored colonies, while *Klebsiella pneumoniae* displayed its typical blue-colored colonies, consistent with initial observations. On EMB agar, *E. coli* exhibited a distinctive green metallic sheen, a hallmark of its lactose-fermenting ability and acid production. *Klebsiella pneumoniae*, although a lactose fermenter, did not produce the metallic sheen but showed mucoid colonies, further confirming its identity.

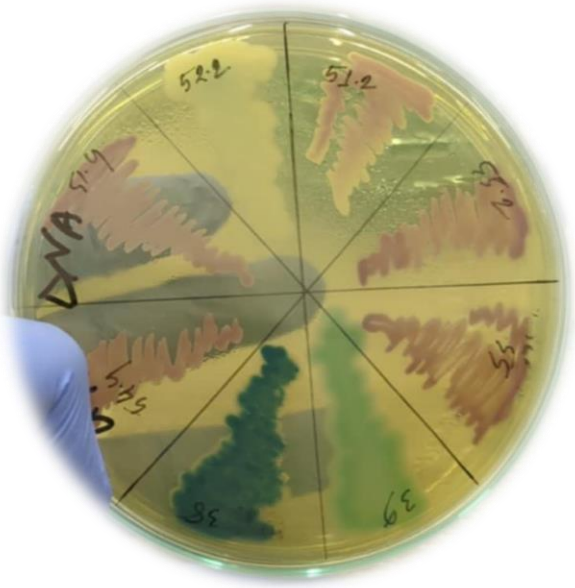

Figure 5: Further confirmation of *E. coli* and *K.Pneumoniae* on HiCrome UTI agar

2.4: DNA Extraction:

DNA Extraction

The DNA extraction procedure began with preparing 1X Tris-EDTA buffer as the base solution for cell lysis. First, 400 µL of 1X TE buffer was added to an Eppendorf tube. A loopful of the bacterial culture was then inoculated into the tube containing the buffer. The sample was then vortexed to completely mix the bacteria with the TE buffer. After initial mixing, the sample was centrifuged for 10 minutes at 13,000 rpm at 25°C to pellet the bacterial cells. After centrifugation, the supernatant was removed, and 400 µL of 1X TE buffer was added into the Eppendorf tube. Then, the sample was again vortexed to resuspend the bacterial pellet. The samples were now to be given a heat treatment to burst the bacterial cells and release their genomic DNA. The tubes were in a heat block at 100°C for 10 minutes. This heat treatment will ensure proper lysis of the bacterial cell wall and membrane, releasing the DNA into the solution. After this step with the heat block, the samples cooled at ambient temperature for 5 minutes. The cooled samples were centrifuged at 13,000 rpm for 10 minutes to separate the remaining debris or any cellular component from the extracted DNA. Following these centrifugation steps, 200 µL of DNA supernatant was transferred to a fresh Eppendorf tube. While transferring, utmost care was taken not to disturb the pellet at the bottom of the tube. This supernatant now contained the extracted DNA and would be ready for further analysis. This method ensured the efficient isolation of high-quality DNA from the bacterial cells for downstream applications such as PCR or sequencing.

Targeted Genes and Primers

In the present investigation, virulence-specific genes of *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were chosen to screen the samples for pathogenic strains of blood. Since septicemia and bloodstream infections are closely related to these pathogens, targeting the

specific genes related to their pathogenicity became critical. For instance, *E. coli* is mainly known for causing infections such as diarrhea infection and urinary tract infections, and its pathogenic types cause severe sepsis. This study intended to detect the positive samples for pathogenic E. coli, Klebsiella pneumoniae, and Staphylococcus aureus strains in blood to determine the bacterial etiology of septicemia.

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′				
	Nuc gene. Encodes	5'-GCG ATT GAT	275bp	Amplification was done by initial denaturation at 95°C	2%	(Al-Sha

Staphylococcus aureus	thermostable nuclease specific to *S. aureus*	GGT GAT ACG GT-3'		for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing temperature of primers was 55°C for 45 sec and extension at 72°C for 1 min. The final extension was conducted at 72°C for 10 min		miri et al., 2021)
		5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'				
Escherichia coli	*Gnd* gene. encodes 6-phosphogluconate dehydrogenase	5′-GACCTCG GTTTAGT TCACAGA -3′	585 bp	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s and extension for 1 min followed by a final extension at 72°C for 5 min.	1.5 %	(Hos sain et al., 2021)
		5′-CACACGC TGACGCT GACCA-3′				

Table 1: List primers and conditions for this study

2.5: PCR (Polymerase Chain Reaction)

It is a laboratory technique for rapidly producing millions to billions of copies of a segment of Segment DNA. This procedure quickly and easily makes numerous copies, which helps in tests like molecular biology, forensic analysis, and medical diagnostics too (Britannica, T. Editors of Encyclopaedia 2023). Within a few hours, rounds of replication using a PCR machine (The Applied Biosystems 2720 Thermal Cycler). To carry out the process, each primer was added to a 25 μL PCR mixture that includes 5 μL of nuclease-free water, two μL of forward primer, two μL of reverse primer, 12 μL of PCR master mix

(Emerald Amp), and 4 μL of template DNA. The mixture was vortexed for 3-5 sec to ensure correct mixing. Following that, PCR was carried out while preserving the PCR settings for each target gene. All the PCR products were to be sorted at - 20°C for additional examination after the PCR ended

2.6: Agarose Gel Electrophoresis:

Agarose gel electrophoresis is considered one of the most effective methods for isolating.

DNA fragments ranging in size from 100bp to 25kp. The biomolecules are separated by size in the agarose. A gel matrix uses an electric field to push charged molecules across the material.

The agarose gel electrophoresis technique is one of many available for figuring out DNA size. In this An electric current causes DNA to move through a strongly cross-linked agarose substrate.

The molecule will move to the positive since the DNA phosphates are negatively charged in the solution. (red) pole. Three variables influence the speed at which DNA migrates through a gel, which gives ideas on the size of the DNA, as well as confirming the presence of it as a particular DNA ladder, were Used. A gel is prepared for the experiments by boiling the required agarose

powder (mostly 1.5-2%) with 2000 μL Tris-acetate-EDTA Buffer and 98% distilled water. Next, four μL ethidium bromide an

The intercalating agent is used as a fluorescent tag. The already-done PCR products were then loaded into Agarose gel with the help of autoclaved tips. Here, TAE (Tris-acetate-EDTA) was used for running. A buffer helps the DNA to migrate to the positive electrode. Each time, agarose gel electrophoresis It was performed using five μL of 100 bp ladder (Biolab) and 100 V of electricity. As the DNAs are colored with MIDORI Green, it allows the tracking of the DNA profession through the gel. After the DNA runs through the gel, it is stained with a substance that binds mainly. DNA molecules and either reflect a specific color when exposed to light in the visible spectrum or fluoresce a particular color when observed under UV light. Thus, with the help of the used DNA Ladder, the band sizes are then figured out and noted down.

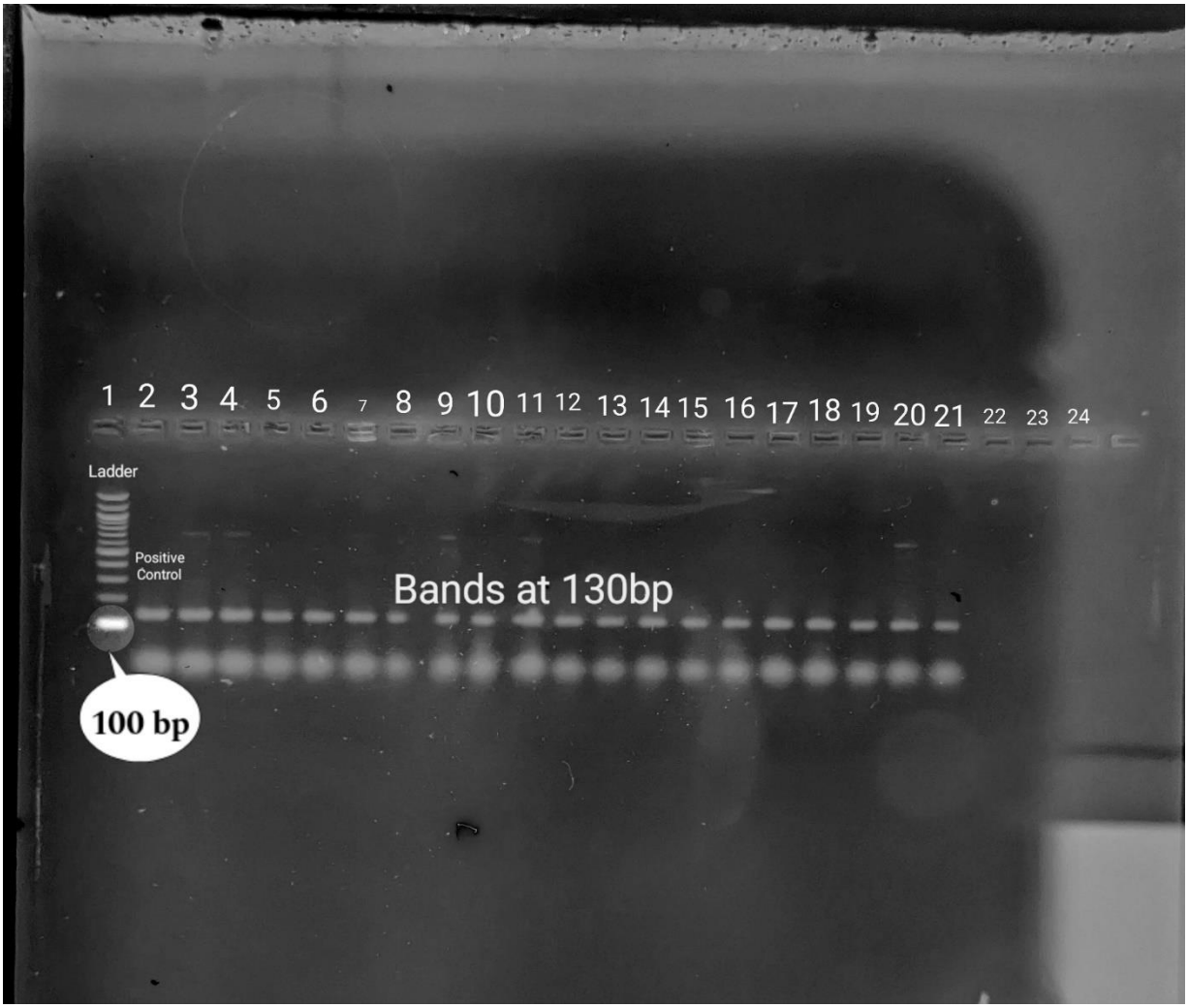

Figure 6: *Klebsiella Pneumoniae* after the agarose gel electrophoresis showing band from the 3rd well to the 21st well.

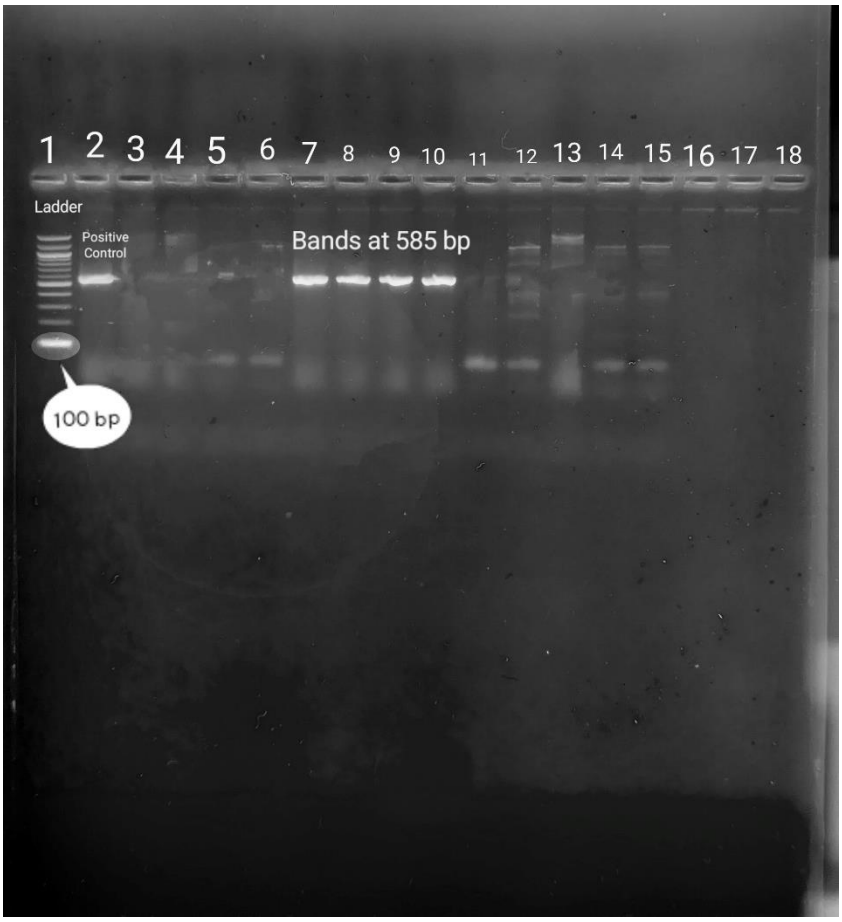

Figure 7: *E. coli* after agarose gel electrophoresis showing bands from the 7th well to the 9th well.

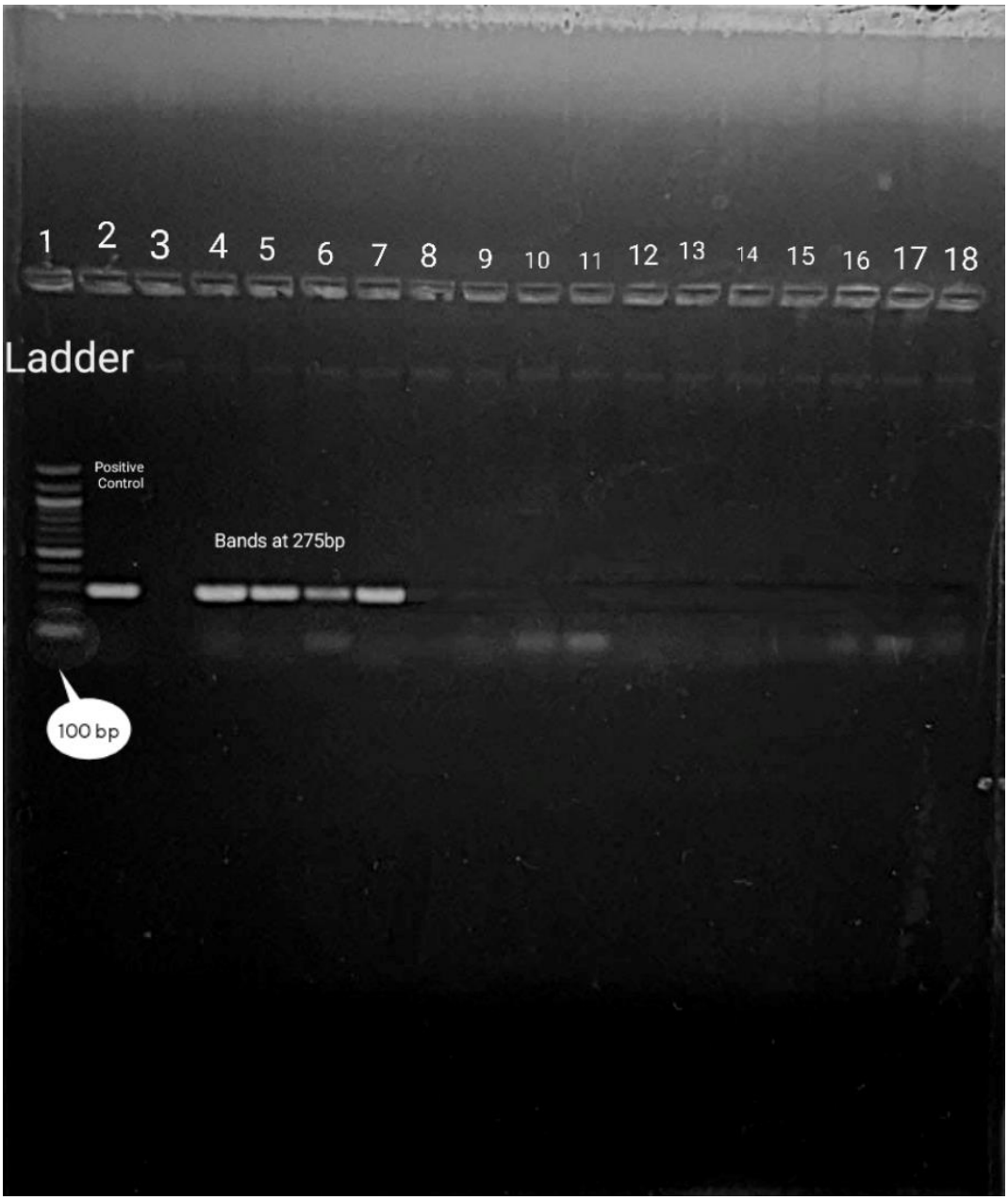

Figure 8: *Staphylococcus aureus* after agarose gel electrophoresis showing bands from the 4th well to the 7th well

2.7: Antibiotic susceptibility testing:

Antimicrobial susceptibility profiles were determined using the Kirby-Bauer disk diffusion technique, which is quicker and more practical than the broth dilution technique. This method is universally used for determining susceptibility and resistance among aerobes and facultative anaerobes against many classes of antibiotics.

Standard antibiotic disks placed on Mueller-Hinton agar plates were used to test the sensitivities of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus isolated strains. Each strain was subjected to various antibiotics to determine the respective resistance or sensitivity pattern. Consequently, inhibitory zones around each antibiotic disk were measured to determine the efficiency of the antibiotics in inhibiting bacterial growth.

This technique proved very useful in elucidating the pathogens' susceptibility pattern, thus guiding proper therapeutic options. The data on these tests are imperative for clinicians and public health officials to meet the challenges of antimicrobial resistance and ensure effective treatment modalities.

Muller Hinton Agar (MHA):

This is mainly designed for antibiotic susceptibility tests used in the disk diffusion method.

i.e., the Kirby-Bauer disk diffusion method. The Clinical and Laboratory Standards Institute has recommended it as the ideal medium for AST primarily because of its medium’s nonselective and non-differential nature (Vasylevskyi S. et al., 2018). Due to the presence of starch, toxins caused by the bacteria are absorbed by it, preventing They refrain from interfering with antibiotics. Moreover, it shows good reproducibility from batch to batch. As it is a loose agar, it facilitates better antibiotic diffusion. Hence, MHA has been used. For the AST so that proper results come out from the study

Selected Antibiotic Disks

Antibiotics belong to a class of drugs with diverse activities that affect different things in bacteria. These activities make it possible for another antimicrobial resistance mechanism to occur in bacteria. To establish broad characteristics of the antimicrobial susceptibility of *Escherichia colli*, *Klebsiella pneumoniae, and Staphylococcus aureus*, twelve to sixteen antibiotics were used in this study. These antibiotics include those from different classes and have different modes of action, which allows consideration of the resistance levels of the isolates.

A table from the excel spreadsheet are given below with the details collected from the data:

Antibiotic Group	Antibiotic Name	Disc Code	Disc Potency (μg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Carbapenems	Imipenem	IPM10	10	≥23	20-22	≤19
	Meropenem	MEM10	10	≥23	20-22	≤19
Beta-lactams	Amoxicillin	AMC30	30	≥18	14-17	≤13
	Ceftriaxone	CTR30	30	≥21	16-20	≤15
	Cefixime	CFM5	5	≥19	15-18	≤14
	Ceftazidime	CAZ30	30	≥18	15-17	≤14
	Cefepime	CPM30	30	≥18	15-17	≤14

	Oxacillin	CRO30	30	≥13	45608	≤10
	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
	Azithromycin	AZM30	30	≥18	14-17	≤13
Tetracyclines	Tetracycline	TE30	30	≥19	15-18	≤14
Phenicols	Chloramphenicol	CL10	10	≥18	14-17	≤13
Quinolones	Ciprofloxacin	CIP5	5	≥21	16-20	≤15

Table 2: Concentrations and diffusion zones of the antibiotics.

Inoculation and Isolation on Muller Hinton Agar Plates

This included preparing a bacterial suspension to the McFarland Standard before inoculating bacterial pathogens on Mueller-Hinton Agar (MHA) plates for isolation. First, 5-6 mL of normal

saline was autoclaved into a test tube. In an aseptic fashion, 3 to 5 bacteria colonies from a newly streaked NA were picked up by an inoculating loop and then placed in the saline. The test tube was then vortexed vigorously until the cloudiness of the suspended material was equivalent to what was termed in the 0.5 McFarland Standard, equal to approximately 1.5 x 10^8 CFU/mL of bacteria. This standard is essential to guarantee a standardized and uniform bacterial density fit for antimicrobial susceptibility testing.

When the required turbidity was achieved, a fresh autoclaved cotton swab was placed in the bacterial suspension, allowing the growth of bacteria to facilitate colony counts; the swab was then rolled against the inner surface of the test tube to remove excess suspension. The swab prepared from the bacterial sample was streaked over the surface of a sterile Mueller Hinton Agar (MHA) plate to spread over the complete petri plate. The cotton swab was rotated multiple times, where at each angle, the plate was rotated to provide a homogenous lawn of bacterial growth.

When the lawn was done, sterile forceps were used to transfer the antibiotic discs to the surface of the MHA plate. To avoid contamination of bacterial specimens, the forceps used in positioning the antibiotic discs were sterilized by wiping them with 70% ethanol and then flamed before picking each antibiotic disc. The antibiotic discs were equally gently placed onto the agar so that they made good contact with the bacterial lawn. Following the placement, the plates were then incubated at the 37-degree Celsius temperature at which the bacteria grew and inhibited the formation of the inhibition zone. Due to the summation of these factors, the precise and standardized method of inoculating the bacterial isolates provided a standard bacterial density and relatively accurate results for the antimicrobial susceptibility of the bacteria present.

Zone of Inhibition:

After incubating for a day, when the MHA plates are taken out, bacterial growth is seen around each disc. If the antibiotic is effective on the tested organism, then there will be 'no growth' visible around it. Similarly, there will be antibiotics which will not be as productive in that case; there it will be a visible zone of Inhibition. When such cases acquire the zone of inhibitions are measured with the help of a scale, which helps to give a clear idea of the acceptable development restraint zones around the discs. (Clinical and Laboratory Standards Institute, 1997, 1999).

Using a standard table by Oxoid Limited (England), the results are then determined whether they are sensitive, intermediate, or resistant. The National Antimicrobial Resistance recommends it.

Chapter 3

Result

3.1: Pathogen Detection and Prevalence in Cultured Septicemia Cases

This study analyzed 56 blood samples to identify bacterial pathogens associated with septicemia. Of these, 33 samples (58.9%) tested positive for bacterial growth, indicating the presence of pathogenic organisms.

Out of the 33 positive samples:

- Twenty-five samples (75.8%) were identified as infected with *Klebsiella pneumoniae* (*KP*), making it the most prevalent pathogen detected.
- Four samples (12.1%) were infected with *Staphylococcus aureus* (*Staph A*), a significant Gram-positive organism linked to bloodstream infections.

- Four samples (12.1%) were infected with *Escherichia coli* (*E. coli*), a known Gram-negative pathogen associated with severe septicemia cases.

These findings underscore the predominance of *Klebsiella pneumoniae* as the leading cause of bloodstream infections among the analyzed samples, followed by *Staphylococcus aureus* and *Escherichia coli*. The remaining 23 samples (41.1%) showed no bacterial growth under the testing conditions, indicating the absence of these targeted pathogens or potential non-bacterial causes of infection.

3.2: Positive result based on Gender:

The 33 positive blood samples were further analyzed to determine the distribution of bacterial infections based on gender. Among the positive cases:

- Thirty samples were from male patients, indicating a significantly higher prevalence of bloodstream infections in males.
- Three samples were from female patients, showing fewer infections among females.

The gender distribution highlights a predominance of septicemia cases among male patients in this study. This gender-based disparity may be influenced by factors such as differences in exposure to healthcare settings, underlying health conditions, or behavioral and biological variations that affect susceptibility to bacterial infections. Further investigation into these factors could provide valuable insights into the observed trend.

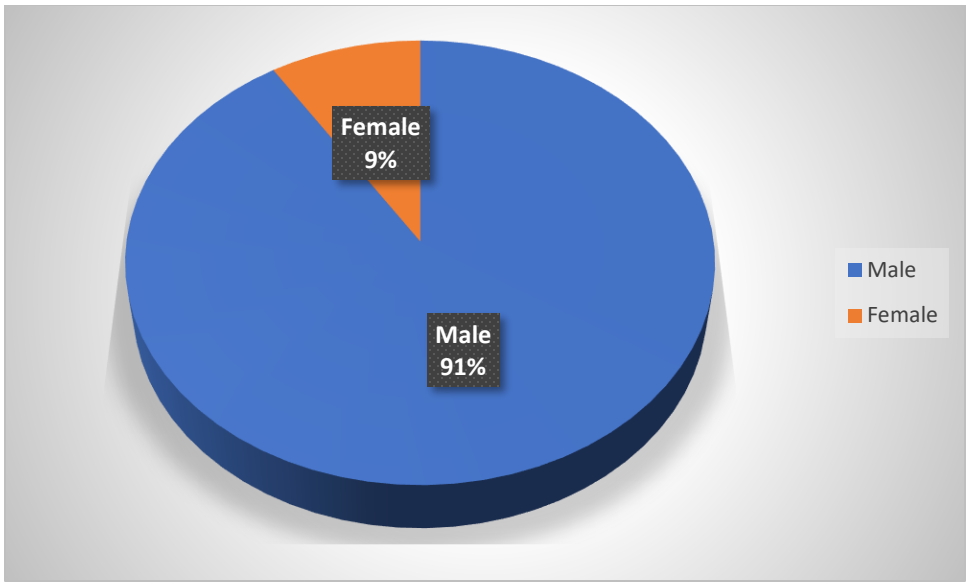

Figure 9: A graph showing the percentage of infected males and females in the total sample.

3.3 Positive Results Based on Age

The distribution of positive cases based on age shows that the infections were observed across a wide age range, from 26 to 94 years. The data was grouped into age brackets for better understanding:

- 20–29 years: 1 case 3%
- 30–39 years: 6 cases 18%
- 40–49 years: 10 cases 31%
- 50–59 years: 8 cases 24%
- 60–69 years: 5 cases 15%
- 70+ years: 3 cases 9%

The results indicate that the majority of positive cases were in the 40–49 age group, accounting for 30.3% of all infections, followed by the 50–59 age group at 24.2%. The lowest prevalence was observed in the 20–29 age group. These findings highlight that middle-aged and elderly individuals are more commonly affected by bloodstream infections in this study.

3.4: Antibiotic Susceptibility Profiling of Isolated Pathogens

Antibiotic Susceptibility Analysis of *Klebsiella Pneuomoniae:*

Out of all the positive *Klebsiella pneumoniae* isolates, most strains showed comparatively high susceptibility to antibiotics, except for Ampicillin, Erythromycin, and Colistin, which demonstrated significant resistance. The data table provided below shows the effectiveness of these 12 antibiotics against the isolates . From the table, it is evident that Ciprofloxacin (CIP5), Imipenem (IPM10), and Meropenem (MEM10) showed high susceptibility, with 96% to 100% of the isolates being susceptible. Conversely, Ceftazidime (CAZ30), Cefixime (CFM5), and Chloramphenicol (CPM30) displayed high resistance levels, with 88% - 92% of the isolates showing resistance. Ceftriaxone (CTR30) exhibited moderate resistance, with 60% of isolates resistant and 20% intermediate. Regarding intermediate resistance, Kanamycin (AK30) and Tetracycline (TE30) had the highest levels, with 8% of isolates showing intermediate susceptibility. In comparison, Piperacillin-Tazobactam (PIT100/10) showed 12% intermediate resistance, which is lower than other antibiotics. One key finding is the absence of intermediate Imipenem (IPM10) resistance, demonstrating 100% susceptibility highlighting its effectiveness against the tested isolates. However, Erythromycin (AZM15/30) showed a significant resistant

pattern (20%) with only 64% susceptibility, underscoring the reduced effectiveness of this antibiotic.

These results indicate that while many isolates remain susceptible to common antibiotics like Ciprofloxacin, Meropenem, and Imipenem, there is significant resistance in certain classes, particularly beta-lactams and Erythromycin. This highlights the need for continued surveillance and antibiotic stewardship to prevent further resistance development.

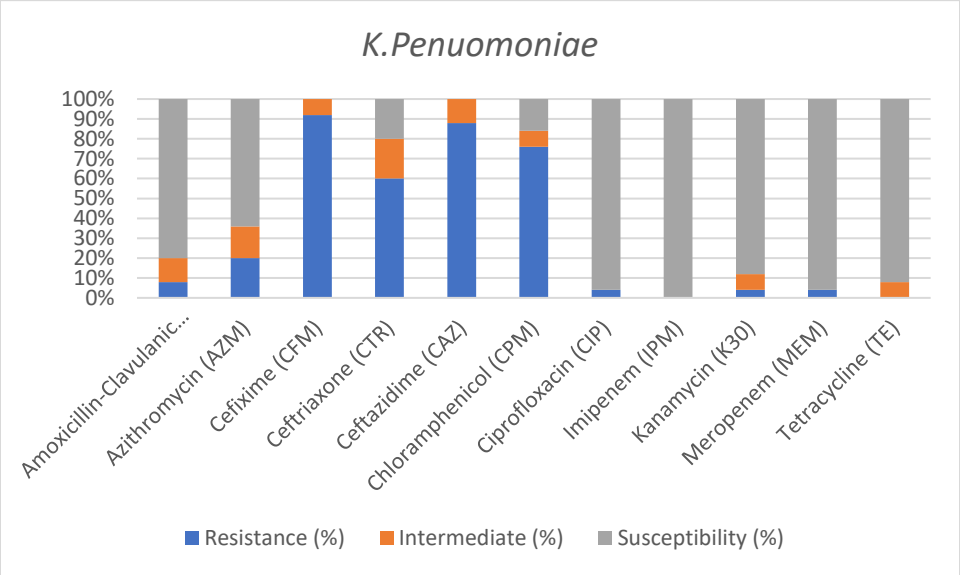

Figure 10: A graph showing the resistance, intermediate and sensitive results of *K.Pneumoniae*

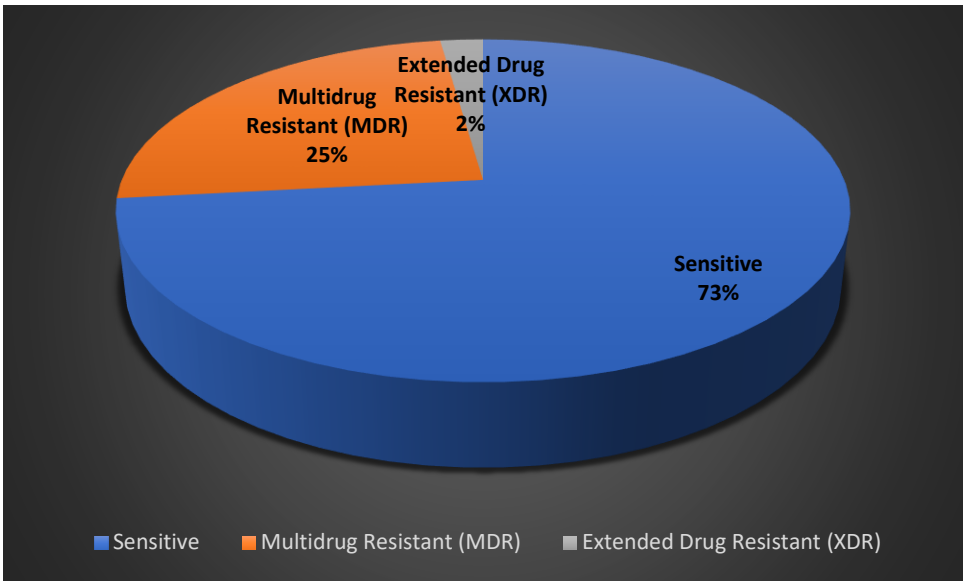

Figure 11: A graph showing the MDR, Sensitive and XDR isolate percentage of *K.Pneumoniae*

Antibiotic Susceptibility Analysis of *E. coli*

The analysis of the 14 antibiotics tested against the isolate reveals 100% susceptibility to critical antibiotics, including Ampicillin, Imipenem, Ciprofloxacin, Tetracycline, Meropenem, and Kanamycin (AK), making these highly effective options for treatment. However, significant resistance was observed for Erythromycin, Cefixime, and Chloramphenicol (CPM), each showing 100% resistance, indicating they are ineffective against the isolate. Antibiotics such as Chloramphenicol (CL), Gentamicin, and Ceftriaxone exhibited intermediate susceptibility, suggesting partial efficacy and limited reliability as standalone treatments. Based on these findings, 71.43% of the antibiotics were classified as sensitive, while 21.43% displayed multidrug resistance (MDR). One antibiotic (Chloramphenicol [CPM]) was categorized as extended drug-resistant (XDR), demonstrating complete resistance. These results underscore the importance of

focusing on highly effective antibiotics while avoiding those with high resistance to ensure optimal treatment outcomes

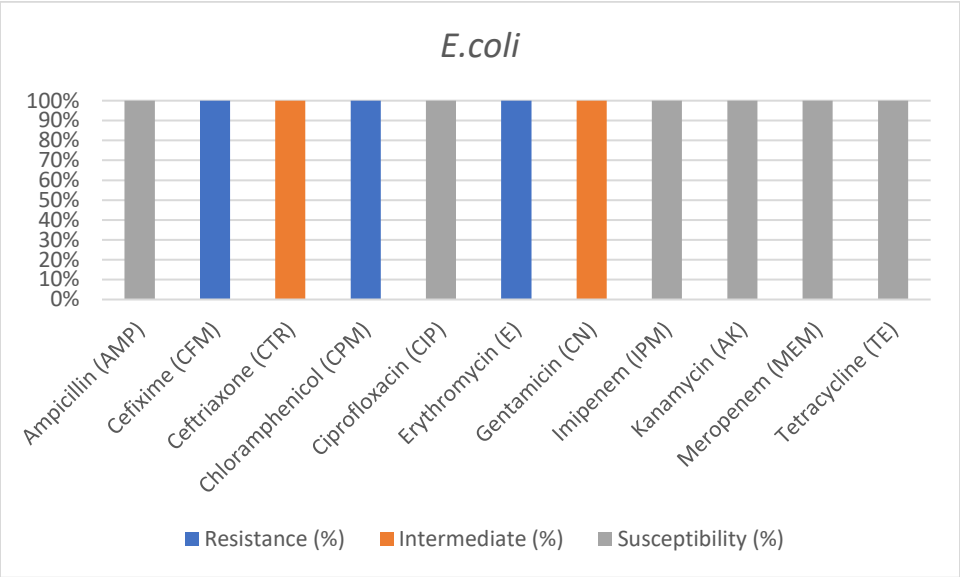

Figure 12: A graph showing the resistance, intermediate and sensitive results of *E. coli*

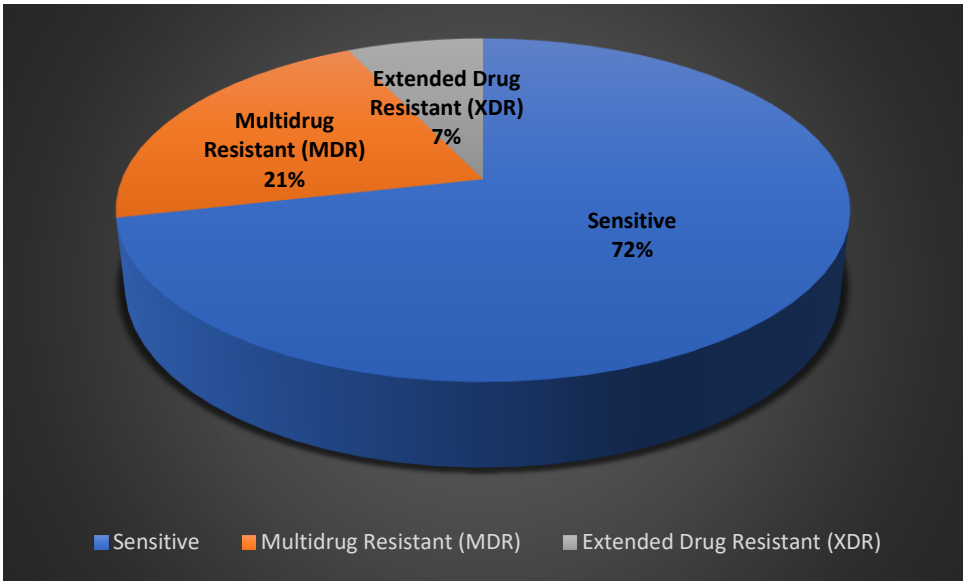

Figure 13: A graph showing the, Sensitive and XDR percentage of the isolates of *E. Coli*

Antibiotic Susceptibility Analysis of *Staphlococcus A*

The antibiotic susceptibility testing of 4 positive Staphylococcus aureus isolates with AST results for 12 antibiotics shows that all these antibiotics (Kanamycin, Imipenem, Amoxicillin-Clavulanic Acid, Meropenem and Ciprofloxacin) are effective with 100% susceptibility. But a high incidence of resistance was observed for Cefixime (CFM), Erythromycin (E15), Ceftazidime (CAZ), which had 100% resistance and also Ampicillin (AMP), Ceftriaxone (CTR) had 75% resistance. Intermediate resistance ranged from 25-50% from the samples to Azithromycin (AZM), Tetracycline (TE), and Ceftriaxone (CTR), and this could be attributed to partial efficacy. When the test antibiotics' reactions were evaluated, 66.67% were sensitive, 25% were MDR, and 8.33% were XDR. These results underscore the importance of using carbapenems, fluoroquinolones, and aminoglycosides and limiting beta-lactams and erythromycin for treating Staphylococcus aureus infections.

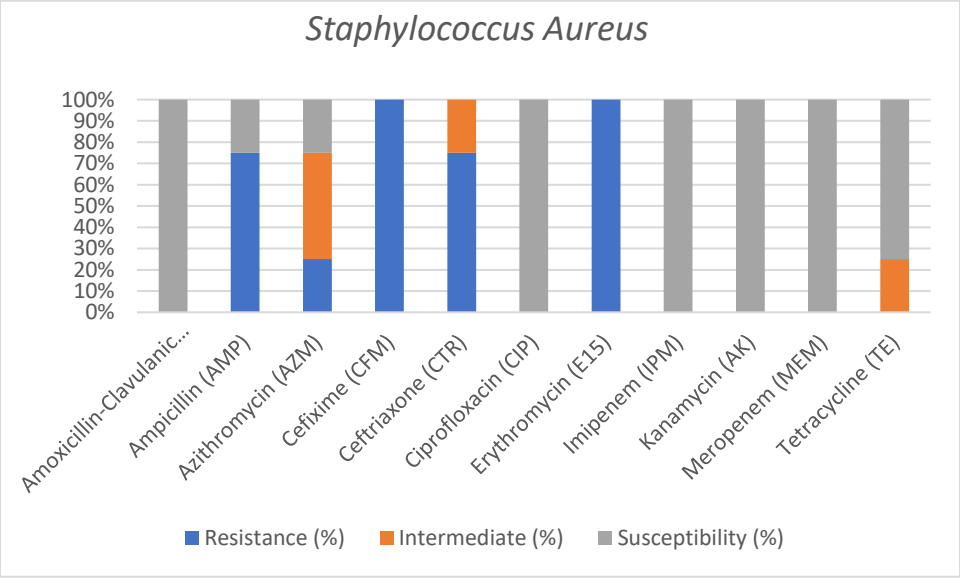

Figure14: A graph showing the resistance, intermediate and sensitive result of *Staph A.*

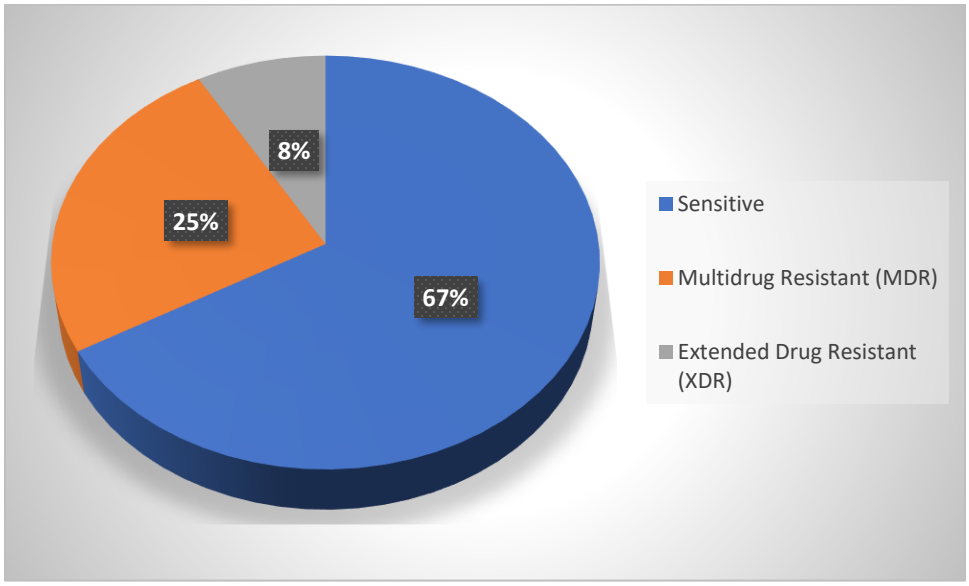

Figure 15: A graph showing the MDR, XDR, and sensitive result of the *Staph A.* isolates.

Chapter 4

Discussion:

The current study analyzed 56 blood samples, from which a positivity rate of 58.9% for BSIs was established. The findings are in agreement with the fact that, globally, BSIs continue to be one of the major concerns in healthcare, causing significant morbidity and mortality worldwide. Recent global estimates ascribe 11 million deaths due to sepsis annually, accounting for 20% of all global deaths (Rudd et al., 2020). BSIs are driven by increasing AMR, further complicating treatment options. The global impact of AMR is highest in LMICs; poor infection control practices, delayed diagnoses, and irrational antibiotic use are the usual characteristics in these settings. Indeed, from the observations of the Mortality from Bacterial Infections Resistant to Antibiotics (MBIRA) study conducted across sub-Saharan Africa, BSIs caused by gram-negative *Enterobacterales*, such as *Klebsiella* pneumonia and Escherichia coli, significantly increase mortality at the distal endpoints of resource constraints (Antimicrobial Resistance.Collaborators, 2022; Biehle et al., 2015) Well-implemented Antimicrobial Stewardship Programs's, coupled with better diagnostic capabilities in high-income countries, have eased the burden of AMR in BSIs. However, in resource-poor settings in Bangladesh, AMR rates in BSIs are still pathetically high due to poor health infrastructure, uncontrolled antibiotic use, and scarce data coming from surveillance programs. The results of this study were dominated by Klebsiella pneumoniae (75.8%), which agrees with many global reports indicating this organism is one of the most common causes of hospital-acquired BSIs (WHO 2023; Peters et al. 2019). These findings on the high prevalence of *K. pneumoniae* in the current study are supported by findings from other LMICs, including Egypt and India, where carbapenem-resistant *K. pneumoniae* rates exceed 50%. Meanwhile, *Staphylococcus aureus* remains a concern worldwide. At the same time, its methicillin-resistant form, MRSA, was

attributed to increased mortality and prolongation of hospitalization in both LMICs and developed countries. It is similarly correct that worldwide, a large percentage of BSIs are due to *Escherichia coli* and are increasingly resistant to beta-lactams and fluoroquinolones. E. coli isolates were found in this study in 12.1 % of cases; findings from Europe and North America are substantially the same, revealing scant treatment alternatives because of resistance patterns. (Cheah et al., 2013) .The incidence of this study was good concerning age and BSIs, as all the positive cases were detected in patients above 40 years of age. Several factors contribute to increased susceptibility in the elderly population, such as immunosenescence, comorbid conditions, and invasive medical procedures. Similarly, reflected worldwide, older adults belong to those who report the highest risk for BSIs and their complications (Simon et al., 2015). The male preponderance, as high as 90.9% in this study, also reflects the global findings, where hormonal and behavioral factors contribute to higher infection rates in males. (Rudd et al., 2020) The results of AST in the current study revealed high resistance rates to beta-lactams and fluoroquinolones. Overall, carbapenem-resistant *K. pneumoniae* and *E. coli* isolates are related to mortality, which exceeds 40% worldwide. This is further heightened by limited access to effective antibiotics in LMICs, which further increases treatment delays and enhances the mortality burden. Various international organizations, including the WHO, call for enhanced ASPs, diagnostics, and international collaborations to deal with the rising menace of AMR. In comparative terms, high-income countries have shown that stringent infection control policies with earlier interventions significantly reduce the burden of AMR infection.

Chapter 5:

Conclusion:

The present study was undertaken to know the bacteriological profile and antimicrobial susceptibility patterns of bloodstream infection in patients suspected of septicemia. Of the 56 blood samples, 33 were positive, with the isolation rate of Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli in order. Klebsiella pneumoniae was the predominant pathogen, contributing to 75.8% of the positive isolate, followed by Staphylococcus aureus and Escherichia coli, contributing to 12.1% of infections. These findings put in perspective the high burden of septicemia with a predominance among middle-aged and elderly and the fact that all were male patients. The high prevalence of Klebsiella pneumonia, concomitantly with its well-documented association with multidrug resistance, is a critical concern corresponding to a much-heightened antimicrobial resistance challenge. Staphylococcus aureus and Escherichia coli underscore the necessity of infection control and targeted surveillance. Susceptibility testing showed variable resistance patterns, thereby underlining the need for tailored therapeutic approaches based on local resistance data. These results point again to the urgent need for antimicrobial stewardship programs to apply the best use of antibiotics and reduce the spread of resistant strains. The study adds knowledge about the epidemiology of septicemia and points out further research needs concerning its challenges. Therefore, more extensive sample-size studies regarding a wide range of pathogens are genuinely needed, along with higher molecular diagnostic methods, so that future studies may better understand the dynamics of bloodstream infection to improve the outcome. Also, public health strategies about early diagnosis, proper use of antibiotics, and strict infection control practices significantly reduce the clinical burden associated with septicemia.

This study adds to the emerging literature related to bloodstream infections and lays the foundation for devising effective ways to combat septicemia, with a view to improving health outcomes.

Chapter 6

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