Isolation and Identification of Respiratory Pathogen from Sputum Sample Assessment Antibiotics Resistance: Single Centered Cross Sectional Study

By

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

"Isolation and Identification of Respiratory Pathogen from Sputum Sample Assessment Antibiotics Resistance: Single Centered Cross Sectional Study" submitted by
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Ethics Statement

To complete this study, the data is authentic and collected from Microbiology Department of National Institute of Diseases of the Chest and Hospital (NIDCH). The data collected from June,2024 to August, 2024. Most importantly, confidentiality and anonymity of the patient's identity were strictly maintained, with data stocked safely and accessible only to authorized personnel. To ensure participant privacy, there are no identifying data was added in this study report.

Abstract

Respiratory tract infections are general health threats that are magnified by increased cases of antibiotic resistance. The purpose of this study was to isolate respiratory pathogens from sputum samples to determine their resistance profile in a single center cross sectional study. In all, 909 samples (indoor and outdoor patients) were analyzed by standard microbiological techniques and the AST was performed. Among 909 samples,413 growth were found where 195(47.32%) were *Klebsiella pneumonia* and 183(44.31%) were *Pseudomonas aeruginosa*. These were the two most common causative organisms in the study. Furthermore, we also found about 7.58% of cases (31) to be *Streptococcus*, 0.24% of cases (2) to be *Escherichia coli* , 0.24% of cases(1) to be *Staphylococcus* and the same for antibacterial. A thorough analysis was performed on data collected from June of 2024 to August of the same year. The present study emphasizes the importance of periodic screening of resistance profiles ensuring that health professionals adhere to appropriate antibiotics prescription to curb the resistant strains. For example, *Klebsiella* Sensitivity of amikacin was 71.79% but its resistance of piperacillin was 29.23%. Similarly, *Pseudomonas* resistance trend for Piperacillin was also similar to *Klebsiella* which was 29.23% and for Meropenem the resistance showed 13.11% which was very alarming. Again, Pseudomonas showed strong effectiveness for amikacin which was around 71.79% sensitive. Thus, according to our findings, the most effective antibiotics are Amikacin, Gentamicin and Meropenem which showed high efficacy for our researched organisms. In contrast, the least effective antibiotics are Clindamycin, Cefixime, Ceftriaxone and Azithromycin which faced widespread resistance, inhibiting their utility in severe infections. This work adds important baseline information to aid health professionals in clinical management and infection prevention planning.

Keywords: Respiratory tract infections, standard microbiological techniques, Antibiotics sensitivity test (AST).

Dedication

This thesis is in honor of my lovely family and friends for their support, encouragement and love throughout the course of this process. To my friends and family thank you for your support throughout as I have followed my dream.

This work is also dedicated to all my mentors/teachers, without their directions and effective knowledge, this work would not be possible.

Finally, for all the patients and health care providers who cope with the difficulties of the infectious diseases daily, this research should serve as a part of the advancements and solutes.

Acknowledgment

First and foremost, I would like to thank Almighty Allah for providing the chance to complete this research work successfully as well as submit this paper.

I would like to extend my heartiest and deepest thanks to the Chairperson of the Department of Mathematics and Natural Sciences, Brac University **Md. Firoze H. Haque, PHD**, Associate Professor **Dr. Munina Haque** for permitting me and encouraging me to finish this thesis work without any hassle.

I would also like to thank my academic supervisor Associate Professor **Dr. Fahim Kabir Monjurul Haque** for his encouragement, help and useful guidance in the process of my research. I want to acknowledge their expertise and engagement which made the completion of this study a great success.

I also thank my professors and teachers who have informed and guided myself and who have influenced my further evolution academically. I am thankful to the technical staff and other researchers who have helped at my times of need and gave me moral support.

Last but not least, I express my appreciation to the academic society to which belongs all the credit for the researches carried out in this field. This thesis is a reflection of all the knowledge and contributions within the field.

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List of Acronyms

Abbreviation	**Elaboration**
CS	Culture Sensitivity
AMR	Antimicrobial Resistance
MDR	Multi-Drug Resistant
XDR	Extensively drug resistant
ABR	Antibiotic Resistance
WHO	World Health Organization
P. aeruginosa	*Pseudomonas aeruginosa*
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
AST	Antibiotic Susceptibility Test
FMT	Fecal Microbial transplantation
PCR	Polymerase Chain reaction

Chapter 1

Introduction

1.1 Background

Respiratory tract infections (RTIs) is a major global health problem, particularly in vulnerable populations, including children, the elderly and persons of compromised immune system. These infections (mostly due to bacterial pathogens such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Streptococcus pneumonia*) are associated with severe disease and increased morbidity and mortality (Rodriguez-Morales et al., 2020; SMART Program, 2016–17). The added difficulty of dealing with RTIs has been the increased prevalence of antibiotic resistant pathogens. Contributing to the growing prevalence of resistance is the misuse and overuse of antibiotics and for questions regarding treatment options due to limited localized data of resistance patterns, in addition to a rising healthcare costs (SMART Program, 2016–2017; Tropical Medicine and Infectious Disease, 2024). In addition to not being effective in treating the illness, antibiotic resistance also means more expensive and complicated health treatments are required. To address these challenges this study aims to isolate and identify respiratory pathogens for sputum samples in a single-centered cross sectional study. It also aims to evaluate their patterns of antibiotic resistance, an important piece of data to shape empirical treatment strategies and to improve antibiotic stewardship. These insights are essential for the tailoring of local infection control strategies and stalling straining spread .
(Sah & Rodriguez-Morales, 2024)

1.2 Challenges of treating antibiotics resistance bacteria

A significant global public health problem is antibiotic resistance. The reasons of the problem are living in hospital, nursing home, long-term care facility environments due to their proximity to patients, chronic disease and exposure to heavy doses of antibiotics. Pathogen such as *Staphylococcus aureus* (MRSA- A type of *Staphylococcus aureus* bacteria that has become resistant to methicillin and other common antibiotics such as penicillin and amoxicillin, known as Methicillin-Resistant *Staphylococcus aureus*), *Escherichia coli* and *Klebsiella pneumonia* are becoming more resistant to common antibiotics with infections becoming harder to treat (Nicolle et al. 1996). The nursing homes have high resistance rates to antibiotics such as gentamicin and fluoroquinolones, according to studies and negative bacteria are common in such homes. Risk factors include invasive devices such as urinary catheters; MRSA infections also have risen. *Serratia marcescens* (a gram negative species of bacteria in the family Enterobacteriaceae.) is of special worry because of its resistance to most antibiotics, with very few treatment options available (Hong et al., 2013). Resistance is spreading because antibiotics are being overused too much in healthcare or in agriculture. When resistant bacteria persist in the environment they can conceivably spread by contaminated food and direct contact. As per Li et al. (2024), antibiotic resistance somehow could lead to up to 10 million deaths annually by 2050, if not checked. The only way to reduce resistance and improve patient outcome is to tackle it together with global cooperation and better antibiotic stewardship.

1.3 Antibiotics

Origin

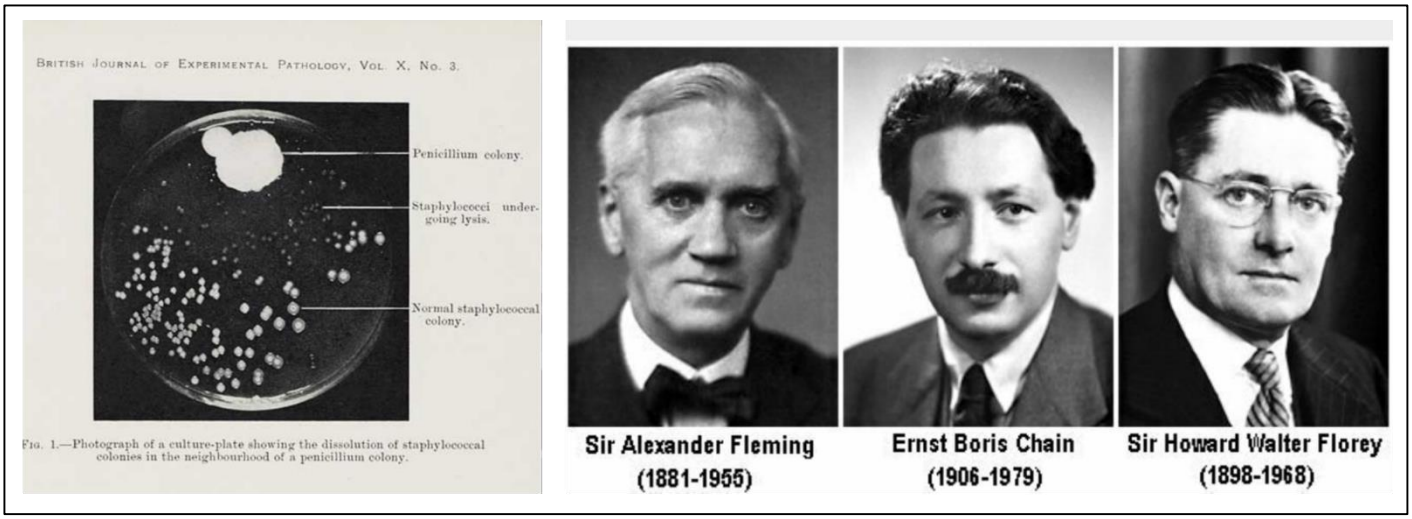

Figure 01: Original report of penicillin antimicrobial activity in the British Journal of Pathology 1928. The discovers and developers of penicillin.

Alexander Fleming discovered penicillin in 1928, which started with the history of antibiotics. The first antibiotic capable of fighting bacterial diseases the like pneumonia and sepsis (Nature, 2017) was Penicillin. This discovery amounted to a major breakthrough in the medical world and the other classes of antibiotics—sulfonamides, tetracyclines—allowed us to treat some other infections. (Dutta, 2022). In the mid 20th century, antibiotics became relatively widely available (especially from World War II on) and we entered the 'Golden days of Antibiotics', the deaths from bacterial infection dramatically dropped, until now. Such new antibiotics as streptomycin and cephalosporins helped fight against diseases causes by tuberculosis, bacterial infections (Dutta, 2022).But in time, however, the overuse of antibiotics bred out bacteria that were resistant to these drugs. This problem began in the 1940's with bacteria like ***Staphylococcus aureus*** becoming resistant to penicillin. Further antibiotics being used (News-Medical, 2023). In fact, antibiotic resistance is now a significant global health threat upon

which there is no available silver bullet. But antibiotics have saved millions of lives and their overuse, in healthcare and in agriculture, has spawned resistant bacteria. In response to all of this, researchers are searching for new antibiotics and different treatments, like bacteriophage therapy or antimicrobial peptides, as well as promoting the judicious use of current ones (News-Medical, 2023). Antibiotics: a story of how they have saved lives while also demonstrating just how important it is to use antibiotics responsibly if we are to keep them safe for future generations.

Usual Classification of Antibiotics

They usually fall into their own antibiotic classes. Antibiotic class is a group of drugs with similar chemical and pharmacologic property. Differences in their chemical structures may not be visible and the two may have similar drugs killing the same or closely related bacteria. Below are the most commonly used classes:

- **Penicillin**

The targeting for penicillin is peptidoglycan, a major component of the bacterial cell wall and one that is needed for cell wall formation. The germs were able to rupture the weakened wall. Additionally, they are good for treating diseases like pneumonia and strep throat (AG Scientific, 2024; Anderson, 2023)

- **Cephalosporin.**

Like **penicillin**, the cephalosporin prevents formation of the bacterial cell wall. They are divided into five generations, with the newer generations such as ceftaroline are effective against MRSA. Meningitis, urinary tract infections, pneumonia. Example: Cephalexin and Ceftriaxone. (Prestinaci,2015)

: ● **Macrolides**

They limit bacterial protein synthesis by binding to the 50S ribosomal subunit and protein biosynthesis. Skin infections, respiratory tract infections and chlamydia are treated by them. For example, azithromycin and erythromycin. (Dutta, 2022).

• **Fluoroquinolones.**

This antibiotic suppresses the bacterial DNA gyrase and topoisomerase IV. Also, two enzymes that are used in DNA replication. These are used to treat serious infections, bacterial pneumonia and urinary tract infections. For example, Ciprofloxacin and Levofloxacin (Prestinaci,2015).

• **Tetracyclines**

They suppress bacterial protein synthesis by binding to the 30S ribosomal subunit and preventing tRNA from binding. They cure acne, Alzheimer's, Lyme and malaria. For example, Doxycycline and Minocycline . (Dutta, 2022).

• **Aminoglycosides**

Aminoglycosides attach to the 30S ribosomal subunit, permanently and produce errors in mRNA translation resulting in error in the produced protein. An antibiotic used to treat severe gram negative bacterial infections, such as sepsis. For example, Gentamicin , Streptomycin (Prestinaci,2015).

• **Sulfonamides**

Dihydropteroate synthase, the enzyme needed to make folic acid, is blocked by sulfonamides. It prevents bacterial development, Bronchitis, urinary tract infections and malaria. For instance, Sulfamethoxazole and Sulfadiazine.

• Carbapenems.

Carbapenems suppresses the bacterial cell wall building by executing against penicillin-binding proteins. They treat infectious diseases resistant to multiple drugs like sepsis and pneumonia. For example, Imipenem and Meropenem (Prestinaci,2015)

Alternative Therapeutic Solutions

Antibiotic resistance is growing to the point of a serious threat to the global public health more. Infections are coming to be difficult, or even impossible to treat with conventional antibiotics. To address this challenge, alternative strategies are being developed to address the challenge and alternative strategies are focusing on innovative and non-traditional treatments. This includes approaches that try and either directly attack resistant bacteria or change the environment in which pathogens resist. Innovative approaches to combating resistant pathogens have been identified as antimicrobial peptides (AMPs), nanoparticles, essential oils, antisense antimicrobial therapeutics and fecal microbiota transplantation (FMT). Natural or synthetic AMPs, using high speed and broad spectrum efficacy, can disrupt bacterial membranes or vital processes, but their clinical potential is restricted by costly production and toxicity. Antimicrobial agents could be delivered using precision, efficiently targeting infection sites and rendering more difficult to develop resistance, with toxic and environmental impacts muted by multifactorial mechanisms using nanoparticles (e.g., silver or gold). Although standardization and toxicity issues must be addressed, broad spectrum activity for essential oils, such as thyme and eugenol and synergism with antibiotics are seen. Antisense

antimicrobial therapeutics consist of nucleic acid molecules which inhibit expression of specific bacterial genes, with the potential of high specificity, low resistance, but need of further development in the delivery methods to provide stability in a biological environment. Transplantation of healthy donor microbiota (FMT) restores gut microbiota balance in the face of challenging success in recurrent Clostridioides difficile infections and evidence for possible application in inflammatory and metabolic disorders, but also exposes donor safety, procedural standardization and infection risk. The potential of each approach lies in themselves, but practically and clinically, they need to be further optimized to overcome limitations.

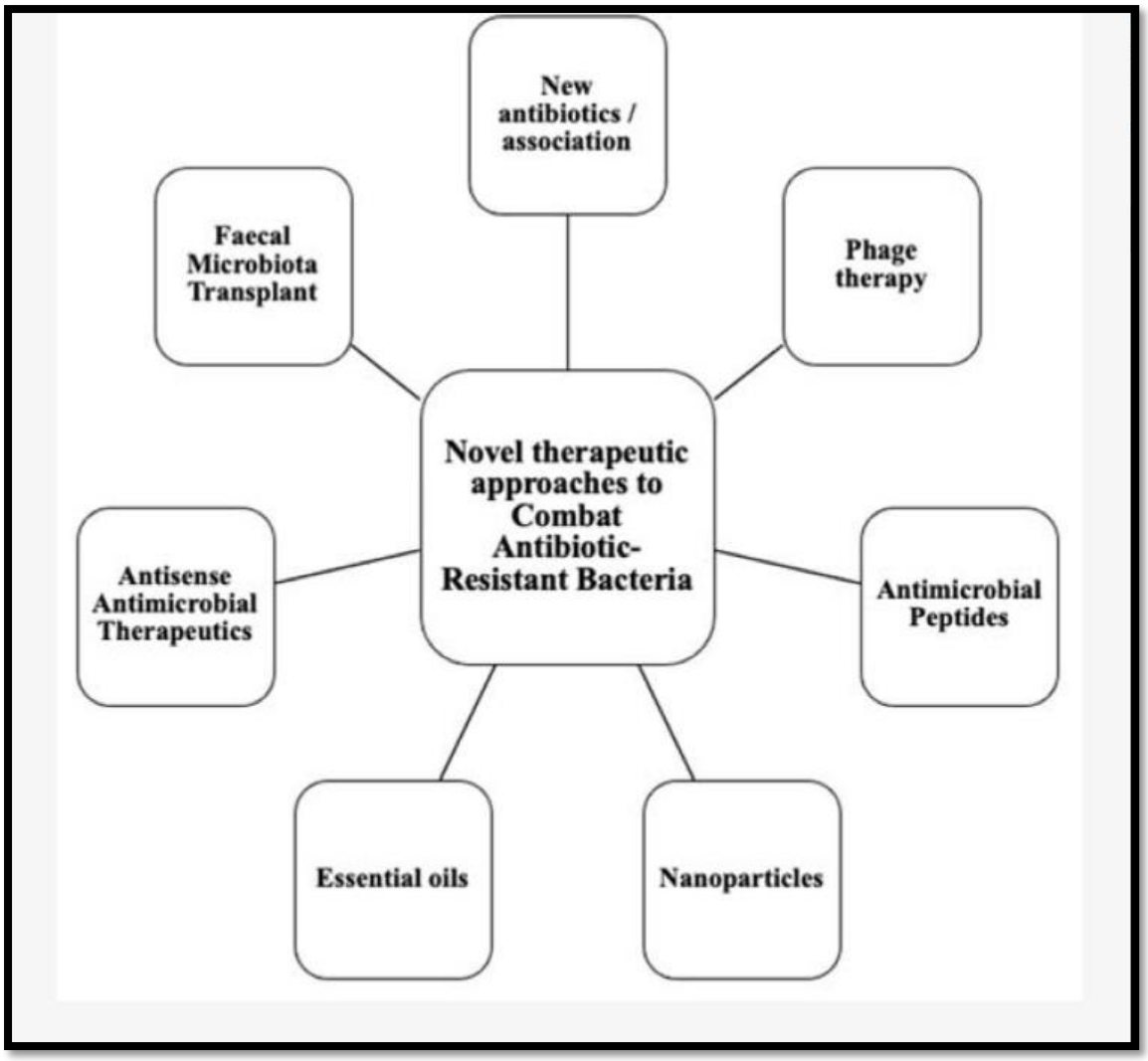

Figure 02: Alternatives therapeutic approaches to conventional antibiotics. MDPI, 2024

1.4 Literature Review

The medical fraternity has been a challenge against antibiotic resistance, it has had a big impact on disease management. This development of antibiotic resistance is due to the over use of antibiotics and also due to doctor's irrational prescription of antibiotics to the masses. In the first year of the covid 19 pandemic, almost 29,400 deaths were attributed to healthcare associated illnesses. Antibiotic resistance has also worsened during the pandemic: 71 percent of hospitalized COVID-19 patients in China received antibiotics. The research also demonstrates the dangers of antibiotic resistance when it comes to treating the disease: Children treated with antibiotics for COVID-19 were more likely to suffer fatal outcomes. (Nadgir,2023).

Bacteria are becoming resistant to antimicrobials worldwide and this has led to consequences of infections either able or impossible to treat with antibiotic therapies, behavior that is designated as an; antimicrobial resistance. This problem is fueled by the overuse of broad spectrum antibiotics without identifying which bacteria. Resistant bacteria are spread due to poor infection control practice. Such updated data provides essential information for efficient treatment decisions as well as for developing antibiotic stewardship programs in hospitals(Thanh Quang Nguyen,2024).Antibiotic resistance is being caused by the misuse of antibiotics in hospitals and on farms. So, the control of it includes better antibiotic use policies and focusing on human medicines. To control this, including better antibiotic use policies and focusing human medicine should be ensured. Though antibiotics such as tetracycline and amoxicillin are often used, even for prevention, this often increases resistance. Last-resort antibiotics such as colistin pose such a concern. Efforts to control this include better antibiotic use policies and focusing human medicine. Antibiotics like tetracycline and amoxicillin are commonly used, sometimes even for prevention, which may increase resistance. The use of last-resort antibiotics like colistin is especially concerning (Ajayi, A. O,2024)

Chapter 2

Materials and Method

2.1 Sample collection and Materials

This experiment is conducted in NIDCH Hospital with 909 patients sample (indoor and outdoor).

This test is conducted by body fluid such as;

- Sputum

Materials;

- Sterile inoculating swab
- Culture medium MacConkey
- Sterile workspace
- Incubator
- Personal protective equipment (gloves, lab coat, mask, etc.)
- Labels (patient info, others)

4.2 Sample Inoculation & incubation

First of all, with patient details the culture was labeled. Sample was collected in a small container. A cotton swab used to take sample from the sample container and careful about contamination. Streak the collected sample across the surface of the agar media in a zigzag style or quadrant streak method to differentiate colonies. For this, fresh swab was used. Then, with appropriate temperature (35-37°C) the inoculated plates were inserted into the incubator. All the culture were allowed to grow within 18-48 hours.

4.3 Result & Microorganisms identification

MacConkey is used for differential culture media as it a selective media and basically utilized to isolate Gram-negative bacteria. Also, MacConkey makes the difference between lactose fermenting and non-fermenting bacteria. Moreover, it is basically applied to detect members of Enterobacteriaceae family. For example, *E. coli*, *Klebsiella* and *Salmonella*.

Translating Results on MacConkey Agar:

Lactose Fermenters (LF):

Lactose fermenting bacteria give Pink to red colonies on the media. Because, the bacteria has the ability to ferment the lactose and produced an acidic byproduct, this makes the pH lower. As a result, the color change indicates medium in the pH indicator which is neutral red. For Example,

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Enterobacter cloacae*

Non-Lactose Fermenters (NLF):

Non Lactose fermenting bacteria gave Colorless or pale colonies. Reason for this is, this type of bacteria does not ferment lactose. As a result, no acid formation has occurred and the pH indicator remain unchanged**.** For Example;

- *Salmonella spp.*
- *Shigella spp.*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*

No Growth:

No bacterial growth can also be found on MacConkey media due to insufficient conditions given in the media. Another reason can be, the presence of bile salt and crystal violet in the agar to stop the growth of microorganisms. For example, gram positive bacteria.

Figure 03: Growth and no growth on MacConkey agar media

2.4 Biochemical Tests

Questions about microbial identity can be asked of microbiologists beyond what the tests tell them about behavior of the microbe.Many of the biochemical tests for microbial identification are quite simple. A microbiologist drops hydrogen peroxide onto a smear of bacteria spread on a microscope slide to test whether bacteria contains a catalase enzyme. If the bacteria contain catalase, the mixture bubbles, the hydrogen peroxide decomposes into water and oxygen.The catalase test in the clinic distinguishes between the Gram positive catalase-positive Staphylococci and the Gram positive catalase negative Streptococcus.

Organism	Motility	Indole	Urease	Catalase	Oxidase	MR	VP	Citrate utilization	Glucose Ferm.	Sucrose Ferm.	Lactose Ferm.	Gas Prod.	H_2S Prod.	Hemolysis
Klebsiella pneumoniae	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	Gamma
Pseudomonas aeruginosa	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	Beta
Escherichia coli	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	variable	+ve	+ve	-ve	Alpha/Beta/ Gamma
Streptococcus spp.	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	.	-ve	Beta
Staphylococcus epidermidis	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	Gamma
Staphylococcus aureus	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	Beta
Salmonella spp.	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	Gamma
Bacillus cereus	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	.	+ve	Beta
Bacillus spp.	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	Alpha/Beta/ Gamma
Listeria monocytogenes	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	Beta

Table 01: Different Organisms Biochemical Test Interpretation

Catalase Test

Materials Needed: Inoculation loop, clean glass slide, 3% hydrogen peroxide solution, bacterial culture.

Steps:

- Put some bacterial colony on a fresh glass slide with a sterile loop.
- Directly to the bacterial sample, add 1-2 drops of 3% hydrogen peroxide.
- Look for immediate (bubbling or effervescence) that indicates the release of oxygen gas.

Positive: bubbles (i.e.,) include: *Staphylococcus spp.*

Negative: No Bubbling, e.g., *Streptococcus spp.*

Oxidase test

The oxidase test is a biochemical process for determining the enzyme known as cytochrome c oxidase among bacterial species. This enzyme is a key element of the electron transport chain during aerobic respiration, cytochrome c oxidase catalysis the transfer of electrons to molecular oxygen to form water. The oxidase test fixes the bacteria according to the formation of this enzyme and is beneficial in the labelling of gram-negative bacteria more specifically pertinent to clinical microbiology (Sievers, 2001).

Procedure of the Oxidase Test

The procedure for the oxidase test is simple and can be completed in a short time, usually within 1-2 minutes:

Inoculation: An overlay of bacteria from a fresh bacterial colony is placed on oxidase test paper or filter paper. A small loop of bacterial suspension can also be used alternatively. The reagent used is an artificial electron donor, tetramethyl-p-phenylenediamine, which is impregnated in the surface of the test paper.

Reagent Application: If cytochrome c oxidase is in the bacterial cells there will be a reagent in the reagent on the test paper or filter paper that will react with the cytochrome c oxidase.

Observation: After a few seconds, 1 minute or less, look for color change. Because cytochrome c oxidase will be oxidized on the paper, there will be a purple or blue color present if cytochrome operates c oxidase is present. On a negative result, no color change is seen.

Positive Result: In a few seconds (up to one minute) a purple or blue color means cytochrome c oxidase is present.

Negative Result: If my paper doesn't change color (or if it's the color of the paper throughout – typically pale), then the test is negative, indicating that the enzyme is not present.

Oxidase-Positive Organisms:

Pseudomonas spp.: *Pseudomonas aeruginosa* and many other species are oxidase positive. This is a geological hallmark characteristic of the genus. Opportunistic pathogen *Pseudomonas aeruginosa* is renowned, implicated in respiratory tract, urinary tract and wound infections.

Neisseria spp.: Genera such as Neisseria (neisserias), which contains pathogens, such as *Neisseria gonorrhea* (gonorrheal), *Neisseria meningitides* (meningitis), are oxidase positive. When used to distinguish Neisseria species from other gram negative diplococci the test is most useful.

Oxidase-Negative Organisms:

E. coli: One of the most common Enterobacteriaceae members is Escherichia coli. This is a cytochrome c oxidase negative or an oxidase negative organism, it does not produce Cyto Brave c oxidase. The test can differentiate E. coli from oxidase positive organisms such as Pseudomonas.

*Klebsiella sp*p.: *Klebsiella s*pecies (including *Klebsiella pneumoniae*) are also oxidase negative. These bacteria are also Enterobacteriaceae member but do not produce cytochrome c oxidase, like E. coli.

Enterobacter spp.: Enterobacter species (for example, *Enterobacter cloacae*) are oxidase negative as are *E. coli* and *Klebsiella*. Enterobacter can be differentiated from oxidase positive organisms by the oxidase test.

Indole Test

Materials Needed: Kovac's reagent, sterile inoculating loop, incubator, test organism, tryptophan broth.

Steps:

- Streak the bacterial culture on tryptophan broth using sterile loop.
- Add the broth to the incubator at 37°C from 24-48 hours.
- Incubate and add 5–10 drops of Kovac's reagent to the culture tube.
- Look for a red or pink ring rising in the top of the broth.

Positive: For e.g. red or pink layer (*Escherichia coli*).

Negative: Not yellowish (e.g., *Klebsiella pneumoniae*) with no color change.

Voges-Proskauer (VP) Test

Materials Needed: Test organism, sterile inoculating loop, MR-VP broth, Barritt's reagent A (αnaphthol), Barritt's reagent B (40% potassium hydroxide).

Steps:

- With a sterile loop stab MR-VP broth with the bacterial culture.
- The broth incubated in 37°C for 48 hours.
- Add 12 drops of Barritt's reagent A (α-naphthol) and 4 drops of Barritt's reagent B (potassium hydroxide) then incubate.
- Shake gently and leave tube undisturbed for 15-30 min.
- Look for red or pink color in the broth.

Positive*: Klebsiella spp*.: Red or pink color.

Negative: Does not change in color or turn copper (e.g. *E. coli*).

Urease Test

Materials Needed: Incubator, sterile inoculating loop, urea agar slant or broth, test organism.

Steps:

- Streak the bacterial culture upon the urea agar or broth slant or broth and grow at 37°C as recommended.
- Boil the medium and incubate at 37°C for 24 – 48 hours.
- Wait for color change in medium.

Positive: Alkaline (pH due to ammonia, e.g., *Proteus spp. or Klebsiella spp*.); bright pink.

Negative: Currently, it will not trigger a color change or yellowish color (i.e. *Escherichia coli).*

2.5 Antibiotics Sensitivity test

This test is conducted to detect antimicrobial agent for separated patients which is specifically effective. The aim of this test to identify possible resistance of drug in usual pathogens and for certain infections to ensure susceptibility to drugs of choice.

Equipment:

- Solid MHA medium
- Cotton swab
- Antibiotic discs
- Millimeter scale
- Incubator
- Autoclave machine

Sample:

- Single colony from organism X

Procedure:

Preparation of Bacterial Suspension

- A single colony is taken from several isolated colonies of organism X with the help of cotton swab.

Inoculation of Mueller Hinton Agar

The plates were allowed to come to room temperature before use.

- The Mueller-Hinton agar media plate was streaked uniformly with the help of a swab to create a bacterial lawn.
- To get uniform progress, the media plate was streaked into a single direction with the help of swab, then rotated 90° and streaked back in that approach.
- The three times rotation was performed,
- On the lawned surface, Antibiotic disks inserted by using a sterile forceps. Additionally, enough space is kept between them. The disk placed correctly so that it could not displaced
- At a temperature, the inoculated plates were kept into an incubator for overnight

Reading and Interpreting Zone Sizes

- After overnight incubation, Zone size means the areas of no bacterial growth around the disk were measured with the help of millimeters ruler.
- To enter the zone sizes a chart which is called Kirby Bauer was used.
- For each antibiotic, there were three classifications such as; Resistant, Intermediate or Sensitive found based on the CLSI recommendations chart on the limits stated.

Figure 04: AST assay on MHA agar *Pseudomonas*.

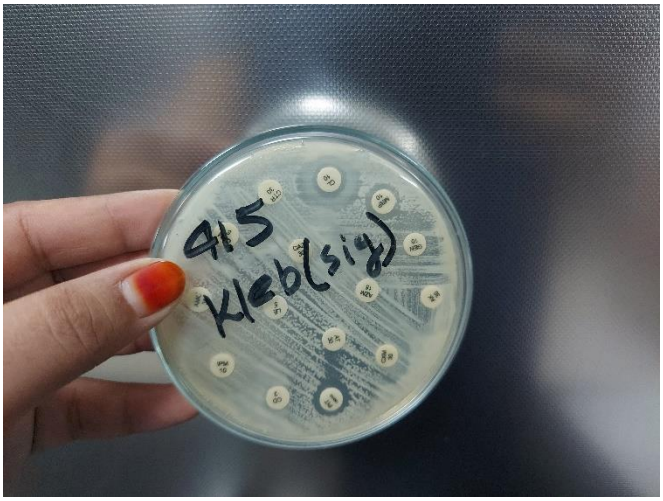

Figure 05: Antibiotics sensitive and resistance of

Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretative Criteria		
					Sensitive mm or more	Intermediate mm	Resistant mm or less
Gentamicin	Aminoglycoside	Gram-positive and Gram-negative	GEN	10	15	13-14	12
Meropenem	Carbapenem	Gram-positive and Gram-negative	MEM	10	23	20–22	19
Cefepime	Cephalosporin	Gram-positive and Gram-negative	CPM	30	25	19–24	18
Piperacillin tazobactam	Penicillin and beta-lactamase inhibitor	Gram-positive and Gram-negative	PIT	100/10	21	18–20	17
Imipenem	Carbapenem	Gram-positive and Gram-negative	IMI	10	23	20–22	19
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
Tigecycline	Glycylcyline	Gram-positive and Gram-negative	TGC	15	18	15-17	15
Aztreonam	Monobactam	Gram-negative	AT	30	21	18–20	17
Clindamycin	Lincosamide	Gram-positive bacteria	CL	2	≥21	15–20	≤14
Levofloxacin	Fluoroquinolone	Broad-spectrum	LE	5	≥17	14–16	≤13
Cefixime	Cephalosporin	Gram-negative bacteria	CFM	5	≥19	16–18	≤15
Amoxicillin	Penicillin	Gram-positive/Gram-negative	AMC	25	≥20	17–19	≤16
Cefuroxime	Cephalosporin	Gram-positive/Gram-negative	CXM	30	≥18	15–17	≤14
Ceftriaxone	Cephalosporin	Broad-spectrum	CTR	30	≥21	14–20	≤13
Sulbactam	Beta-lactamase Inhibitor	Broad-spectrum	SUL	10	≥15	12–14	≤11
Cefoperazone	Cephalosporin	Gram-negative bacteria	CULB	75	≥21	16–20	≤15
Cephalexin	Cephalosporin	Gram-positive bacteria	CEF	30	≥18	14–17	≤13
Sulbactam	Beta-lactamase Inhibitor	Broad-spectrum	SUL	10	≥15	12–14	≤11
Cefoperazone	Cephalosporin	Gram-negative bacteria	CULB	75	≥21	16–20	≤15
Cephalexin	Cephalosporin	Gram-positive bacteria	CEF	30	≥18	14–17	≤13
Clindamycin	Lincosamide	Gram-positive bacteria	CD	2	≥21	15–20	≤14
Ceftazidime	Cephalosporin	Gram-negative bacteria	CAZ	30	≥21	18–20	≤17

Table 02: List of Antibiotics used During the Experiment

	Drug Groups	Drugs	Gram negative (Y=Yes,N=No)	Gram Positive (Y=Yes,N=No)
1	Aminoglycosides	Amikacin (AK)	Y	Y
		Gentamicin (GEN)	Y	Y
2	Penicillin	Piperacillin (PIT)	Y	N
		Amoxicillin (AMC)	Y	Y
3	Cephalosporin's	Cefixime (CFM)	Y	Y
		Cxm - Cefuroxime	Y	Y
		CULB - Cefuroxime	Y	Y
		Ceftriaxone (CTR)	Y	Y
		Ceftazidime (CAZ)	Y	y
		Cefepime (CEF)	Y	Y
		Cefoperazone (CRP)	Y	Y
4	Carbapenems	Meropenem (MRP, MEM, MER)	Y	Y
		Imipenem (IPM)	Y	Y
5	Monobactams	Aztreonam (AT)	Y	N
6	Macrolides	Azithromycin (AZR, AZM)	Y	y
7	Fluoroquinolones	Levofloxacin (LE)	Y	N
8	Lincosamides	Clindamycin (CL, CD)	Y	N
9	Sulfonamides	Sulfamethoxazole (SUL)	Y	Y
10	Tetracyclines	Tigecycline (TGC)	Y	Y

Table 03 : Antimicrobial panels: The antibiotic class, the name of the antibiotics, and their spectrum is mentioned.

Chapter 3

Result

The sputum sample was collected from in a container of 909 patients. The 615 males represent 67.56% and the 287 females 31.57% representing females aged between 2 and 91 years. This study is mostly age wise distribution shown in Table A1 as most of the participants are.Ages 51 to 60 old adults are mostly affected aged 25.4% (Table 1).

Age	**Percentage Distribution among Age Groups**	**Number of Patients (N = 909) (%)**	*Klebsiella(N=195)*	*Pseudomonas (N=183)*	***E. coli*** *(N=2)*	*Staphylococcus (N=1)*	Acinetobacter *(N= 1)*	***Streptococcus (N=31)***
0-10	1.1	**10**	**1(0.5)**	**2(1.1)**	**0**	**0**	**0**	**1(3.2)**
11-20	6.6	**60**	**12(6.2)**	**7(3.8)**	**0**	**1(100)**	**0**	**2(6.5)**
21-30	9.8	**89**	**19(9.8)**	**11(6.0)**	**2(100)**	**0**	**0**	**2(6.5)**
31-40	15.5	**141**	**26(13.5)**	**26(14.2)**	**0**	**0**	**0**	**4(12.9)**
41-50	16.7	**152**	**32(16.6)**	**38(20.8)**	**0**	**0**	**0**	**6(19.4)**
51-60	25.4	**231**	**42(21.8)**	**44(24.0)**	**0**	**0**	**1(100)**	**10(32.3)**
61-70	17.3	**157**	**42(21.8)**	**34(18.6)**	**0**	**0**	**0**	**4(12.9)**
71-80	5.3	**48**	**14(7.3)**	**12(6.6)**	**0**	**0**	**0**	**1(3.2)**
81-90	2	**18**	**4(2.1)**	**8(4.4)**	**0**	**0**	**0**	**1(3.3)**
91-100	0.2	**2**	**1(0.5)**	**1(0.5)**	**0**	**0**	**0**	0
Total		**909**	**195**	**183**	**2**	**1**	**1**	31

Table 04: Age wise distribution of most prevalent microorganisms.

In a population of 909 patients, a detailed examination of the distribution of bacterial infections by age groups is given in the table. It categorizes patients into 10-year intervals from 0-10 years to 91-100 years and reports the prevalence of infections caused by six bacterial species: Acinetobacter, *Streptococcus, E. coli, Pseudomonas, Klebsiella* and *Staphylococcus*. This data gives information of trends in distribution and prevalence of these infections by different groups of age.

Klebsiella were isolated of all the bacteria. *Pseudomonas* was found to be the most common constituting more than 40% of all micro-organisms. On the other hand, *E.coli, Staphylococcus* and Acinetobacter caused a total of 8.54% of all organisms presented and *E.coli, Staphylococcus, Streptococcus* and Acinetobacter were the least prevalent, accounting for a total of 45.43% of all organisms. The prevalence of *Streptococcus* was low compared to other Microorganisms, about 7.58%, of the isolated organisms. In addition, this study identified that approximately 21.8 % of *Klebsiella* were found to be the most common constituting more than 40% of all microorganisms. On the other hand, *E.coli, Staphylococcus, Streptococcus* and Acinetobacter were the least prevalent, accounting for a total of 8.54% of all organisms. Compared to other microorganisms, the prevalence of streptococcus was low, accounting for around 7.58% of the isolated organisms. Moreover, this study found that about 21.8% of *Klebsiella*. In the case of individuals aged 51 years and older, 24% of cases were caused by *pseudomonas* infection. In addition, this study demonstrated that infections with *Streptococcus* had a prevalence of 32.3% in persons between the ages of 51 and 60 (Table 01).

My findings illustrate the need for age specific vulnerability based targeted healthcare strategies. Less old adults but middle aged adults, especially those aged 51-60 need the surveillance and preventive measures on *Klebsiella* and *Pseudomonas*. At the same time, younger patients seem harder hit and therefore, are candidates for the standard preventive care. Elderly patients have relatively low infection rates, and more work is needed to determine the

factors leading to their low rates and ensure they receive the appropriate care. Overall the table elucidates major age related patterns of bacterial infections and serves as a guide to design clinical and preventive interventions tailored to age specific needs.

Antibiotics	*Klebsiella ,N=195*		*Psedumonus ,N=183*		*Streptococcus , N=31*		*E.coli,N=2*		*Staphylococcu,N=1*		*Acinetobacter ,N=1*	
	R %	S %	R %	S %	R %	S %	R	S	R	R	R	S
Amikacin	36(18.46)	140(71.79)	26(14.21)	97 53.01	12 38.71	16 51.61	0	1(50)	0	1(100)	1(100)	0
Clindamycin	41(21.03)	92(47.18)	15(8.2)	32(17.49)	9 29.03	2 6.45	0	1(50)	0	0	1(100)	0
Piperacillin	57(29.23)	140 (71.79)	54 29.51	128 69.95	12 38.71	19 61.29	0	2(100)	0	1(100)	1(100)	0
Levofloxacin	53(27.18)	75 (38.46)	45 24.59	82 44.81	8 25.81	3 9.68	0	1(50)	0	0	1(100)	0
Gentamicin	47(24.1)	147 (75.38)	40 21.86	143 78.14	12 38.71	19 61.29	0	2(100)	0	1(100)	1(100)	0
Meropenem	25(12.82)	42 (21.54)	24 13.11	20 10.93	1 3.23	3 9.68	0	0	0	0	0	0
Aztreonam	36(18.46)	104 (53.33)	26 14.21	97 53.01	24 77.42	1 3.23	2(100)	0	1(100)	0	0	0
Cefixime	23(11.79)	4 (2.05)	128 69.95	6 3.28	19 61.29	0 0	1(50)	0	0	0	1(100)	0
Amoxicillin	102(52.31)	20 (10.26)	102 55.74	11 6.01	12 38.71	1 3.23	0	1(50)	0	0	1(100)	0
Imipenem	13(6.67)	6 (3.08)	128 69.95	23 12.57	25 80.65	5 16.13	2(100)	0	1(100)	0	1(100)	0
Cefuroxime	98(50.26)	2 (1.03)	97 53.01	4 2.19	10 32.26	1 3.23	1(50)	0	0	0	1(100)	0
Meropenem	51(26.15)	100 (51.28)	55 30.05	112 61.2	12 38.71	13 41.94	1(50)	1(50)	0	1(100)	1(100)	0
Clindamycin	112(57.44)	1 (0.51)	98 53.55	0 0	22 70.97	0 0	0	1(50)	1(100)	0	0	0
Ceftazidime	41(21.03)	0 0	55 30.05	3 1.64	0 0	0 0	1(50)	0	0	0	0	0
Aztreonam	126.15)	0 0	18 9.84	0 0	2 6.45	0 0	0	0	0	0	0	0
Meropenem	12(6.15)	0 0	11 6.01	1 0.55	7 22.58	0 0	0	0	0	0	1(100)	0
Azithromycin	55(28.21)	1 (0.51)	59 32.24	5 2.73	5 16.13	1 3.23	1(50)	0	0	0	1(100)	0
Ceftriaxone	40(20.51)	25 (12.82)	68 37.16	17 9.29	7 22.58	0 0	0	1(50)	0	0	1(100)	0
Sulbactam	5(2.56)	3 (1.54)	13 7.1	1 0.55	4 12.9	0 0	0	0	0	0	1(100)	0
Cefoperazone	2(1.03)	1 (0.51)	4 2.19	0 0	0 0	0 0	0	0	0	0	0	0
Cephalexin	5(2.56)	3 (1.54)	6 3.28	6 3.28	4 12.9	0 0	0	0	0	0	1(100)	0
Tigecycline	4(2.05)	6 (3.08)	19 10.38	0 0	4 12.9	1 3.23	0	0	0	0	1(100)	0
Cefepime	2(1.03)	3 (1.54)	3 1.64	2 1.09	0 0	0 0	0	0	0	0	0	0

Table 05: Sensitivity profile of the most prevalent isolated organisms.

Levels of antibiotic resistance find their way into the data. *Klebsiella* shows resistance to Clindamycin (57.44%), Amoxicillin II (52.31%) and Cefuroxime (50.26%), however shows less resistance to Imipenem (6.67%) and Sulbactam (2.56%). Amoxicillin (55.74%) and Meropenem (30.05%) have high resistance, Piperacillin (29.51%) and Gentamicin (21.86%) have moderate resistance and high resistance was shown for Ciprofloxacin. There is extremely high resistance of *Streptococcus* to Aztreonam (77.42%), but lower levels of Gentamicin (38.71%) and Ceftriaxone (22.58%). For Amoxicillin and Aztreonam resistance rates in *E. coli* are lower but nevertheless worth mentioning. Nineteen last resort antibiotics, such as Tigecycline and Cefepime, all have negligible resistance rates, between 1.03 to 3.08 percent resistance against pathogens, which suggest the possibility of therapeutic activity. These results highlight the importance of evidence based antibiotic use and strengthened infection control measures to prevent the spread of resistant bacteria.

The sensitivity data showed that different bacterial pathogens respond to antibiotics differently. While high sensitivity was demonstrated towards Gentamicin (75.38%), Piperacillin (71.79%) and Amikacin (71.79%) for *Klebsiella* and it showed lower sensitivity to Imipenem (3.08%) and Cefuroxime (1.03%). Gentamicin (78.14%) was highly sensitive to *Pseudomonas*, as is Piperacillin (69.95%), while sensitivity to Meropenem (10.93%) and Cefuroxime (2.19%) were much lower. Amikacin (51.61%) and Meropenem (41.94%) showed moderate sensitivity to *Streptococcus* but the sensitivity of Cefixime (0%) for *Streptococcus* was reduced. Some effectiveness of these drugs was shown by Aztreonam, Sulbactam and *E. coli* exhibited 50% sensitivity. Amikacin, Aztreonam and Imipenem showed 100% sensitivity to *Staphylococcus*, though limited data. In the end, Cefepime and Tigecycline showed varying sensitivity to the *Klebsiella* and *Pseudomonas* with 1.54 % and 3.08 % sensitivity respectively. This variability within pathogen demonstrates the importance of relying on sensitivity data for to optimize treatment planning and antibiotic stewardship.

The *Klebsiella* showed a wide antibiotic sensitivity, highest among Gentamicin (75.38%) and Piperacillin (71.79%) which suggested that this is as an effective treatment for these diseases. *Klebsiella* infections showed sensitivity rates of 71.79% for amikacin as well. Meropenem (51.28%) and Aztreonam (53.33%) were moderately sensitive while drugs including Imipenem (3.08%) and Cefuroxime (1.03%) were very poorly effective. These findings are valuable in affirming that Gentamicin or Amikacin are key antibiotics to address *Klebsiella*; these should not be thrown out for less effective antibiotics.

Amoxicillin and Aztreonam had moderate resistance for *E. Coli* with sensitivity rate of 50% for both. However, Aztreonam (77.42%), Amikacin (51.61%) and Meropenem (41.94%) showed high sensitivity to *Streptococcus*. Multiple antibiotics, including Amikacin and Meropenem had 100% sensitivity against *Staphylococcus* making it a very treatable infection. Though not extensively elaborated on, Acinetobacter possessed minimal resistance to key antibiotics like Amikacin, therefore may be susceptible – at least to some level – to certain treatments.

To identify the usefulness of the drugs used, we executed an AST test, using antibiotic disks. Incubation overnight shows the result and helps us to gain knowledge about useful drug. Also, determine the number of resistant and sensitive antibiotics.

Chapter 4

Discussion

AMR remains a major public health crisis with increasing global prevalence, as will be shown in this study of antibiotic resistance. This work identifies *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as the most prevalent pathogens with high resistance to aminoglycosides, beta lactams and carbapenems, which correlates with the global trends reported by World Health Organization (WHO). However, these antibiotics resistant organisms are a serious challenge particularly in low and middle income countries, where access to robust diagnostics and antimicrobial stewardship programs is limited, this makes the problem more complex.

A study in India showed that *Pseudomonas* is sensitive to penicillin, cephalosporin, carbapenem and monobactam. Among these group, piperacillin and ticarcillin (penicillins), ceftazidime (cephalosporin), cefepime (cephalosporin), aztreonam (monobactam), imipenem, meropenem and doripenem (carbapenems) are most effective and commonly used in the treatment of *Pseudomonas aeruginosa*. (Pachori et al., 2019).However the experimental data shows that Amikacin,Piperacilin,Gentamicin are most sensitive, which is more than 50%.

A research was based on reports from 14 countries (China, Egypt, Russia, Taiwan, India, Iran, Sudan, the US, Brazil, Ethiopia, Pakistan, Bangladesh, Madagascar, and Italy) on four continents (Asia, Africa, Europe and the Americas).It revealed the prevalence of antibiotic resistance for Klebsiella . We have seen high resistance rates against many different classes of antibiotics. Respectively, ampicillin-sulbactam had 45.3% resistance making them ineffective. For example, 38.1% of cephalosporin cefazolin and 26.7% of cefuroxime were resistant. Large proportions of resistance were found to third generation cefotaxime (65.8%) and ceftazidime (57.1%) and cefepime (51.3%). Imipenem (45.7%), meropenem (51.0%) and

ertapenem (40.6%) were spared their last resort carbapenem status.(Beig et al., 2024).On the other hand , from our experiment Amikacin(71.79%),piperacillin(71.79%),Gentamicin(75.38%),Azteronam(53.363%).Meropenem(51.28/%) are sensitive to the *Klebshilla* and Amosicilin(52.31%),Cefurosime(50.26%),Clindamycin(57.44%) are resistant to the *Klebshilla.*

Globally Clindamycin resistance is present on approximately 22% of *Streptococcus pneumonia* isolates.(Cherazard et al., 2017) Specifically, there is high prevalence of penicillin resistance among *S. pneumonia* isolates within Europe, particularly in France and Spain. In European countries such as France, Spain, Belgium and Italy, macrolide resistance has recently become an important issue for *S. pneumonia* also as the extent of resistance to macrolide has exceeded that to penicillin in these countries.(Adam, 2002) .From the given data *Streptococcus* is resistant to Amikacin(51.61%),Piperacillin(69.95%),Gentamicin(61.29%) and sensitive to Aztreonom(77.42%),Cefixim(61.29%),Imipenem(86.65%),Clindamycin(701.97). Piperacillin is a member of penicillin and our data shows the highest resistance to the organism .

In a study , 10 were from Africa, 13 were from Asia, 1 was from Europe, 8 were from South America and 1 was a multiple location.Pooled prevalence of commensal *E. coli* from healthy individuals in community .A high prevalence was seen for some of the commonly prescribed antibiotics in these countries like ampicillin (72%,), cefotaxime (27%,), chloramphenicol (45%), ciprofloxacin (17%), tetracycline (67%). *(Nji et al., 2021).*

On the contrary, *E.coli* is most resistant to Aztreonam and Imipenem and sensitive to Piperacillin and Gentamicin .

Carbapenem resistant *Klebsiella pneumoniae* (CRKP) has become a global nightmare because of the intense spread of this organism among healthcare settings. As with *Pseudomonas aeruginosa*, resistance in *P. aeruginosa* is mirrored by international data which find rates ranging from 10–30%, according to geographical region. The findings of age specific trends in rates of infection revealed that inflammation in middle aged adults is most prevalent and emphasize the need for demographic specific prevention and treatment strategies.

Regional disparities in data collection and intervention are evident in global initiatives as with the WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS). For instance, Europe's EARS-Net reports comparable assessment of AMR patterns across countries, facilitating specifically the Netherlands reducing its resistance rates to extremely low levels with stringent antibiotic policies.

In contrast, most of Africa and South Asia's low and middle income countries (LMICs) simply lack the infrastructure to monitor resistance patterns correctly, resulting in under reporting and treatment challenges.

The world is increasingly interested in alternative therapies. Research into antimicrobial peptides (AMP) directed to resistant bacteria has been pioneered in the United States while bacteriophage therapy has successfully treated MDR Pseudomonas in chronic wound infections in Belgium. Trials across China and India have shown promise with nanoparticle delivery of gold and silver particles, which can provide targeted approaches with a utilized resistance potential. Fecal microbiota transplantation (FMT) is increasingly utilized in high

income countries for the treatment of recurrent Chloridoids difficile infections and perhaps a role of gut microbiota associated resistance. (Prestinaci,2015)

Both amikacin and gentamicin showed efficacy but give early indication that alternative therapies are urgently needed. Globally, more and more innovative approaches are emerging, such as bacteriophage therapy, antimicrobial peptides (AMPs) and fecal microbiota transplantation (FMT). For example, AMPs have wide and rapid spectrum of activity against resistant bacteria, yet their use is currently hampered by obstacles in cost effective production. One approach is to use nanoparticle-based therapies that target infections more effectively, and which reduce the likelihood of resistance. While they still struggle with environmental impact and toxicity. The study also underlines the urgent requirement for integrated global surveillance systems. Comprehensive resistance monitoring often cannot be conducted in the infrastructure that exists within LMICs such as the study region, which leaves large gaps in data collection and reporting. Additionally, the WHO's Global Antimicrobial Resistance and Use Surveillance System (WHO GLASS) is a platform for promoting data sharing, as well as standardization within and between national surveys. Community driven initiatives to tackle the burden of infectious diseases go above and beyond technical solutions such as hygiene, sanitation and infection control. Public awareness and education campaigns specifically on minimizing antibiotic misuse have a major role in decreasing antibiotic misuse a major cause of resistance across the globe.

Chapter 5

Conclusion

Antimicrobial resistance (AMR) is a significant global health challenge, driven by the misuse and overuse of antibiotics and further complicated by limited surveillance and treatment options. This study highlighted the predominance of bacteria, particularly *Klebsiella*

pneumoniae and *Pseudomonas aeruginosa*, which exhibited resistance to beta-lactams and carbapenems, raising concerns about the efficacy of last-line treatments. *Streptococcus* and *Staphylococcus*, also showed worrying resistance patterns, including reduced susceptibility to Clindamycin.

However, while many challenges existed for antibiotic choice, Amikacin and Gentamicin remained highly sensitive to the infection. The findings highlight the importance of antibiotic stewardship, rapid diagnostic tools and infection control measures especially in middle aged adults, who were most affected. Still limited to a single center, the study serves as an invaluable insight into resistance patterns and provides an urgent call to arms for novel therapeutic means, such as bacteriophage therapy and a willingness to cooperate internationally to tackle AMR. By doing innovative research and clinical interventions around pathogens we can mitigate the impact of pathogens and have better patient outcomes.

Reference

1. A Guide to the 8 Most Common Classes of Antibiotics. (n.d.). Agscientific.com. https://agscientific.com/blog/8-most-common-classes-of-antibiotics.html

2. Adam, D. (2002). Global antibiotic resistance in Streptococcus pneumoniae. Journal of Antimicrobial Chemotherapy, 50(suppl 1), 1–5. https://doi.org/10.1093/jac/dkf801

3. Ajayi, A. O., Odeyemi, A. T., Akinjogunla, O. J., Adeyeye, A. B., & Ayo-ajayi, I. (2024). Review of antibiotic-resistant bacteria and antibiotic resistance genes within the one health framework. Infection Ecology & Epidemiology, 14(1). https://doi.org/10.1080/20008686.2024.2312953

4. Anderson, L. (2023, July 19). Antibiotics Guide. Drugs.com; Drugs.com. https://www.drugs.com/article/antibiotics.html

5. Beig, M., Nahal Majidzadeh, Mahshid Khazani Asforooshani, Rezaie, N., Abed, S., Elham Hajagha Gholizadeh Khiavi, Sholeh, M., & Shadi Aghamohammad. (2024). Antibiotic resistance rates in hypervirulent Klebsiella pneumoniae strains: a systematic review and meta-analysis. Journal of Global Antimicrobial Resistance. https://doi.org/10.1016/j.jgar.2024.06.018

6. Chang, R. Y. K., Nang, S. C., Chan, H.-K., & Li, J. (2022). Novel antimicrobial agents for combating antibiotic-resistant bacteria. Advanced Drug Delivery Reviews, 187, 114378. https://doi.org/10.1016/j.addr.2022.114378

7. Creative Biolabs. (2022). Mode of Actions and Targets for Antibacterial Drugs - Creative Biolabs. Www.creative-Biolabs.com. https://www.creative-biolabs.com/drug-discovery/therapeutics/mode-of-actions-and-targets-for-antibacterial-drugs.htm

8. Cherazard, R., Epstein, M., Doan, T.-L., Salim, T., Bharti, S., & Smith, M. A. (2017). Antimicrobial Resistant Streptococcus pneumoniae: Prevalence, Mechanisms, and Clinical Implications. American Journal of Therapeutics, 24(3), e361–e369. https://doi.org/10.1097/MJT.0000000000000551

9. Dutta, S. S. (2022, June 21). The History of Antibiotics. News-Medical.net. https://www.news-medical.net/health/The-History-of-Antibiotics.aspx

10. Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. Journal of Infection and Public Health, 10(4), 369–378. ScienceDirect. https://doi.org/10.1016/j.jiph.2016.08.007
11. Halawa, E. M., Fadel, M., Al-Rabia, M. W., Behairy, A., Nouh, N. A., Abdo, M., Olga, R., Fericean, L., Atwa, A. M., El-Nablaway, M., & Abdeen, A. (2023). Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Frontiers in Pharmacology, 14, 1305294. https://doi.org/10.3389/fphar.2023.1305294
12. Hong, P.-Y., Al-Jassim, N., Ansari, M., & Mackie, R. (2013). Environmental and Public Health Implications of Water Reuse: Antibiotics, Antibiotic Resistant Bacteria, and Antibiotic Resistance Genes. Antibiotics, 2(3), 367–399. https://doi.org/10.3390/antibiotics2030367
13. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, Present and Future. Current Opinion in Microbiology, 51(1), 72–80. https://doi.org/10.1016/j.mib.2019.10.008
14. Mackie, T. J., Collee, J. G., & Mccartney, J. E. (2007). Mackie and McCartney practical medical microbiology. Elsevier.
15. Nadgir, C. A., & Biswas, D. A. (2023). Antibiotic Resistance and Its Impact on Disease Management. Antibiotic Resistance and Its Impact on Disease Management, 15(4). https://doi.org/10.7759/cureus.38251
16. Nicolaou, K. C., & Rigol, S. (2017). A brief history of antibiotics and select advances in their synthesis. The Journal of Antibiotics, 71(2), 153–184. https://doi.org/10.1038/ja.2017.62
17. Nicolle, L. E., Strausbaugh, L. J., & Garibaldi, R. A. (1996). Infections and antibiotic resistance in nursing homes. Clinical Microbiology Reviews, 9(1), 1–17. https://doi.org/10.1128/cmr.9.1.1
18. News-Medical. (2018, October 15). Mechanisms of Antibiotic Action. News-Medical.net. https://www.news-medical.net/life-sciences/Mechanisms-of-Antibiotic-Action.aspx
19. Nji, E., Kazibwe, J., Hambridge, T., Joko, C. A., Larbi, A. A., Damptey, L. A. O., Nkansa-Gyamfi, N. A., Stålsby Lundborg, C., & Lien, L. T. Q. (2021). High prevalence of antibiotic resistance in commensal Escherichia coli from healthy human sources in community settings. Scientific Reports, 11(1), 3372. https://doi.org/10.1038/s41598-021-82693-4
20. Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health, 109(7), 309–318. https://doi.org/10.1179/2047773215y.0000000030

21. Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes & Diseases, 6(2), 109–119. https://doi.org/10.1016/j.gendis.2019.04.001

22. Rodriguez-Morales, A. J., Gallego, V., Escalera-Antezana, J. P., Méndez, C. A., Zambrano, L. I., Franco-Paredes, C., Suárez, J. A., Rodriguez-Enciso, H. D., Balbin-Ramon, G. J., Savio-Larriera, E., Risquez, A., & Cimerman, S. (2020). COVID-19 in Latin America: The implications of the first confirmed case in Brazil. Travel Medicine and Infectious Disease, 101613. https://doi.org/10.1016/j.tmaid.2020.101613

23. Thanh Quang Nguyen, Bo Eun Heo, Jeon, S., Ash, A., Lee, H., Moon, C., & Jang, J. (2024). Exploring antibiotic resistance mechanisms in Mycobacterium abscessus for enhanced therapeutic approaches. Frontiers in Microbiology, 15. https://doi.org/10.3389/fmicb.2024.1331508

24. World Health Organization. (2023, November 21). Antimicrobial resistance. World Health Organization; World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance