Bacteriological analysis and antibiotic resistance in patients with suspected septicemia in Dhaka, Bangladesh

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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

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Declaration

It is hereby declared that

1. The thesis submitted titled **"Bacteriological analysis and antibiotic resistance in patients with suspected septicemia in Dhaka, Bangladesh"** is our own original work while completing a 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. We have acknowledged all main sources of help.

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In Spring, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology in Fall, 2024

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Ethics Statement

This study was conducted in strict accordance with the highest ethical standards in research. Blood samples were collected from patients at four branches of Islamic Bank Hospital in Dhaka, Bangladesh, with full adherence to established ethical guidelines. Ethical approval was sought and granted from the review boards of all four hospital branches prior to the initiation of the study. These institutional review boards meticulously reviewed and approved the research protocols to ensure compliance with the National Guidelines on Health Research and the International Guidelines for Ethical Considerations in Research.

Furthermore, the study underwent review and received approval from the Ethics and Research Review Committee of the Faculty of Mathematics and Natural Sciences, MNS, at BRAC University, Dhaka, Bangladesh. The research was conducted under the supervision of Dr. Fahim Kabir Monjurul Haque, with all aspects of the study aligning with the ethical guidelines set forth by BRAC University.

Informed consent was obtained from the patients prior to the collection of samples. patients were thoroughly informed about the study's objectives, the procedures involved, potential risks, and the benefits. Data confidentiality and patient’s anonymity were rigorously maintained, with all personal identifiers being removed during data analysis.

This robust ethical framework underpins the study's integrity and validity, ensuring adherence to internationally recognized norms and principles for ethical research.

Acknowledgement

We would like to begin by expressing our deepest gratitude to Almighty Allah for granting us the opportunity to undertake and complete this research. Our heartfelt thanks go to our parents whose unwavering support has been invaluable from the inception to the completion of this study.

We are also deeply grateful to the Chairperson of the Department of Mathematics and Natural Sciences, **Md. Firoze H. Haque**, and to all the faculty members of BRAC University for their continuous support. Special thanks to our supervisor, **Dr. Fahim Kabir Monjurul Haque**, Associate Professor in the Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University. His expert guidance, constructive feedback, and unwavering support were crucial to the success of this research. This work would not have reached its full potential without his dedicated mentorship and insightful critiques.

Lastly, I want to show my heartfelt appreciation to my mentor, **Fahim Faisal Nakib**, for his constant clear direction and guidance. His guidance helped me ease up and navigate the journey while enlightening and enriching the experience.

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Abstract

The objective of our study was to isolate bacteria from septicemia patient's blood and subsequently analyze their antibiotic resistance pattern. We collected 80 blood samples from four different branches of Islami Bank Hospital (Mirpur, Kakrail, Motijheel, and Nayapaltan) in Dhaka, Bangladesh. Isolating one bacterial colony from a blood culture plate of selective media. Then, based on colony morphology, selecting the bacterial colonies and subculturing them for presumptive identification. Confirming their identity through biochemical tests (catalase test, oxidase test) and molecular test (polymerase chain reaction) followed by gel electrophoresis (for visualizing the band size of targeted microorganisms). Among the 80 samples, 66 samples were culture positive. From positive cultures, 65.2% were ***Salmonella typhi***, 13.6% were ***Klebsiella pneumoniae***, 12.1% were ***Escherichia coli***, and 9.1% were ***Staphylococcus aureus***. 22% were gram-positive and 77% were gram-negative) For antibiotic susceptibility tests (AST), we selected one isolate from each culture-positive sample. Total 8 *Escherichia coli*, 43 *Salmonella typhi*, 9 *Klebsiella pneumoniae*, and 6 *Staphylococcus aureus* isolates were selected and AST was performed using the Kirby-Bauer disc diffusion method. We observed that 90% to 100% of the ***Salmonella typhi*** isolates 89% and 79% were resistant to Nalidixic acid and Ampicillin respectively. 68%, 79% and 39 % of ***Klebsiella pneumoniae*** isolates were resistant to Amoxicillin, Cefixime and Erythromycin respectively. 69% and 80% of all ***Escherichia coli*** isolates were resistant to Amoxicillin and Cefixime respectively, and 28% of the isolates were resistant to Azithromycin, Ciprofloxacin, Imipenem, Ceftriaxone. 49% of all ***Staphylococcus aureus*** isolates were resistant to Cefixime, Sulfamethoxazole and Ceftazidime, 28% were resistant to Erythromycin and Ceftriaxone, and 19% were resistant to Imipenem and Gentamicin. In this study, a total of 18 isolates were identified as multi-drug resistant (MDR), including 7 *Salmonella typhi*, 6 *Klebsiella pneumoniae*, 2 *Escherichia coli*, and 3 *Staphylococcus aureus* strains. Among these, 3 isolates were further classified as extensively drug-resistant (XDR), comprising 1 *Salmonella typhi*, 1 *Klebsiella pneumoniae*, and 1 *Staphylococcus aureus* isolate.

Keywords: Septicemia**,** ***Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus,*** Multi-drug resistant (MDR), Extensively drug-resistant (XDR).

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List of Acronyms

PCR	Polymerase Chain Reaction
AST	Antibiotic Susceptibility testing
MHA	Muller-Hinton Agar
MDR	Multi Drug Resistant
XDR	Extensive Drug Resistant
XLD	Xylose Lysine Deoxycholate
EMB	Eosin Methylene Blue
MAC	MacConkey Agar
MSA	Mannitol Salt Agar

CHAPTER 1

1.1: Introduction

Sepsis is a hazardous organ dysfunction caused by an immune response to infection. And so it is a leading cause of death, especially in low income countries like Bangladesh. In developing countries like Bangladesh, India or Pakistan, the leading causes of infection are malaria, pneumonia and diarrhea. Here, sepsis dominates because of higher malnutrition, lack of certified doctors, poor healthcare infrastructure, and late sepsis detection. If left untreated, sepsis can manifest in more severe forms like septic shock, multiple organ failure, and finally death. That's why it is important to detect sepsis as soon as possible. We also need more information to design the most appropriate treatment method for each sepsis case. (Garbern et al., 2024) At present, special colorimetric assays can detect septicemia at early stages, by tracking special biomarkers in blood. This is a very important discovery for medical science. Because a patient's life depends on the earliest possible availability to treatment. (Dashtian et al., 2024). This has been confirmed in a review of 42 studies on 19000 patients and more. All the findings confirm that the death risks of septicemia drop by 16 percent if the patients receive treatment within the first 3 hours of diagnosis. After this time has passed, the chances of death increase by 30 per cent. The results are published in the European Journal of International Medicine (Leung et al., 2024). But sadly, antibiotic resistance is on the rise (confirmed by the World Health Organization). This is a huge obstacle to treatment (Baral et al., 2024). And most of the causative agents of septicemic pathogens are already resistant to the commonly used antibiotics. For example, a study in Turkey shows that deaths of 48% of infected patients are caused by gram negative sepsis. Infections from Methicillin resistant *Staphylococcus aureus* cause 36 % of deaths. Carbapenem-resistant pathogens can kill 60 percent of patients on average. (Gucer et al., 2024). Another research in Ethiopia showed that 89 percent of nosocomial pathogens are already resistant to multiple drugs (like ampicillin or amoxicillin.) Among them, 34.7% are resistant to carbapenems and 46.3 percent to beta lactams (Tilahun et al., 2024). These statistics are concerning. So, these pathogens should be studied extensively. And appropriate antibiotics should be designed to stop their growth, so that antibiotic resistance can be slowed down or reversed, if possible. (Baral et al., 2024). Even Bangladesh is trying to design proper treatment plans for septicemia. Experts from other countries are also trying to find solutions in our country, like Dr. Stephanie Garbern from Brown University, United States. She mainly

studied children affected with septicemia in poor and middle-income countries. She even worked with engineers and local doctors to develop an effective diagnostic gadget. It was also trained with a machine learning algorithm, which can collect the person's data (heart rate, oxygen rates etc.) Then the AI can detect early symptoms of sepsis, which doctors might mistake as fever or common cold. She only used cheap, available tools so that poor and middle-class people can access it too. (Garbern et al., 2024). Nevertheless, it is important to find cost effective ways to diagnose and treat septicemia in a third world country like Bangladesh.

Despite the discovery of advanced treatment methods for septicemia, antibiotics are the standard treatment plan because it directly targets the pathogens, who are the main agents of disease. That's why we felt the need to conduct this research- to find the ideal antibiotic to kill each pathogen. But antibiotics are losing their potency because of some malpractices in our Healthcare system. Antibiotic usage is largely unregulated in Bangladesh, especially in rural areas. Without diagnosis, pharmacists and doctors prescribe multiple antibiotics to the customers without diagnosis. These antibiotic sellers don't know the guidelines of using antibiotics. In addition, patients don't complete their medication for the full prescribed time, which buys time for bacteria to develop antibiotic resistant genes (Bepari et al., 2023). Extensive studies have been conducted on this pattern of emergence. A review article analyses 46 studies from 2014 to 2018, which reveals high prevalence of antibiotic resistance among common pathogens. (Ahmed et al., 2019).

Regarding the growing resistance to antibiotics, it is essential to conduct regular studies to identify the bacteria causing septicemia among Bangladeshi patients and assess their antibiotic resistance. This helps to monitor the changes in antibiotic resistance. Additionally, this will provide doctors and authorities with insights to develop treatment strategies that prescribe efficient antibiotics for septicemia patients.

1.2: Objectives

The objectives of this study were to isolate the bacteria causing septicemia from septicemia-suspected patients in Dhaka city and to analyze their antibiotic resistance patterns.

CHAPTER 2

Materials and Methods

2.1 Study design

The study was conducted among clinically suspected septicemia patients who were admitted to hospitals in Dhaka, Bangladesh. Samples were collected from four branches of Islami Bank Hospitals; Mirpur, Motijheel, Kakrail and Naya paltan & 80 samples were collected from these branches. The blood sample collection duration was from December,2024 to April, 2025. Blood samples were collected from patients of various age groups and both genders who were admitted to various wards including the Intensive Care Unit (ICU), Medicine, Pediatrics, and Emergency wards. Written consent was taken prior to sample collection, from either patients or the legal guardians of patients.

2.2 Sample collection

Venous blood samples were taken from clinically confirmed septicemia patients upon admission to hospitals that were chosen for the study. Venipuncture was used to obtain blood samples using sterilized, disposable syringes and needles by professional experts. Before beginning antibiotic treatment, each patient had a blood draw of about 2-3 mL. Blood was immediately put into a heparin tube which was transported to the microbiology lab via a sealed iced box(4°C). The samples were appropriately labeled with the information of patient's ID, date, name, gender and indoor/outdoor patients. After being collected, they were taken to the microbiological lab in 1-2 hours(Nursing et al., 2023).

2.3 Culture to isolate the bacteria

Upon arrival at the laboratory, each blood sample was immediately processed to ensure the integrity of microbial content. A volume of 100 µL of the blood sample was aseptically dispensed and spread onto various culture media. This was carried out using a sterile micropipette and spreader under laminar flow conditions to minimize contamination. The selected media types

included MacConkey (MAC), Xylose Lysine Deoxycholate (XLD), Eosin Methylene Blue (EMB), Mannitol Salt Agar (MSA), HiCrome™ UTI, HiCrome™ KPC Agar media. They were then incubated at 37°C and checked 5-7 days for indications of microbial growth such as changes in medium color and colonies. After up to seven days of incubation, spread plates were observed if there is any growth. Positive cultures that showed evidence of growth were sub cultured onto selective and differential media such as *Salmonella typhi* on SS Agar media, XLD medium, *Escherichia coli* on EMB agar, HiCrome™ UTI Agar, *klebsiella pneumoniae* on KPC, *Staphylococcus aureus* on Mannitol Salt Agar. Thus, expected colony morphology of these organisms has shown in **Table 1**. For 18 to 24 hours, plates were incubated at 37°C, and the visible of bacterial colonies was observed and has shown in the **Figure 1.**

Table 1: Desired colony morphology of isolated bacteria

Organism	**Gram Positive/Negative**	**Media**	**Expected Colony Morphology**
Staphylococcus aureus	Gram-positive	Mannitol Salt Agar	Yellow/white colonies surrounded by the yellow zone
Klebsiella pneumoniae	Gram-negative	HiCrome UTI Agar	Blue, mucoid
Klebsiella pneumoniae	Gram-negative	HiCrome KPC Agar	Bluish-green
Escherichia coli	Gram-negative	HiCrome UTI Agar	Purple colonies
Escherichia coli	Gram-negative	EMB Agar	Purple with a black center and green metallic sheen
Salmonella typhi	Gram-negative	Salmonella Shigella Agar	Colorless with black centered
Salmonella typhi	Gram-negative	Xylose-Lysine-Deoxycholate agar	Red with black centered

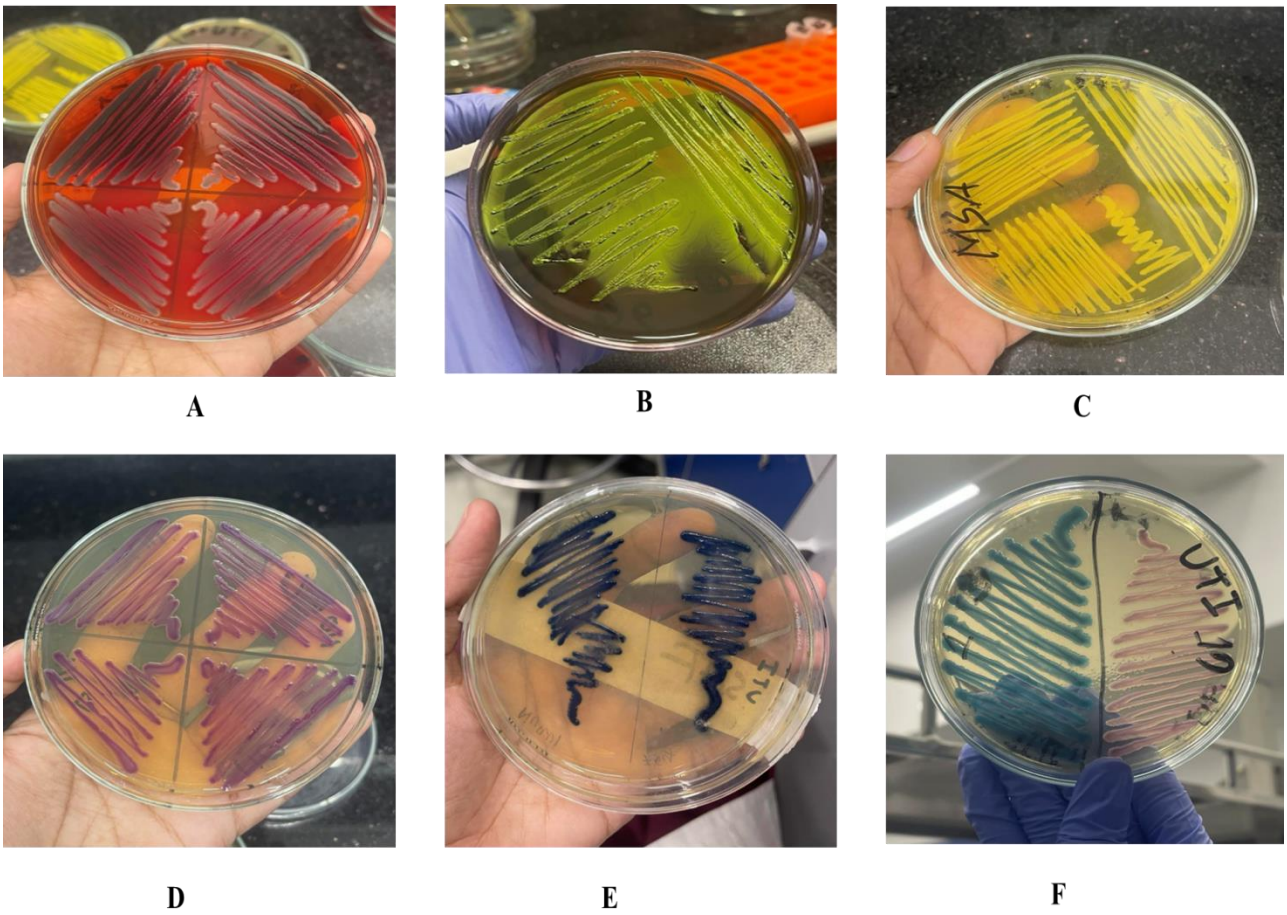

Figure 1 : Different bacterial colonies of different selective media. (**A**) *Salmonella typhi* has given black centered on XLD agar, (**B**) *Escherichia coli* shows green metallic sheen on EMB agar, (**C**) *Staphylococcus aureus* gives yellow colored colony on MSA, (**D**) *Escherichia coli* has given purple colony on HiCrome UTI Agar, (**E**) *Klebsiella pneumoniae* has given blue colony on HiCrome KPC Agar & lastly (**F**) *Escherichia coli* with purple colony & *Klebsiella pneumoniae* with blue colony which distinguish them on UTI media.

2.4 Biochemical tests

Biochemical tests were performed to analyze the metabolic and enzymatic properties of the isolated organisms. Here, catalase, oxidase tests were done for *Staphylococcus aureus, Escherichia coli, Salmonella typhi & Klebsiella pneumoniae.* **Table 2** shows the biochemical test results for the isolated bacteria.

Catalase test method:

1)Spread the bacteria on an agar plate and incubate it overnight (18-24 hours) under the proper conditions.
2)Using a sterile inoculating loop, select bacteria from a single colony and transfer them to a microscope slide.
3)After adding one drop of 3% H2O2 to the bacteria, the suspension will give the result whether it is positive or negative.

- **Positive test result:** Gas formation (O_2) in the form of bubbles shows that the bacterium has a catalase.
- **Negative test result:** No Gas formation (O_2) in the form of bubbles.

Oxidase test method:

1)On a piece of filter paper, apply two drops of the oxidase reagent.
2)Using a sterile loop to transfer bacteria from one colony onto the oxidase reagent area. The result can be observed after 30 sec.

- **Positive test result:** Dark blue-purple color change within 10-30 sec.
- **Negative test result:** No color change or color change after more than 30 sec (*VETBact*, n.d).

(A) **(B)** **(C)**

Figure 2.4: Positive results of catalase tests of *Salmonella typhi & Escherichia coli* **(A)**, *Klebsiella pneumonia* & *Staphylococcus aureus* **(B)** is shown in the above figure. Negative results of oxidase tests of ***Salmonella typhi, Escherichia coli, Staphylococcus aureus & Klebsiella pneumonoiae (C).***

Table 2: Biochemical test results of the organism

Organism	Catalase	Oxidase
Staphylococcus aureus	+ve	-ve
Escherichia coli	+ve	-ve
Salmonella typhi	+ve	-ve
Klebsiella pneumoniae	+ve	-ve

2.5 DNA extraction

DNA was extracted for the purpose of identifying the organism by visualizing their band production in gel electrophoresis followed by PCR. For the isolation of high-quality bacterial genomic DNA, a freshly grown subculture was prepared using nutrient-rich growth media. As a base 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 inoculated with a loopful of the bacterial culture. After that, the sample was then vortexed to thoroughly mix the TE buffer with the bacterial cells for 15 seconds. To pellet the bacterial cells, the sample was centrifuged for 10 minutes at 13,000 rpm and 25°C after initial mixing. After centrifugation, the supernatant was discarded and 400 µL of 1X TE buffer was added to the Eppendorf tube along with the pellet. The bacterial pellet was then resuspended by vortexing the sample once 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 machine. 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. Lastly, 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 additional examination. Then store the DNA at -20° temperature. For use in PCR or sequencing, this technique guaranteed the effective extraction of high-quality DNA from the bacterial cells (Moore et al,2005).

2.6 PCR (Polymerase Chain Reaction)

To confirm the identity of the bacterial isolates, Polymerase Chain Reaction (PCR) was performed. This molecular method was utilized to detect organism-specific genetic sequences.

PCR method

PCR cycle is carried out 25-40 times in order to amplify the DNA according to the organisms. To initiate the PCR process, each reaction mixture was prepared in a final volume of 13 µL. The reaction mixture included 3 µL of nuclease-free water, 1 µL of forward primer, 1 µL of reverse primer, 6 µL of Emerald Amp PCR Master Mix (Takara Bio Inc.), and 2 µL of template DNA. All components were carefully added into sterile PCR tubes. Following preparation, the tubes were vortexed gently for 3–5 seconds to ensure thorough mixing of reagents. The mixtures were then briefly centrifuged to collect contents at the bottom of the tubes before being loaded into a thermal cycler (Applied Biosystems 2720 Thermal Cycler). PCR amplification was carried out under standardized cycling conditions. Typically, 25–35 amplification cycles were performed which involved denaturation, primer annealing, and extension steps—allowing exponential amplification of the target DNA region. The total run time for the PCR ranged from 1.5 to 3 hours, depending on the specific protocol and target DNA fragment length. Upon completion, all amplified PCR products were stored at –20°C to preserve sample integrity for downstream applications such as gel electrophoresis or sequencing (Lorenz, 2012).

Targeted genes and their primers:

This study focused on detecting virulence-associated genes specific to *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus,* and *Salmonella Typhi* in order to screen blood samples for the presence of pathogenic strains, making it essential to identify the genetic markers associated with their pathogenicity. For example, these four microorganisms are prominent etiological agents in septicemia, each with distinct virulence factors. **Table 3** shows the list of targeted genes & primers of these organisms. This investigation aimed to detect these bacterial pathogens in blood samples by targeting their specific virulence genes, thereby contributing to a better understanding of the microbial causes of septicemia.

Table 3: List of targeted genes and of their primers

Name of bacteria	Primer Designation	Targeted genes	Primer Sequence (5′to 3′)	Product size	PCR conditions	References
Klebsiella pneumoniae	KP pf-F	16S-23S rRNA Internal Transcribed Spacer (ITS)	ATTTGAAGAGGT TGCAAACGAT	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 final extension 72°C.	(Kot et al., 2023)
	KP pr1-R	16S-23S rRNA Internal Transcribed Spacer (ITS)	TTCACTCTGAAG TTTTCTTGTGTTC			
Escherichia coli	ECO- F	*malB* (maltose operon)	GACCTCGGTTTA GTTCACAGA	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.	(Lindsey et al., 2017)
	ECO-R	*malB* (maltose operon)	CACACGCTGACG CTGACCA			
Salmonella typhi	Salmonella-F	invA	GTATTGTTGATT AATGACATCCG	403 bp	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30s, annealing at 60°C for 30s, and extension for 1 min at 72°C followed by a final extension at 72°C for 10 min.	(Ranjbar et al., 2016)
	Salmonella-R	invA	ATATTACGCTAC GGAAACACGTT			
Staphylococcus aureus	NUC-F	nuc	GCGAT GATGGTGATACG GTT	279 bp	4 min at 94°C and then 34 cycles of 1min at 94°C for the denaturation step and 45s at 55°C for the annealing & extension at 72°C for 45s followed by final extension at 72°C For 10min.	(Gogoi et al., 2024)
	NUC-R	nuc	AGCCAAGCCTTG ACGAACTAAAGC			

2.7 Agarose gel electrophoresis

After performing Polymerase Chain Reaction, the resulting DNA fragments were analyzed through Agarose gel electrophoresis method. This technique was employed to visualize DNA fragment sizes and confirm the presence of DNA by comparison with a DNA ladder. To prepare the gel, agarose powder (typically 1.5–2%) is mixed with 2000 µL of 50x Tris-acetate-EDTA (TAE) buffer and distilled water (98%) and heated until fully dissolved. Ethidium bromide, an intercalating agent that fluoresces under UV light, is added at a concentration of 4 µL to enable DNA visualization. PCR products are carefully loaded into the wells of the prepared gel using sterilized (autoclaved) micropipette tips. The gel is run using a 10x TAE buffer as the running buffer, facilitating the movement of DNA toward the positive pole. For each run, 4 µL of a 100 bp DNA ladder (Biolab) is used as a reference and electrophoresis is carried out at 100 volts at 400 amp. Once the electrophoresis is complete, the gel is observed under UV light. The DNA bands become visible due to binding of the fluorescent stain, which emits specific colors. Band sizes are then determined by comparing them to the bands in the DNA ladder, and the results are recorded accordingly (*Addgene: Protocol - How to Run an Agarose Gel*, n.d.). Below the four types of band sizes have been shown in **Figure 2(a), 2(b), 2(c), and 2(d).**

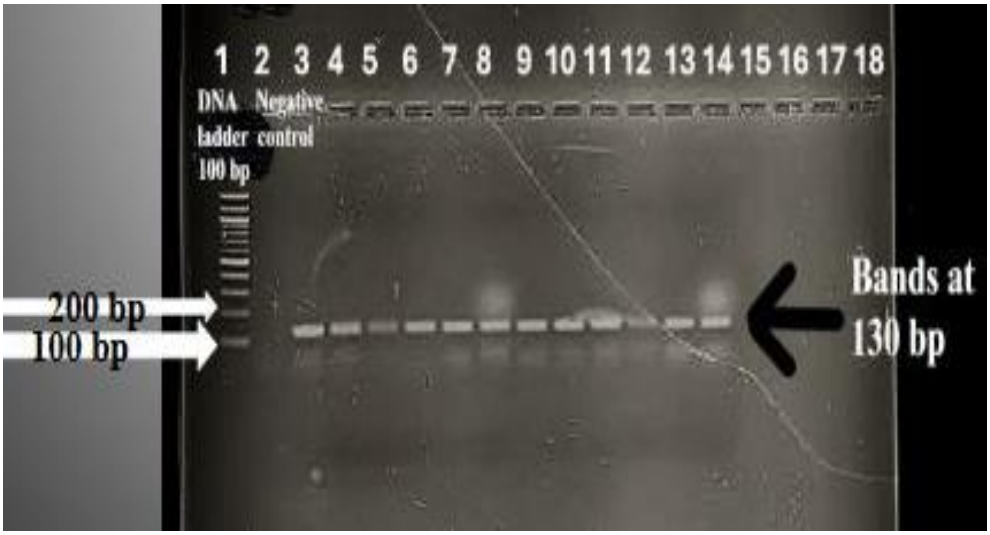

Figure 2(a): Agarose gel electrophoresis of PCR assay of *Klebsiella pneumoniae* isolates. Here, first lane is 100 bp DNA ladder, 2nd lane is negative control and well 3-14 are positive samples at 130 bp. The image was captured using a camera while the agarose gel was positioned under a UV transilluminator. The complete original image was utilized to generate the figure panel.

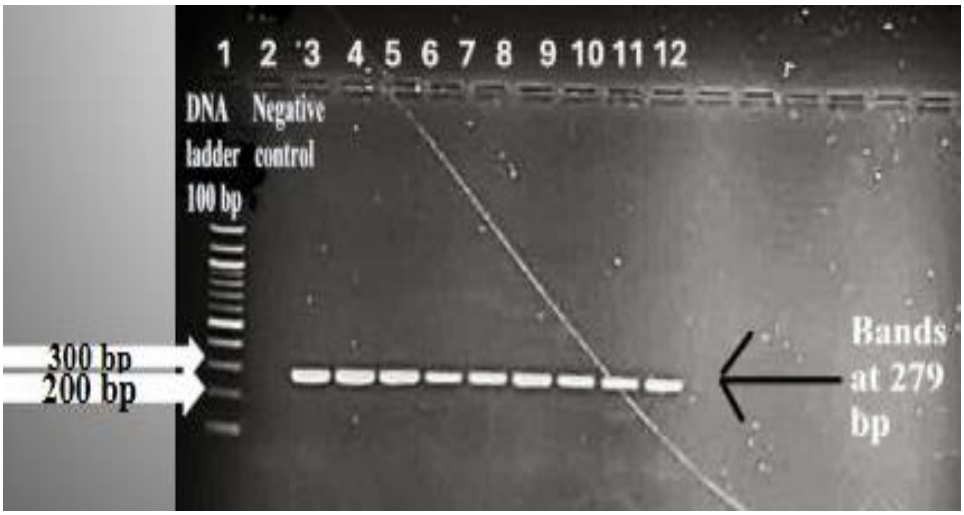

Figure 2(b): Agarose gel electrophoresis of PCR assay of *Staphylococcus aureus* isolates. Here, first lane is loaded with a 100 bp DNA ladder, the 2nd lane is negative control and lanes 3-11 are positive samples at 279 bp. The image was captured using a camera while the agarose gel was positioned under a UV transilluminator. The complete original image was utilized to generate the figure panel.

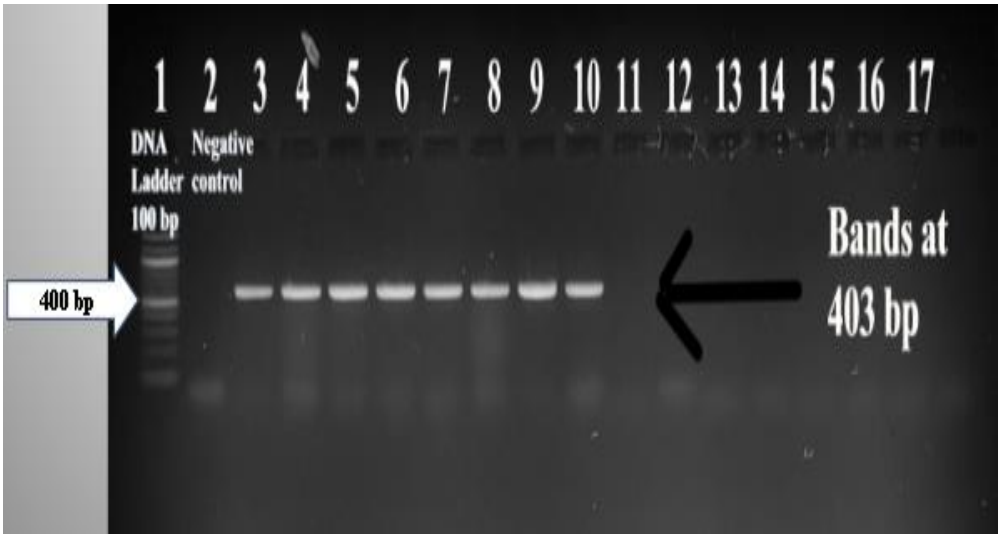

Figure 2(c): Agarose gel electrophoresis of PCR assay of *Salmonella typhi* isolates. Here, first lane is 100 bp DNA ladder, 2nd lane is negative control and lane no. 3-10 are positive samples at 403bp. The image was captured using a camera while the agarose gel was positioned under a UV transilluminator. The complete original image was utilized to generate the figure panel.

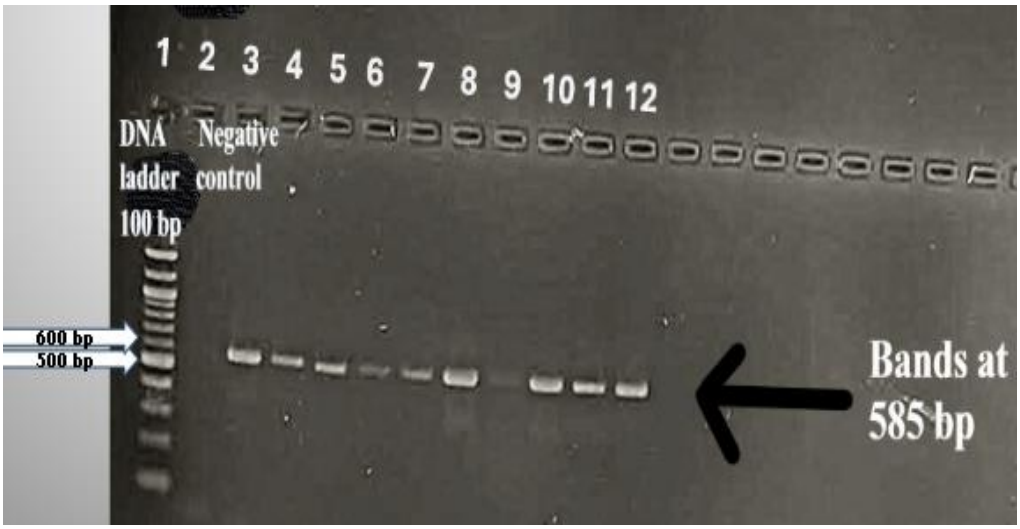

Figure 2(d): Agarose gel electrophoresis of PCR assay of *Escherichia coli* isolates. Here, first lane is a 100 bp DNA ladder, the second lane is negative control and well 3-12 are positive samples at 585 bp. The image was captured using a camera while the agarose gel was positioned under a UV transilluminator. The complete original image was utilized to generate the figure panel.

2.8 Antibiotic susceptibility testing

The Antibiotic Susceptibility Test (AST) was performed using the Kirby-Bauer disk diffusion method to determine the effectiveness of various antibiotics against the isolated bacteria. Each bacterial isolate from the culture plates was sub-cultured on freshly prepared nutrient agar to obtain a fresh and pure culture before performing AST. Mueller-Hinton Agar (MHA) media was freshly prepared for antibiotic susceptibility testing. A 0.9% sodium chloride (NaCl) solution was also prepared by dissolving the appropriate amount of NaCl in distilled water and briefly heating it. Using a sterile glass pipette, 5–6 mL of the NaCl solution was dispensed into each test tube. A bacterial suspension was prepared by picking colonies from a pure culture and adjusting the turbidity to match the 0.5 McFarland standard. The suspension was then evenly spread over the surface of a Mueller-Hinton agar plate using a sterile cotton swab to ensure a uniform lawn of bacterial growth. After allowing the plate to dry for a few minutes, antibiotic discs were placed on the agar surface using sterile forceps. The plates were incubated at 35–37°C for 16–18 hours (Jan Hudzicki, 2009).

Zone Of inhibition:

Following 24 hours of incubation, the Mueller-Hinton Agar (MHA) plates were examined for bacterial growth surrounding the antibiotic discs. If an antibiotic effectively inhibits the test organism, a clear area devoid of bacterial growth—known as the zone of inhibition—will be observed around the disc. In contrast, if the antibiotic is less effective or ineffective, bacterial growth will extend closer to the disc, resulting in a smaller or no visible inhibition zone. The diameter of each zone of inhibition is measured using a ruler or scale to accurately assess the degree of bacterial growth suppression. These measurements are then interpreted using standardized reference charts provided by Oxoid Limited (England), which classify the bacteria as sensitive, intermediate, or resistant to the antibiotics tested. This interpretation method aligns with the guidelines recommended by the National Antimicrobial Resistance Monitoring System and the Clinical and Laboratory Standards Institute (CLSI, 1997, 1999). **Table 4**: shows the concentration and interpretive criteria for diffusion zones of the antibiotics.

Table 4: Concentrations and interpretive criteria for diffusion zones of the antibiotics.

Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretative Criteria		
					Sensitive mm or more	Intermediate mm	Resistant mm or less
Azithromycin	Macrolide	Gram-positive and Gram-negative	AZM	15	18	14–17	13
Amikacin	Aminoglycoside	Gram-positive and Gram-negative	AK	30	17	15–16	14
Colistin	Polymyxin E	Gram-negative	CL	10	-	11-17	-
Cefepime	Cephalosporin	Gram-positive and Gram-negative	CPM	30	25	19–24	18
Meropenem	Carbapenem	Gram-positive and Gram-negative	MEM	10	23	20–22	19
Imipenem	Carbapenem	Gram-positive and Gram-negative	IMI	10	23	20–22	19
Nalidixic Acid	Quinolone	Gram-negative	NA	30	19	14–18	13
Streptomycin	Aminoglycoside	Gram-positive and Gram-negative	S	10	15	12-14	11
Ampicillin	Beta-lactamase	Gram-positive and Gram-negative	AMP	10	17	14–16	13
Amoxicillin	Beta-lactam (Penicillin)	Gram-positive and Gram-negative	AML	10 µg	17	14-16	13
Vancomycin	Glycopeptide	Gram-positive	VA	30	17	15–16	14
Linezolid	Oxazolidinones	Gram-positive	LZ	30	23	21–22	20
Tigecycline	Glycylcycline	Gram-positive and Gram-negative	TGC	15	18	15-17	15
Tetracycline	Tetracycline class	Broad-spectrum (Gram-positive and Gram-negative)	TE	30	15	12-14	11
Ciprofloxacin	Fluoroquinolone	Broad-spectrum Gram-negative	CIP	5	21	16-20	15
Chloramphenicol	Amphenicol	Broad-spectrum (Gram-positive and Gram-negative)	C	30	18	13-17	12
Cefixime	3rd Gen Cephalosporin	Gram-negative	CFM	5	19	16-18	15
Ceftazidime	3rd Gen Cephalosporin	Gram-negative	CAZ	30	18	15-17	14
Erythromycin	Macrolide	Mainly Gram-positive, some Gram-negative cocci	E	15	23	14-22	13
Gentamicin	Aminoglycoside	Broad-spectrum; effective mainly against Gram-negative and some Gram-positive bacteria	CN	10	15	13-14	12
Trimethoprim-Sulfamethoxazole	Sulfonamide combination	Broad-spectrum; many Gram-positive and Gram-negative bacteria	SXT	25	16	11-15	10

CHAPTER 3

Result

3.1 Demographic characteristics of patients

This study included a total of 80 participants, individuals sourced from Islami Bank Hospital from Mirpur, Motijheel, Kakrail, Naya Paltan branches of Dhaka. Among these participants, 15(18.75%) from Islamic Bank Hospital Motijheel branch, while 40 (50%) were from Mirpur branch & the remaining 15(18.75%) & 10(12.5%) patients from Naya Paltan branch & Kakrail branch. The majority of the study's subjects fell within the age range of 40 to 80 years old. Moreover, male participants take more account for this study with a percentage of 56.25% & remaining 43.75% is female participants.Among the patients, most of them acquired infection through community & after that they got admitted to the hospitals. A summary of the demographic characteristics of the study's patients is presented in **Table 5**.

Table 5: Demographic characteristics of patients

Characteristics	Categories	Frequency	Percentage
Number of participants at this study	Islamic Bank Hospital, Mirpur	40	50%
	Islamic Bank Hospital, Motijheel	15	18.75%
	Islamic Bank Hospital, Kakrail	10	12.5%
	Islamic Bank Hospital, Nayapaltan	15	18.75%
Gender	Male	45	56.25%
	Female	35	43.75%
Age(in years)	0-19	10	12.5%
	20-39	15	18.75%
	40-59	20	25%
	60+	35	43.75%
Patient type	In patient	25	31.25%
	Outpatient	55	68.75%

3.2 Prevalence of positive isolates in septicemia patients

Among 80 samples, bacterial growth was observed in 66 samples. The distribution of the bacteria in these culture-positive samples are shown in **Figure 3**. The data indicates that *Salmonella typhi* is the most prevalent pathogen, responsible for 65.2% of the positive isolates, with 43 out of 80 samples yielding positive results. *Klebsiella pneumoniae* follows with 9 positive isolates, representing 13.6% of the total, while *E. coli* is identified in 8 isolates, accounting for 12.1%. *Staphylococcus aureus* appears in 6 isolates, making up 9.1% of the cases. All infections were due to a single organism. These findings reflect the distribution of bacterial pathogens in septicemia cases and emphasize the dominance of *Salmonella typhi* in the observed patient population.

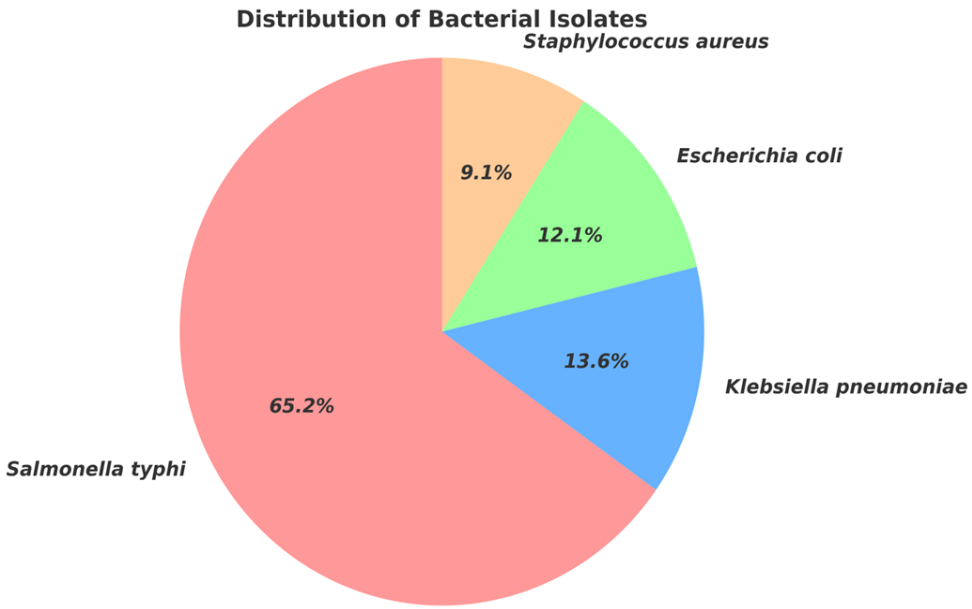

Figure 3: Prevalence of isolated bacteria in 66 culture-positive samples of suspected septicemia patients. This pie chart shows the distribution of isolated pathogens identified in septicemia patients. *Salmonella typhi* at 65.2% of the total isolates, followed by *Klebsiella pneumoniae* at 13.6%, *Escherichia coli* at 12.1%, and *Staphylococcus aureus* at 9.1%.

3.3 Antibiotic resistance & sensitive profile of the positive isolated organisms

In this study, a total of 80 isolates have been selected to identify the antibiotic resistance of different organisms. The Kirby-Bauer Disc Diffusion test was performed on each isolate. A total of 8 isolates of *Escherichia coli*, 43 isolates of *Salmonella typhi*, 9 isolates of *Klebsiella pneumoniae*, and 6 isolates of *Staphylococcus aureus* were examined for antibiotic susceptibility. **Figure 4(a), 4(b), 4(c), 4(d)** demonstrates all antibiotic susceptibility profiles for specific bacteria. **Figure 5** shows the MDR, XDR & susceptible of all the isolates. In order to get the best possible treatment results, these findings highlight the significance of concentrating on highly efficient antibiotics while avoiding those with high resistance.

Antibiotic Susceptibility Analysis of *Salmonella typhi*:

Out of the all-positive *Salmonella typhi isolates* showed relatively high antibiotic susceptibility, with the exception of Nalidixic acid (89%) & Ampicillin (79%), which demonstrated substantial resistance. The efficiency of these 12 antibiotics against the isolates is displayed in the graph **Figure 4(a)** below. From the graph, it is evident that Chloramphenicol, Ceftazidime, Gentamicin, Ceftriaxone and Meropenem showed high susceptibility, with 90% to 100% of the isolates being susceptible.

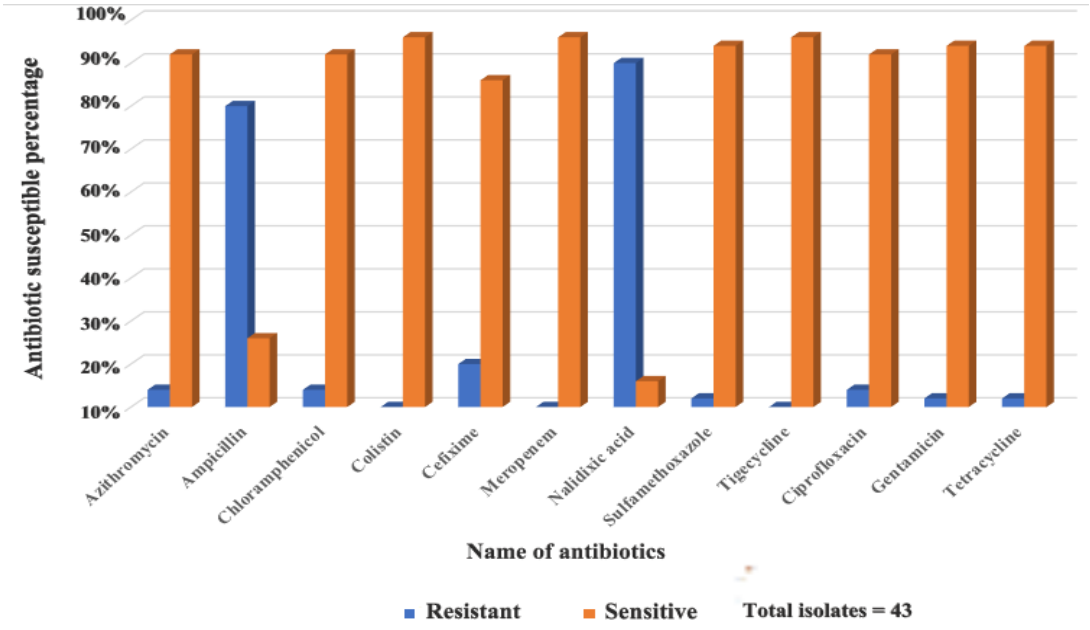

Figure 4(a): Antibiotic Susceptibility Profiling of *Salmonella typhi*. Total isolate number was 43.

Antibiotic Susceptibility Analysis of *Klebsiella Pneumoniae:*

Most of the positive *Klebsiella pneumoniae* isolates showed in the relatively high antibiotic susceptibility, with the exception of Amoxicillin (68%), Cefixime (79%) which demonstrated substantial resistance & Erythromycin (39%) comparatively lower resistance. The efficiency of these 12 antibiotics against the isolates is displayed in Figure **4(b)** below. From the graph, it is evident that Ciprofloxacin, Azithromycin, Gentamicin, Tetracycline, Tigecycline and Meropenem showed high susceptibility, with 75% to 98% of the isolates being susceptible.

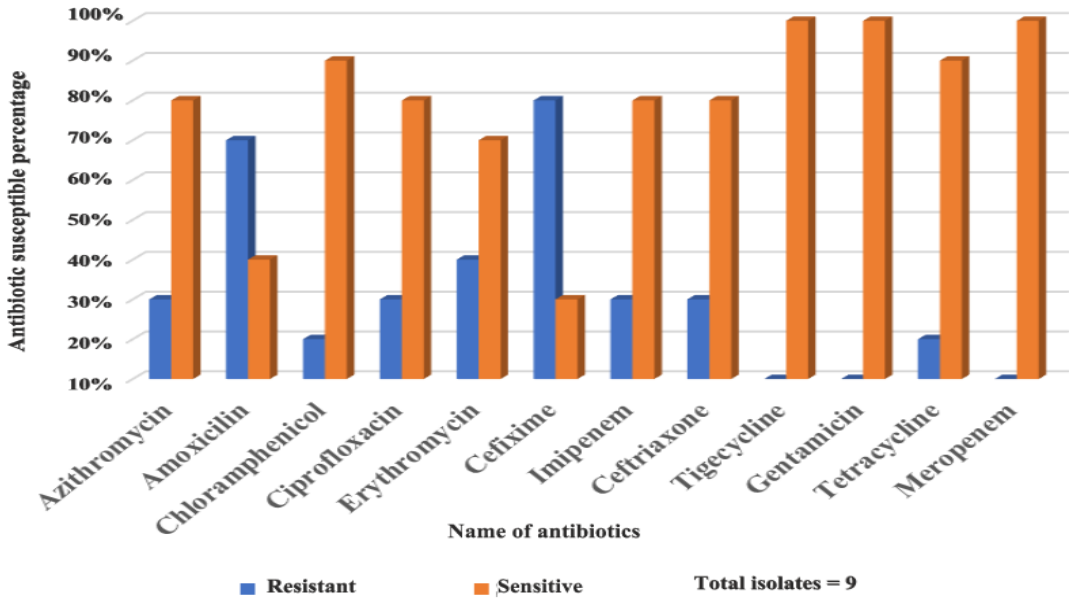

Figure 4(b): Antibiotic Susceptibility *Klebsiella pneumoniae.* The total number of isolates was 9

Antibiotic Susceptibility Analysis of *Escherichia coli:*

Out of the all-positive *Escherichia coli* isolates showed relatively high antibiotic susceptibility, with the exception of Amoxicillin (80%) and Ampicillin (99%), which demonstrated substantial resistance. Cefixime (58%), Ceftriaxone (28%) and Tetracycline (38%) resistance of positive isolates. The efficiency of these 12 antibiotics against the isolates is displayed in the graph **Figure 4(c)** below. From the graph it is evident that Azithromycin, Chloramphenicol, Ciprofloxacin, Imipenem, Colistin, Tigecycline, Gentamicin showed high susceptibility, with 100% of the isolates being susceptible.

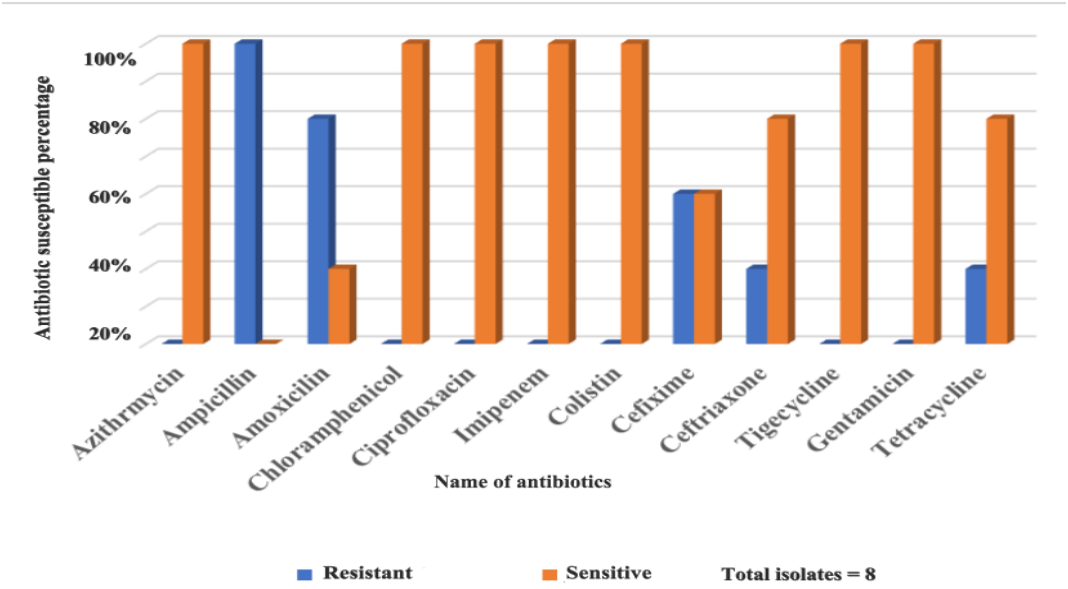

Figure 4(c): Antibiotic Susceptibility Profiling of *Escherichia coli*. The total number of isolates was 8.

Antibiotic Susceptibility Analysis of *Staphylococcus aureus*:

Out of all positive *Staphylococcus aureus isolates* showed relatively high antibiotic susceptibility, with the exception of Cefixime, Sulfamethoxazole and Ceftazidime showed 49% of resistance. Then Erythromycin and Ceftriaxone showed 28% resistance among positive isolates. Imipenem and Gentamicin showed 19% of resistance which is comparatively lower. The efficiency of these 12 antibiotics against the isolates is displayed in the graph **Figure 4(d)** below. From the graph it is evident that Azithromycin, Ciprofloxacin, Tigecycline, Vancomycin, Tetracycline and Imipenem showed high susceptibility, with 85% to 100% of the Positive isolates

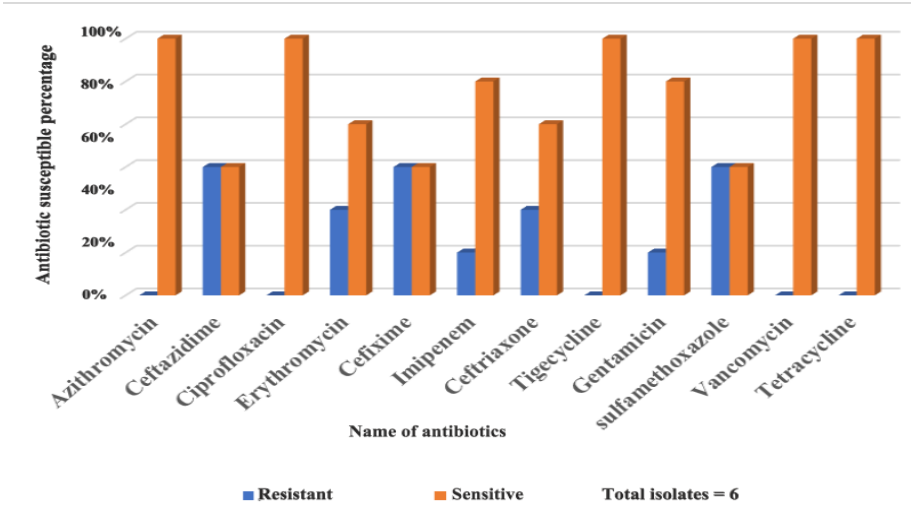

Figure 4(d): The Graph showing the resistance and sensitivity to various antibiotics of *Staphylococcus aureus.* The total number of isolates was 6

3.4: Multi-drug resistant bacteria

Among the all-positive isolates of *Salmonella typhi* shows 16.67% for MDR & 2.32% for XDR. MDR in *klebsiella pneumoniae* isolates showed 66.67% with 11% XDR. *Escherichia coli* has shown MDR in 25% but no XDR. Lastly, gram positive bacteria *Staphylococcus aureus* showed MDR in 50% & 16.67% XDR. The graph **(Figure 5)** shown MDR and XDR pattern below,

Organism	MDR	XDR
Salmonella typhi(N=43)	7 (16.67%)	1(2.32%)
Escherichia coli(N=8)	2 (25%)	0
Klebsiella pneumoniae(N=9)	6 (66.67%)	1(11%)
Staphylococcus aureus(N=6)	3 (50%)	1 (16.67%)

Figure 5: The table showing MDR and XDR pattern

CHAPTER 4

Discussion

This study aimed to assess the bacteriological profile and antibiotic resistance patterns of septicemia pathogens among diagnosed patients at the four branches of Islamic Bank Hospital in Dhaka city, Bangladesh. The study's findings revealed that among a total of 80 samples, *Salmonella typhi* was the most prevalent pathogen, accounting for 65.2% of the isolates, followed by *Klebsiella pneumoniae* (13.6%), *Escherichia coli* (12.1%), and *Staphylococcus aureus* (9.1%). This study aligns with the international and local patterns of *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus* being the predominant pathogens in bloodstream infections (BSIs). The prevalence of *Salmonella typhi* in this study (65.2%) is consistent with findings from Kathmandu, Nepal, where *Salmonella typhi* accounted for 71% of blood culture isolates (Simkhada, 2016). The high prevalence of *Salmonella typhi* in both studies could be attributed to the common occurrence of typhoid fever in countries with poor sanitation and water contamination issues. Typhoid fever, which has not yet been eradicated, continues to cause septicemia (Simkhada, 2016); therefore, it is not surprising that in this study *Salmonella typhi* was the leading pathogen with 65.2% of the isolates. Dagnew et al. (2013) reported similar results in Ethiopia and Nepal, where *Salmonella typhi* dominated the septicemia pathogen.

The prevalence of *klebsiella pneumoniae* in our study (13.6%) is much lower compared to the reported for Sub-Saharan Africa (54.4%). *Escherichia Coli* in your study (12.1%) is slightly lower than the study reported in Sub-Saharan Africa (18.4%) (Lester et al., 2019)**.** According to our analysis, the prevalence of *Staphylococcus aureus* was strikingly low at 9.1%, unlike other studies conducted in Bangladesh which noted a higher prevalence where *Staphylococcus aureus* was more often associated with septicemia. More specifically, this is one of the comparisons that can be made regarding the use of bacterial infections in the current study and *Staphylococcus aureus* for past investigations conducted in the same part of the world. In contrast to the studies conducted in Mymensingh, Bangladesh, which reported a prevalence of approximately 20%, the current study showed a lower prevalence of 9.1%. This is likely due to differences in hospital-based settings or the specific antibiotic resistance trends governing the prevalence of *Staphylococcus aureus*

infections in the community. Various reasons explain this, including differences in the patient populations, healthcare settings, or methodologies employed in the studies (Khan et al., 2024).

The study analysis showed that septicemia was primarily in the elderly (60 years and above) which contributed to 43.75% of the cases followed by the 40-59 age group with 25%. This aligns with other global reviewed article where in older adults (≥65) constitute **58–65%** of sepsis cases (Starr & Saito, 2014). Due to septicemia being more common amongst older adults because of the immunosenescence- a weakening immune system due to aging—associated with heightened vulnerability to infections (Opal et al., 2005). Therefore, the geriatric population continues to be at risk to certain community infections notably caused by *Salmonella typhi, Escherichia coli,* and *Klebsiella pneumoniae* which could lead to potentially devastating complications if they are not treated promptly. On the basis of gender, the males in this study outnumbered the females, 56.25% and 43.75% respectively. Male predominance in septicemia cases is consistently reported globally, In Wuhan, study found 61% male, 39% female among 2,111 sepsis patients (Zhao et al., 2025). This disparity also aligns with other studies which demonstrate that men are at higher risk of septicemia because of the differences in immune responses (Pradipta et al., 2013). It is known that women's immune response is enhanced due to the presence of estrogens, thus giving women better defenses against infections, particularly in community settings.

This study highlights the increasing trend of antimicrobial resistance in Bangladesh. Antimicrobial resistance (AMR) remains a critical global health challenge, with developing countries like Bangladesh facing particularly high rates due to factors such as unregulated antibiotic use, and inadequate healthcare infrastructure. In this study, *Salmonella typhi* (65.2%), *Klebsiella pneumoniae* (13.6%), and *Escherichia coli* (12.1%) were the most prevalent pathogens in septicemia patients, reflecting similar patterns observed in other countries. For instance, in Nepal, *Salmonella typhi* was responsible for 71% of bloodstream infections (Simkhada, 2016), while in Ethiopia, *Klebsiella pneumoniae* and *Escherichia coli* were also leading causes of sepsis, with resistance rates of 68% to amoxicillin and 79% to cefixime (Dagnew et al., 2013). In our studies, *Salmonella typhi* showed resistance to nalidixic acid (89%) and ampicillin (79%). Resistance to commonly used antibiotics like ampicillin, azithromycin, and nalidixic acid in this study mirrors trends in India, where *Klebsiella pneumoniae* and *Escherichia coli* show similar resistance patterns to beta-lactams and fluoroquinolones (Khan et al., 2022). However, the study found relatively low

prevalence of *Staphylococcus aureus* (9.1%), which contrasts with other studies in Bangladesh and globally, where *Staphylococcus aureus* typically accounts for a larger portion of septicemia cases (Khan et al., 2024). Additionally, colistin resistance observed in the study aligns with rising concerns in countries like China and India, where colistin resistance is becoming an emerging public health threat, especially in multidrug-resistant Gram-negative infections (Zhu et al., 2021). In contrast, in this study colistin is a more effective antibiotic against *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus.* While resistance rates in high-income countries like the U.S. are comparatively lower, the increasing global spread of MDR and XDR pathogens highlights the urgency of implementing robust infection control, better surveillance systems, and effective antibiotic stewardship programs worldwide (Smith et al., 2023). Multidrug-resistant (MDR) pathogens are a growing global concern, as demonstrated by the high prevalence of MDR *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus* in this study. Isolates of 16.67% *Salmonella typhi* were identified as MDR, with 2.32% classified as extensive drug-resistant (XDR). Additionally, *Klebsiella pneumoniae* isolates were shown 66.67% of MDR, with 11% were XDR, while *Escherichia coli* isolates were shown 25% of MDR, and *Staphylococcus aureus* isolates were shown 50% of MDR, with 16.67% of XDR. Similar findings have been reported in other countries, such as India, where 50% of *Salmonella typhi* isolates were MDR (Khan et al., 2022), and Ethiopia, where 40% of *Klebsiella pneumoniae* isolates were resistant to multiple drugs (Dagnew et al., 2013). In contrast, the prevalence of MDR strains in developed countries like the U.S. is relatively lower, with Klebsiella pneumoniae MDR rates reported around 15-20% (Smith et al., 2023). MDR rate reported in a study from south India, where 24.6% of *Escherichia coli* isolates from bloodstream infections showed multidrug resistance (Babu et al., 2018). The global rise in MDR and extensive drug-resistant (XDR) pathogens highlights the need for improved infection control, antibiotic stewardship, and timely diagnostics to manage AMR effectively in both high-income and low-income countries (Bayot & Bragg, 2024).

CHAPTER 5

Conclusion

Our studies have shown that most of the pathogens are already resistant to antibiotics like Amikacin, Gentamicin, Imipenem, Tigecycline, Linezolid and Vancomycin. Age, gender and immunity might also play a role in the frequency of the onset of sepsis and the effectiveness of antibiotics. In our study, most of the sepsis patients are more than 60 years old (43%) and male(56.25%). The thickness of the peptidoglycan layer on the surface of bacteria may have an impact on their pathogenicity. Our study showed that 77% of the patients were infected by gram negative bacteria, especially *Salmonella typhi*. Nevertheless, data on antibiotic susceptibility is not universal and permanent, because antibiotic susceptibility or resistance varies from strain to strain, and the results will differ with variations in location or continent. In addition, the antibiotics that are currently effective on a pathogen, may not retain the same efficacy after a few years. Some of them may even gain multidrug resistance. This happens because antibiotics pose a selective pressure on microbes, or which they have to survive by evolving with newer antibiotic resistance genes, which spread rapidly within the microbial kingdom (through horizontal and vertical gene transfer). Nevertheless, we need to continue a thorough longitudinal study with larger sample sizes (annually if possible), to validate the findings of the research and get more insights.

CHAPTER 6

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