Prevalence & Antibiotic Susceptibility Patterns Of Pathogens Obtained From The Lower Respiratory Tract Of Patients From NIDCH Dhaka-Bangladesh

By
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A thesis submitted to the Department of Mathematics & Natural Sciences in partial fulfillment of the requirements for the degree of
B.Sc. in Biotechnology

Department of Mathematics & Natural Sciences
BRAC University
December 2019

Declaration

I hereby declare that;

1. The thesis submitted is my 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 that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all of the main sources of help.

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Approval

The thesis **"Prevalence & antibiotic susceptibility patterns of pathogens obtained from the lower respiratory tract of patients from NIDCH Dhaka-Bangladesh"** submitted by Kisuule Gordon (15236015) of Fall, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. In Biotechnology on 24th December 2019.

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Abstract

Lower Respiratory Tract Infections (LRTIs) are very common and the usual way to treat them is by using antibiotics. Recently, treatment has become more complicated since most of the pathogens that cause these infections have become resistant to many antibiotics. This study aimed at examining the antimicrobial activity of commonly used antibiotics against pathogens from the Lower Respiratory Tract and establishing the susceptibility and prevalence patterns of the pathogens. Out of 57 samples, 28 were *Klebsiella pneumoniae,* 25 were *Pseudomonas aeruginosa,* and 4 were *Escherichia coli.* Antibiotic susceptibility tests were done using Kirby – Bauer method, using 8 different antibiotics. The highest resistance was observed against cefixime (96.49 %), followed by cefepime (87.72 %). All *E. coli* isolates were considered multi-drug resistant. For *K. pneumoniae,* 96.43% of the isolates, for *P. aeruginosa,* 96% of the isolates were multi-drug resistant. Findings from this study confirm the dangers posed by antimicrobial-resistant bacteria on human health and also the urgent need to combat the problem of antimicrobial resistance.

Keywords: Lower Respiratory Tract Infections; Antibiotics; Antimicrobial resistance, Multi-drug resistant

Dedicated to my family and my teachers

Acknowledgement

I am so grateful to the Almighty God who has given me good health, strength, and wisdom to allow me to complete my Undergraduate thesis successfully.

I also thank my parents, Kisuule Robert, Namwanga Proscovia, my siblings Kisuule Robert, Kisuule George, Nakalanzi Irene, Namwebe Brenda, for their unconditional support and words of encouragement during the most difficult times.

I would like to thank Professor A. F. M. Yusuf Haider, and Dr. Mahboob Hossain, Coordinator of Microbiology Program of MNS Department of BRAC University for supporting to complete my undergraduate thesis.

My sincere appreciation goes to my respected Supervisors Professor Dr. Mahboob Hossain and Mr. Akash Ahmed for their guidance and helpful criticism throughout the entire period of my thesis.

I would like to also thank the respective lab officers Miss Asma Binte Afzal and Mr. Md Nazrul Islam for the assistance they gave me during my work.

I also extend my appreciation to teaching assistants, Rwezan Kabbir, Romi Marina George and Nawrin for their guidance and assistance during my work

My regards to Ashique-E- Khuda, Tanzila Ahmmed Bonna, Shilpi Akter, Nadira Begum, Md Furkan Mia, Md. Morshed-Al-Mamun for always helping me in the Lab.

I also extend my sincere appreciation to my colleague, Fariba Salahuddin who helped so much in almost all the work and also my friends, Kashfia Hassan, Rian Rafsan, Syeda Maniza Rahman, Rose Prema Gomez, Maliha Tabassum Rashid, Shaeri Nawar, Deepawnita Chackraborty, Samiha Ashreen, Farzana Priyanka, Jamie Radia, and Nitul for their valuable company.

Finally, I extend my gratitude to all my relatives and all my friends from Uganda for their unwavering support.

Kisuule Gordon
December 2019

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List of Acronyms

MDR	Multi Drug Resistant
XDR	Extensively Drug Resistant
PDR	Pan Drug Resistant
et al	And others
CDC	Center for Disease Control
WHO	World Health Organization
CLSI	Clinical and Laboratory Standards Institute
NIDCH	National Institute of Diseases of the Chest and Hospital
LRTIs	Lower Respiratory Tract infections
AMR	Antimicrobial Resistant
COPD	Chronic Obstructive Pulmonary Disease
mg	Milligram
µg	Microgram
ml	Milliliter
MHA	Muller Hinton Agar
NA	Nutrient Agar
Fig	Figure

List of Acronyms

CFM	Cefixime
CPM	Cefepime
CN	Cephalexin
AZM	Azithromycin
AMC	Amoxyllin /clavulanic acid (amox clav)
CL	Colistin
TE	Tetracycline

CHAPTER 1

INTRODUCTION

Chapter 1

Introduction

1.1 Background

Antimicrobial resistance is currently one of the most severe global threats to both human and veterinary medicine (Ahmed et al., 2019). Generally, it is now accepted as a global threat that requires coordinated action across countries and sectors to minimize the emergence and spread of resistant bacteria around the world (Guardabassi, 2017). According to the United States Center for Disease Control and Prevention (CDC), more than 2,000,000 people every year are affected by antibiotic-resistant infections and at least 23 000 dying because of the infections (Prestinaci et al., 2015).

AMR bacteria, most especially those that are responsible for causing Lower Respiratory Infections (LRTIs) have made treatment even more difficult leading to the loss of many lives. Lower respiratory tract infections, excluding tuberculosis, rank third as causes of death worldwide and were responsible for 3,200,000 deaths worldwide in 2015.

In developing countries, lower respiratory tract infections remain the top cause of death. This is linked to poverty, which is associated with malnutrition, overcrowding, and air pollution, which increases the risk of infection with airborne pathogens. Nonetheless, the majority of these infections are avoidable, but due to a lack of resources and access to healthcare people do not have access to immunizations or get timely antibiotics (José, 2018).

Nonetheless, along with the WHO, other organizations such as the Centers for Disease Control and Prevention (CDC), the European Centre for Disease Prevention and Control (ECDC) have also put in much effort to combat this crisis.

Fortunately, several strategies have been put in place to help prevent this problem from getting worse. Among these include; eradicating the use of antibiotics as growth promoters as well as limiting its use for other nontherapeutic applications, educating the public about antimicrobial resistance and also ensuring that MDR (multidrug-resistant) bacteria are prevented from entering the food chain through proper farming methods (Roca et al., 2015; Bloom et al., 2017).

1.2 Explaining Antibiotics and Antibiotic Resistance

In ancient civilizations, people used herbs, honey and also animal feces to treat bacterial infections. But one of the most outstanding of all was the use of moldy bread to treat infections. This method was widely used in ancient Egypt, China, Serbia, Greece and, Rome.

Although still a mystery, there is also a possibility that substances with tetracycline which is an antibiotic used today, could have been used in ancient times as it was evidenced that some of its traces were found in skeletons excavated in Nubia and Egypt (Gould, 2016).

The discovery of penicillin by Alexander Fleming in 1928 changed the course of medicine and since then antibiotics have been used extensively to treat bacterial infections (Gaynes, 2017). Alexander Fleming's discovery was so important and helped to reduce the number of deaths that occurred due to bacterial infections most especially in Europe. More scientists thereafter developed several antibiotics such as sulfonamides, beta-lactams, fluoroquinolones, cephalosporins, etc. However, it was in the golden era of antibiotics (1940-1962) that most of the antibiotics that we use to date were developed and on a very large scale.

1.3 How antibiotics work

To help treat infections, antibiotics use different mechanisms depending on the structure and general characteristics of the bacteria.

The different ways by which different antibiotics function .i.e. by inhibiting the synthesis of bacterial cell walls (e.g. penicillins and cephalosporins); Interfering with the cell membrane of the microorganism hence affecting permeability (e.g. some antifungal agents), Inhibiting protein synthesis by affecting the function of 30S or 50S ribosomal subunits (e.g. tetracyclines, macrolides, and clindamycin), Blocking important metabolic steps of the microorganisms (e.g. sulfonamides and trimethoprim) and also interfering with nucleic acid synthesis (e.g. metronidazole and quinolones) (Soares et al., 2012).

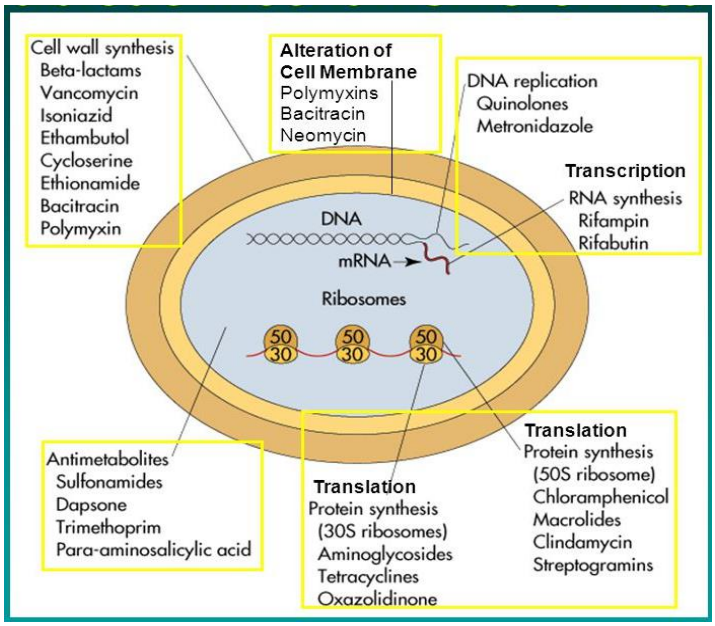


Fig 1: Mechanisms of antimicrobial action of antibiotics

1.4 The cause of antimicrobial resistance

The prime causes of AMR include overuse of antibiotics almost everywhere such as in poultry, animal and aquatic food, poor systems of purchasing antibiotics, movement by humans from one country to another, poor sanitation / hygiene, and release of non-metabolized antibiotics or their residues into the surrounding through manure / feces.

These factors lead to genetic selection pressure for the emergence of MDR bacterial infections in the community. Of recent, a worldwide consumption of antimicrobials in livestock has been noted as the potential cause of serious dangers in both the economic and public health sectors in the future (Van et al., 2015).

1.5 Superbugs and Super resistance

Superbug is a term used to define a microbial organism with enhanced morbidity and mortality, due to multiple mutations leading to high levels of resistance to the antibiotic classes that are specifically recommended for its treatment.

Most of the bacterial pathogens linked with epidemics of human disease have evolved into multidrug-resistant (MDR) forms and have become very difficult to treat with antibiotics. e.g. MDR *M. tuberculosis* which is found in both developing and industrialized countries.

Other serious infections include nosocomial infections caused by *Acinetobacter baumannii, Burkholderia cepacia, Campylobacter jejuni, Citrobacter freundii, Clostridium difficile, Enterobacter spp., Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella spp., Serratia spp., Staphylococcus*

aureus, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *a*nd *Streptococcus pneumoniae.* The treatment options of these superbugs are relatively difficult and periods of hospital care are extended and more costly as well (Davies, 2010).

1.6 Meaning of MDR, XDR, and PDR

Several definitions have been used to categorize patterns of resistance found in resistant antimicrobial bacteria (Magiorakos et al., 2012), leading to a misunderstanding among scientists about the use of these terms. Therefore, following a joint initiative by the European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), experts came together to define the terms MDR, XDR & PDR.

Often, MDR gram-positive and gram-negative bacteria are defined as bacteria that are "resistant to three or more antibiotic classes". Hidron et al (2008) define MDR as resistance to one key antimicrobial agent. MDR can also be defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Extensively drug resistant (XDR) microorganisms are defined by two sets of criteria i.e.; according to the number of antimicrobials or classes or subclasses to one or more key antimicrobial agents (Cohen et al., 2008; Hidron et al., 2008). Also, XDR can be defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) (Magiorakos et al., 2012).

Pan drug Resistant (PDR) is a term that is used to refer to bacteria that are resistant to all antimicrobial agents. However, the definition of pan drug resistant (PDR) is greatly varied from one study to another. Nonetheless, the definition of PDR is not a rigid term and it should be defined as resistant to all antimicrobials regularly tested or non-susceptibility to all agents in all antimicrobial categories (Magiorakos et al., 2012).

1.7 Antibiotic-Resistant organisms and Infections

Currently, patients suffering from antibiotic-resistant infections can be found all over the globe (Golkar et al., 2014). There are also several reports which claim that both resistant gram-positive and gram-negative organisms are responsible for causing most of these infections, leading to many deaths worldwide (Rossolini et al., 2014).

Among the MDR gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and multi-drug resistant *Streptococcus pneumoniae* have been regarded as the serious public threats by the Centers for Disease Control and Prevention. Moreover, MRSA and VRE are the leading causes of healthcare-associated infections in the United States whereby, they are claimed to be responsible for over 12,000 deaths per year. Also, infections due to drug-resistant *S. pneumoniae* which is the leading cause of bacterial pneumonia and meningitis in adults, are estimated to be responsible for about 19,000 excess admissions and 7,000 annual deaths in the United States (Munita et al., 2015).

Among the gram-negative pathogens, most of the serious infections come about in health care settings and most are caused by Enterobacteriaceae (most especially *Klebsiella pneumoniae*), *Pseudomonas aeruginosa,* and *Acinetobacter*. The other gram-negative bacteria that have also become a threat, are extended-spectrum beta-lactamase-producing *Escherichia coli* and *Neisseria gonorrhoeae* (Ventola, 2015).

Infections caused by AMR bacteria can occur in both communities as well as hospital settings. Among community-acquired infections, the most common are; Community Acquired Pneumonia (CAP) which is caused by *S pneumonia*, infections like pharyngitis and scarlet fever that are caused by *S. pyogenes*, bacterial gastroenteritis caused by *Campylobacter jejuni* or *Salmonella ssp,* and Urinary Tract Infections (UTIs) that are caused by *E. coli.* (Felmingham et al., 2002; Goossens et al., 1998; Cunningham, 2000). The resistance of these pathogens to antibiotics such as penicillins, cephalosporins, β - lactams and fluoroquinolones that were previously used to treat them has been reported worldwide (Goossens et al., 1998).

In hospitals, particularly in ICUs, infections caused by antibiotic resistant bacteria are very common. This is due to the exposure to extensive use of antibiotics and a relatively high number of patients that are in frequent contact with healthcare staff, thus risk of cross-infection which happens in the hospitals (Struelens et al., 1998).

Among nosocomial infections, 90% are caused by bacteria such as *Staphylococcus aureus, Streptococcus spp, Bacillus cereus, Acinetobacter spp.,* coagulase-negative *staphylococci, enterococci, Pseudomonas aeruginosa, Legionella* and members of the Enterobacteriaceae family (*E. coli, Proteus mirabilis, Salmonella spp, Serratia marcescens, Klebsiella*

pneumoniae). However, infections caused by mycobacterial, viral, fungal and protozoal agents are very rare (Bereket et al., 2002).

1.8 Respiratory infections

The respiratory tract is a primary attachment site for opportunistic organisms as well as a major location for respiratory pathogens. In patients with compromised immune systems, microorganisms present in the oral cavity cause opportunistic infections and systemic diseases such as bacterial endocarditis, aspiration pneumonia and osteomyelitis. Among the bacteria, infections caused by *Escherichia coli, Klebsiella pneumonia, Haemophilus influenza, Streptococcus pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris* occur quite frequently (Islam, 2011; Felmingham et al., 2002).

Respiratory tract infections can either be of the upper respiratory tract or the lower respiratory tract. The upper respiratory tract consists of; the nose, nasal passages, paranasal sinuses, the pharynx and the portion of the larynx above the vocal codes. These parts mainly aid in breathing and as well as speech. The infections that affect this region include; common cold, laryngitis, pharyngitis / tonsillitis, acute rhinitis, acute rhinosinusitis, and acute otitis media.

The lower respiratory tract consists of the bronchi, bronchioles, and the alveoli. These structures mainly help in gaseous exchange in the lungs. Infections that affect this region include; acute bronchitis, bronchiolitis, pneumonia, and tracheitis.

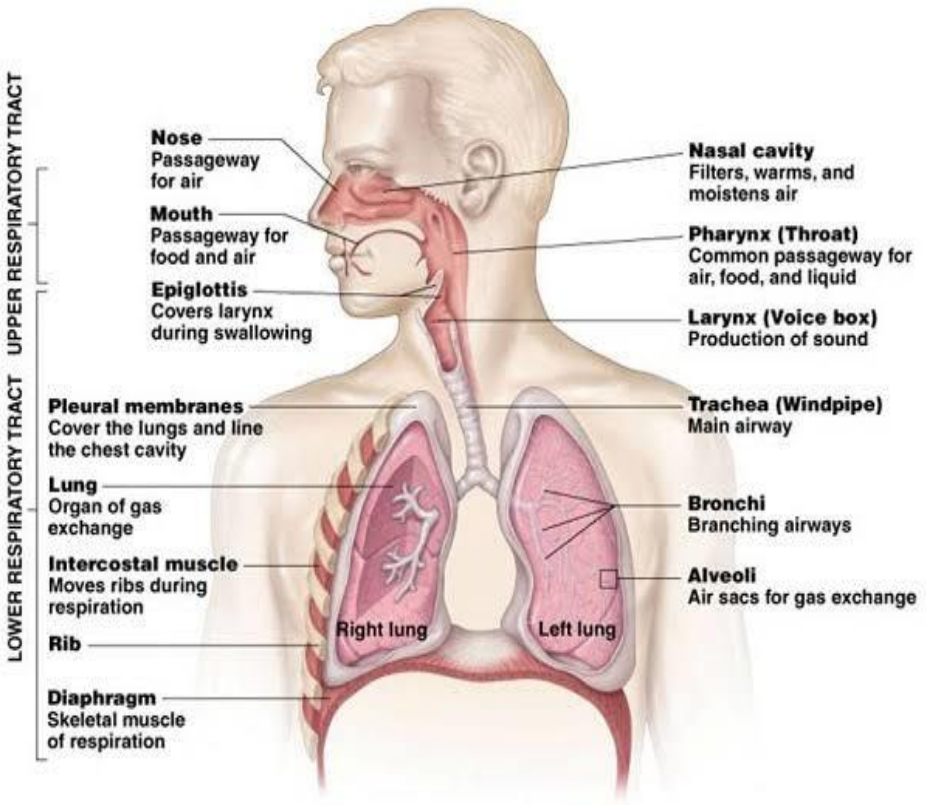


Fig 2: The Human respiratory system

Globally, respiratory infections account for significant morbidity and mortality and amongst all diseases, lower respiratory tract infections have caused more problems on human health compared to ischemic heart disease and diabetes mellitus respectively. (Centre for Clinical Practice at NICE (UK), 2008).

Based on the Global Burden of Disease 2015 study (GBD 2015), chronic obstructive pulmonary disease (COPD) and lower respiratory tract infections (LRTIs) rank third and fourth as the most common causes of death respectively. Among LRTIs, Pneumonia is the

most common and annually, about 24.8 per 10,000 adults are affected by it (Mahashur, 2018).

The most usual way to treat these infections is by antibiotic therapy and it is in Europe where it is done extensively. It was reported that the prescription of antibiotics to treat LRTIs varies from one region to another. For example, in the United Kingdom and France, penicillins are preferred; in Germany, tetracyclines while macrolides are highly used in Spain. However, in Italy, third-generation cephalosporins are used. (Huchon et al., 1996). However due to the emergence of resistant strains of bacteria that cause some of these diseases, the use of antibiotics to treat LRTIs is has become ineffectual.

1.9 Aims and Objectives

a) Examining the antimicrobial activity of commonly used antibiotics against pathogens isolated from the Lower Respiratory Tract.

b) Establishing the susceptibility and prevalence patterns of the pathogens

CHAPTER 2

MATERIALS AND METHODS

Chapter 2

Materials & Methods

2.1 Apparatus and Reagents

Petri Dishes, Test tubes, vials, Glass rods, Bunsen burner, Laminar Air Flow Cabinet, Conical Flasks, Measuring Cylinders, Antibiotic Discs, Autoclave Machine, Incubator machine, Inoculation loop, Weighing balance, Measuring scale.

2.2 Reagents

Sodium Chloride, Distilled water, Glycerol, Media (Muller Hinton Agar, Nutrient Agar), McFarland standard solution.

2.3 Place and Time frame of Research

The study was done at BRAC University in Dhaka, Bangladesh. All the Laboratory work and data analysis were done in the Microbiology Research Laboratory of Department of Mathematics and Natural Sciences (MNS) of BRAC University. The period of the study was from June 2019 to November 2019.

2.4 Sample size

A total of 57 bacterial samples were collected from the microbiology laboratory of the National Institute of Diseases of the Chest and Hospital (NIDCH) in Dhaka.

2.5 Methodology

The research was aimed at observing the effect of various antibiotic agents on the clinical isolates of the pathogens obtained from the lower respiratory tract of patients at the National Institute of Diseases of the Chest and Hospital in Dhaka and then establish the antibiotic susceptibility and prevalence patterns. Thereafter, the samples that were resistant to multiple drugs were identified.

2.6 Collection of samples

Clinical isolates of the bacteria were collected from the Microbiology Department of the National Institute of Diseases of the Chest and Hospital (NIDCH). The collection of the samples was done from June 2019 to November 2019. The isolates were sub-cultured on Nutrient Agar (NA) slants and then taken to the Microbiology Laboratory of BRAC University. The nutrient agar slants were then incubated at 37^{o}C for 24 hours. Thereafter, the samples were transferred to nutrient agar plates by streak plate method and then used overtime from the subcultures. These samples were then stocked in glycerol media and stored at a temperature of -20^{o}C in the refrigerator.

Fig 3: Stock samples of *Pseudomonas aeruginosa*

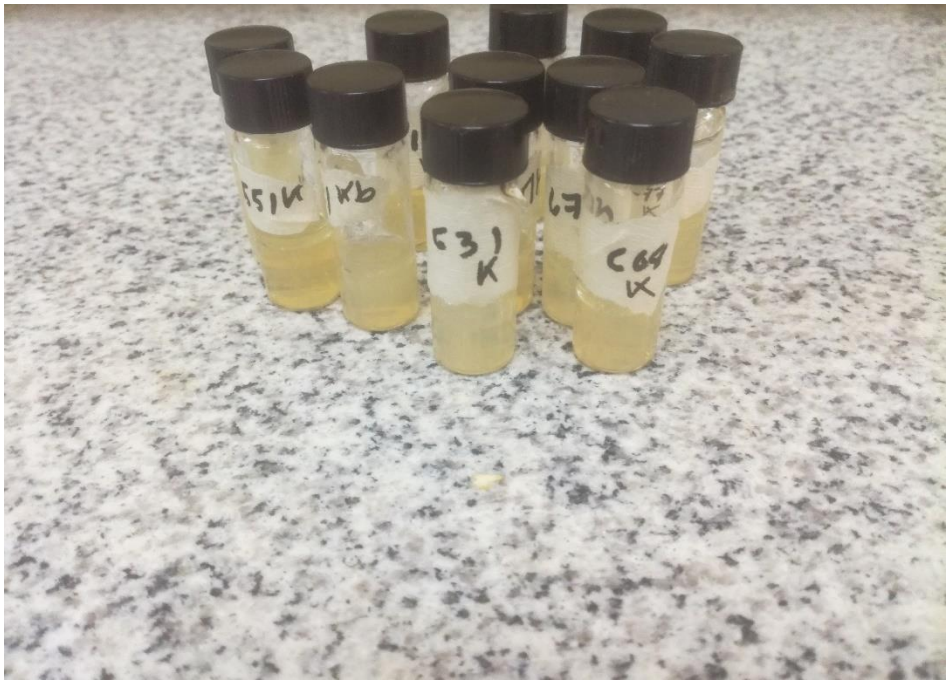


Fig 4: Stock samples of *Klebsiella pneumoniae*

2.7 Collection of antibiotics

All the Antibiotics used in the study were obtained from A_Z pharmacy that is located adjacent to building 1 of BRAC University. The antibiotics used in the study included the following; Amoxicillin-clavulanic acid, azithromycin, cefixime, tetracycline, Colistin, aztreonam, cefepime, and cephalexin.

2.8 Preparation of media

The media used in the study included;

1. Nutrient Agar

2. Muller Hinton Agar

Both media were used during the study and were prepared as per standard protocol and used within a week

2.9 Preparation of Nutrient Agar (NA)

Nutrient agar was used for the cultivation and subculture of the collected pathogens to allow the organisms to grow. Nutrient Agar is popularly used because it allows the growth of a variety of bacteria and fungi. Besides, it also contains many nutrients that are required for the growth of bacteria. In this study, a specific amount of NA was first measured by an electronic balance and then dissolved in the required amount of water in a conical flask by heating over a bunsen burner. The media was then autoclaved at 121^{o}C and 15 psi for a time of 15 minutes. It was then plated, allowed to solidify and was put in the incubator to check for sterility.

2.10 Preparation of Muller Hinton Agar (MHA)

Muller-Hinton Agar is a microbial growth medium used commonly for antibiotic susceptibility testing. Moreover, it is used to study the effects of antibiotic agents and plant extracts on bacterial growth. Also, it is a non-selective and non-differential medium which allows antibiotics to be used favorably.

During the study, a specific amount of MHA was initially measured by an electronic balance and then dissolved in the corresponding amount of distilled water in a conical flask by heating over a bunsen burner till all agar was melted. Thereafter, the top of the flask was covered with aluminum foil paper and then autoclaved at 121^{o} C and 15 psi for 15 minutes. The media was then plated into large sterilized plates where it solidified before storage at 40^{o}C.

2.11 Preparation of physiological saline

Physiological saline consists of a sterilized solution of sodium chloride (NaCl). It is isotonic to body fluids and important for temporary maintenance of living tissue. Besides, it is used as a solvent during the administering of drugs.

0.9g of sodium chloride was dissolved in 100 ml of distilled water and then stirred till all the salt dissolved. 5 ml of saline solution was then transferred to several test tubes and then autoclaved.

The saline was made for the preparation of a bacterial suspension that was matched with the McFarland standard solution.

2.12 Disc Diffusion Method / Kirby- Bauer Test

The disc diffusion test was used to determine the sensitivity of bacteria to antibiotics.

Filter-paper disks, impregnated with an antibiotic agent were used in this test to study how the bacterial growth is affected. This method is cheap and very efficient for primary screening of antimicrobial resistance.

The disc diffusion test was done to determine the antibiotic-resistant pattern of the bacteria and also to categorize to MDR, XDR, and PDR by the guideline of clinical and Laboratory Standards Institute (CLSI). In this study, 8 different antibiotics were used**.** To ensure the consistency of the results, each experiment of a particular sample was performed twice and the resulting average was noted.

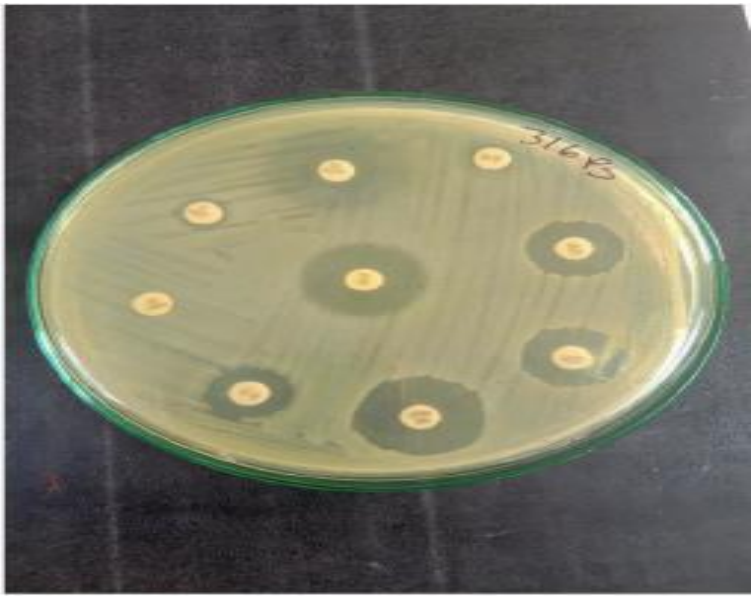


Fig 5: **Disc diffusion test for** ***Pseudomonas aeruginosa***

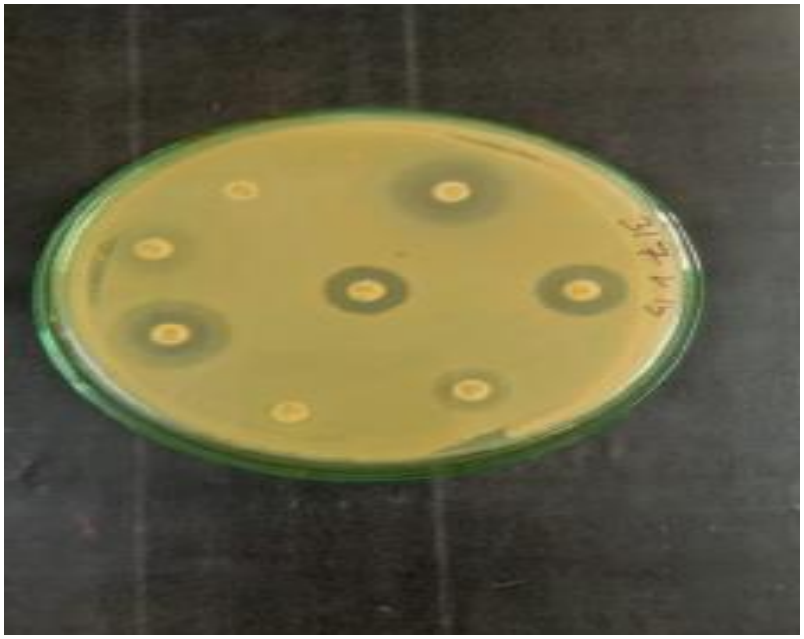


Fig 6: **Disc diffusion test for** ***Klebsiella pneumonia***

CHAPTER 3

RESULTS

Chapter 3

Results

3.1 Prevalence of bacterial pathogens among Lower Respiratory Tract infected patients

A total of 57 bacterial samples were collected from the NIDCH hospital in Dhaka. Since the samples were already tested and confirmed by the health care professionals from the laboratory at the hospital, further tests were not required. Among these, 28 of them were *Klebsiella pneumonia*, 25 were *Pseudomonas aeruginosa* and 4 were *E. coli*. They were obtained from patients that were suffering from respiratory tract infections.

Table 1: Prevalence of bacterial pathogens among Lower Respiratory Tract infected patients (N = 57)

Pathogen	Number (n)	Percentage (%) amongst total isolates
K. pneumonia	28	49.12
P. aeruginosa	25	43.86
E. coli	4	7.02

3.2. Antimicrobial sensitivity and resistance patterns of the pathogens

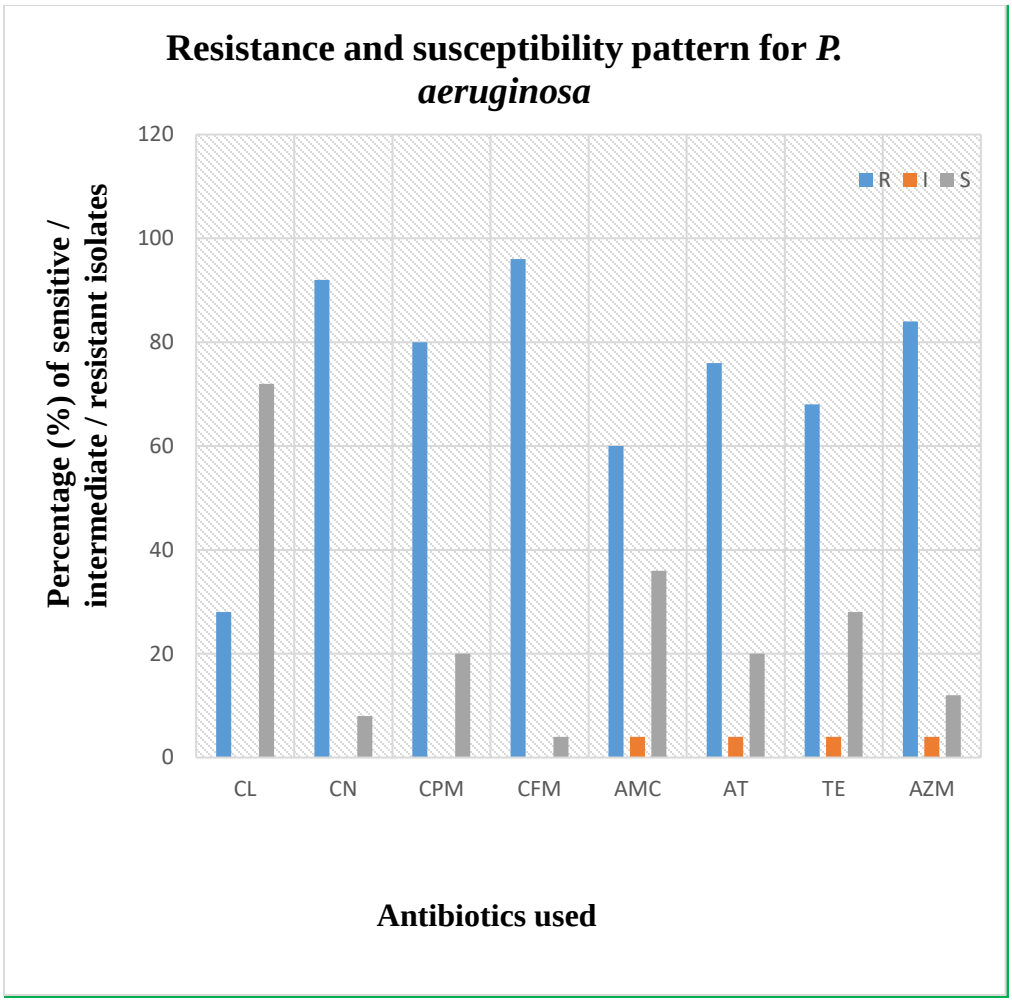
For all the 57 bacterial samples, 8 different antibiotics were consistently used to determine the sensitivity and resistance patterns of the isolates. When the antibiotics were placed on the bacterial cultures in MHA media after a period of 24 hours, clear zones (Zones of Inhibition) were observed. The zones were measured by a scale and the results were recorded carefully. However, for some samples, no clear zone was formed.

The results obtained were then compared to a CLSI (Clinical & Laboratory Standards Institute) chart, which contains information of standard measurements of the respective Zones of inhibition from the CLSI chart and how they should be interpreted. Letters S, I and R were used to show that a particular sample was Sensitive or Intermediate or Resistant to a specific antibiotic.

Table 2: Antibiogram for *P. aeruginosa*

SAMPLE ID	Zone of inhibition (mm)															
	CL		CN		CPM		CFM		AMC		AT		TE		AZM	
	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT
Ps235	18	S	0	R	21	S	18	S	30	S	29	S	17	I	12	R
Ps212	12	R	26	S	0	R	0	R	28	S	0	R	10	R	0	R
Ps216	20	S	0	R	0	R	0	R	30	S	0	R	21	S	12	R
Ps236	15	S	0	R	0	R	0	R	30	S	0	R	21	S	0	R
Ps237	18	S	0	R	0	R	0	R	0	R	22	S	0	R	0	R
Ps400	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps438	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps327	20	S	0	R	11	R	0	R	20	S	0	R	21	S	0	R
Ps300	16	S	0	R	21	S	0	R	23	S	0	R	25	S	20	S
Ps310	19	S	0	R	11	R	0	R	0	R	10	R	0	R	0	R
Ps316	17	S	0	R	22	S	0	R	0	R	14	R	19	S	13	R
Ps309	19	S	0	R	0	R	0	R	19	S	21	S	11	R	0	R
Ps319	16	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps251	11	S	24	S	0	R	0	R	10	R	0	R	23	S	24	S
Ps524	0	R	0	R	12	R	0	R	0	R	0	R	12	R	0	R
Ps666	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps664	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps518	13	S	0	R	0	R	0	R	0	R	0	R	20	S	19	S
Ps520	19	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps545	19	S	0	R	12	R	0	R	19	S	16	I	0	R	12	R
Ps550	22	S	0	R	26	S	0	R	24	S	30	S	0	R	0	R
Ps687	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps522	22	S	0	R	0	R	0	R	0	R	0	R	0	R	0	R
Ps535	15	S	0	R	25	S	0	R	0	R	22	S	0	R	0	R
Ps544	25	S	0	R	0	R	0	R	16	I	0	R	12	R	12	I

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant, CFM =Cefixime, CPM= Cefepime, CN = Cephalexin, AZM = Azithromycin, AMC = Amox clav, CL = Colistin, TE = Tetracycline, AT = Aztreonam



R	**Resistant**
I	**Intermediate**
S	**Sensitive**

Fig 7: Resistance & susceptibility pattern for *Pseudomonas aeruginosa.*

The highest resistance was observed against cefixime, followed by cephalexin, with 96% and 92% of the *Pseudomonas aeruginosa* isolates resistant to these antibiotics respectively. On the other hand, the highest sensitivity was observed with colistin and only 28% of the isolates showed resistance to it.

Table 3: Antibiogram for *K. pneumonia*

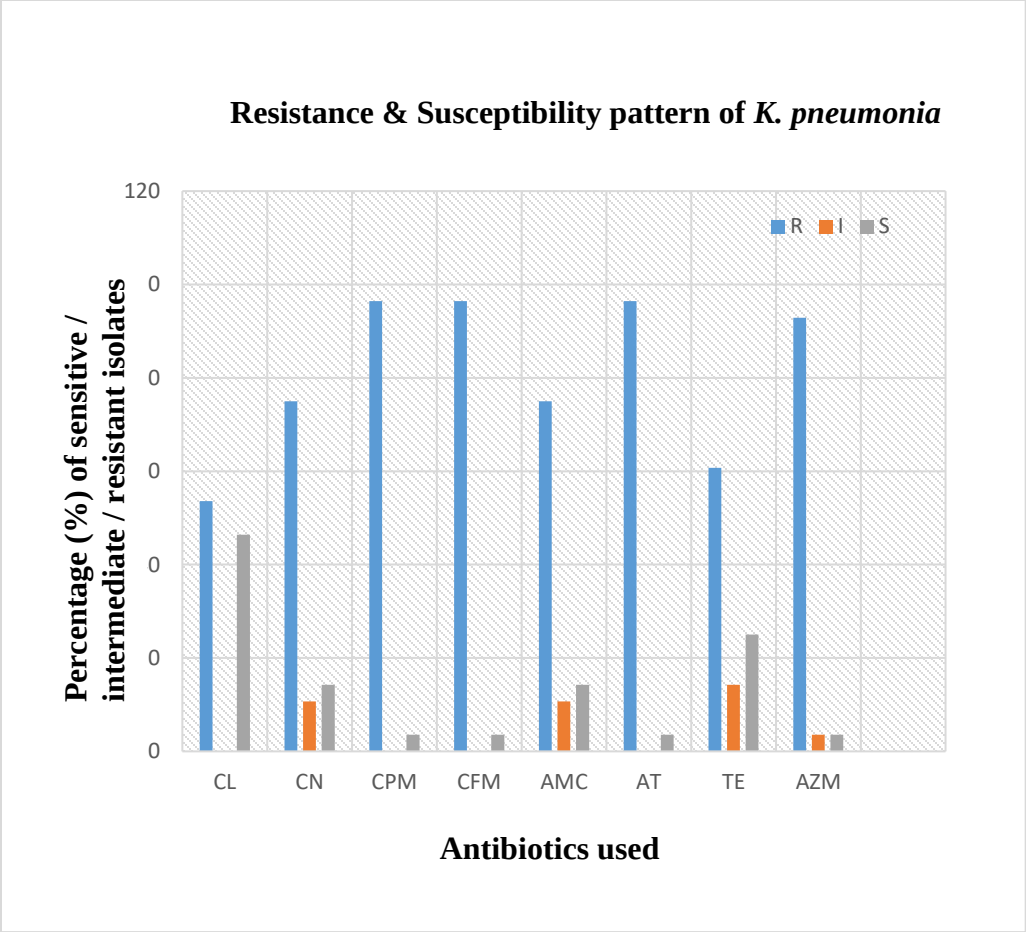
SAMPLE ID	Zone of Inhibition (mm)															
	CL		CN		CPM		CFM		AMC		AT		TE		AZM	
	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT
K215	18	S	15	I	0	R	0	R	0	R	11	R	0	R	0	R
K231	17	S	0	R	0	R	0	R	0	R	0	R	10	R	0	R
K228	12	S	22	S	0	R	0	R	0	R	0	R	25	S	15	I
K230	14	S	30	S	0	R	0	R	11	R	0	R	22	S	0	R
K248	0	R	30	S	0	R	0	R	0	R	0	R	10	R	0	R
K315	14	S	0	R	0	R	0	R	0	R	12	R	12	R	10	R
K246	9	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
K265	14	S	0	R	0	R	0	R	14	I	0	R	0	R	11	R
K245	10	R	0	R	0	R	0	R	0	R	0	R	11	R	0	R
K253	12	S	0	R	0	R	0	R	0	R	13	R	12	R	23	S
K297	18	S	0	R	10	R	0	R	0	R	14	R	17	I	0	R
K317	16	S	0	R	0	R	0	R	18	S	0	R	12	R	12	R
K277	19	S	0	R	0	R	0	R	0	R	0	R	15	I	0	R
K305	15	S	15	I	26	S	22	S	21	S	24	S	20	S	12	R
K430	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
K433	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
K422	0	R	0	R	0	R	0	R	18	S	0	R	0	R	0	R
k307	9	R	19	S	0	R	0	R	22	S	0	R	19	S	0	R
K620	0	R	0	R	0	R	0	R	10	R	0	R	0	R	11	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant, CFM =Cefixime, CPM =Cefepime, CN = Cephalexin, AZM = Azithromycin, AMC = Amox clav, CL = Colistin, TE = Tetracycline, AT = Aztreonam

Table 4: Antibiogram for *K. pneumonia*

SAMPLE ID	Zone of Inhibition (mm)															
	CL		CN		CPM		CFM		AMC		AT		TE		AZM	
	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT
K608	9	R	15	I	0	R	0	R	17	I	0	R	25	S	0	R
K653	0	R	0	R	0	R	0	R	0	R	0	R	17	I	0	R
K706	0	R	0	R	0	R	0	R	13	R	0	R	24	S	0	R
K631	0	R	0	R	0	R	0	R	0	R	0	R	0	R	10	R
K621	14	S	0	R	0	R	0	R	0	R	0	R	0	R	11	R
K677	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
K674	0	R	0	R	0	R	0	R	0	R	0	R	0	R	0	R
K614	16	S	0	R	0	R	0	R	16	I	14	R	0	I	0	R
K647	10	R	20	S	0	R	0	R	10	R	0	R	0	S	0	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant, CFM =Cefixime, CPM = Cefepime, CN = Cephalexin, AZM = Azithromycin, AMC = Amox clav, CL = Colistin, TE = Tetracycline, AT = Aztreonam



R	**Resistant**
I	**Intermediate**
S	**Sensitive**

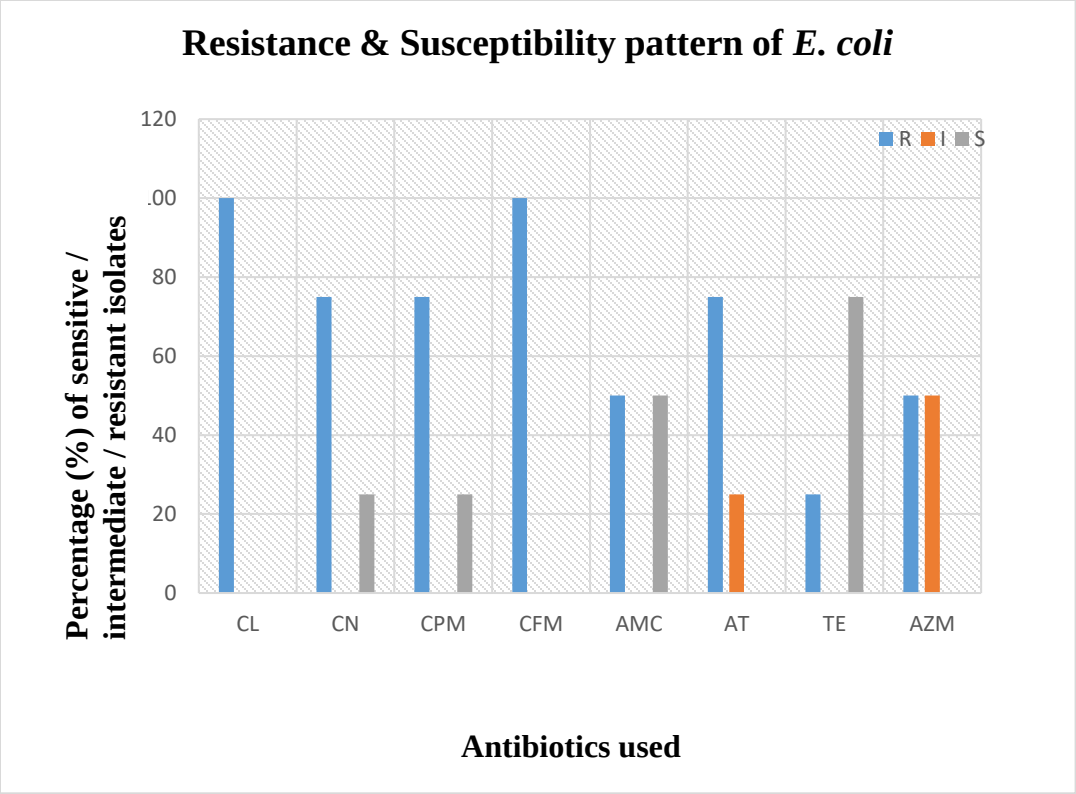
Fig 8: Resistance & susceptibility pattern for *Klebsiella pneumonia.*

The highest resistance was observed against cefixime, cefepime and, aztreonam with 96.3% of the *Klebsiella pneumoniae* isolates resistant to each of these antibiotics respectively. On the other hand, the highest sensitivity was observed with colistin and 53.57% of the isolates showed resistance to it.

Table 5: Antibiogram for *E. coli*

SAMPLE ID	Zone of Inhibition (mm)															
	CL		**CN**		**CPM**		**CFM**		**AMC**		**AT**		**TE**		**AZM**	
	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT	ZI	INT
E209	0	R	0	R	0	R	0	R	0	R	0	R	22	S	0	R
E234	0	R	0	R	0	R	0	R	0	R	10	R	13	R	14	I
E232	10	R	19	S	0	R	0	R	20	S	0	R	27	S	17	I
E551	0	R	0	R	19	S	0	R	25	S	19	I	22	S	0	R

ZI= Zone of Inhibition, INP= Interpretation, S= Sensitive, I= Intermediate, R=Resistant, CFM =Cefixime, CPM = Cefepime, CN = Cephalexin, AZM = Azithromycin, AMC = Amoxy clav, CL = Colistin, TE = Tetracycline, AT = Aztreonam



R	**Resistant**
I	**Intermediate**
S	**Sensitive**

Fig 9: Resistance & susceptibility pattern for *E. coli.*

The highest resistance was observed against cefixime, followed by colistin, with 100% of the isolates resistant for each of these antibiotics. On the other hand, the highest sensitivity was observed with tetracycline and only 25% of the isolates showed resistance to it.

3.3. Prevalence of resistant patterns of the bacteria

From the results obtained from the disc diffusion tests, the percentage of resistance of the samples towards the particular antibiotics was determined. From these values, the most effective of the used antibiotics was determined.

Table 6: Prevalence of resistant pattern of the bacteria

No.	Antibiotic	Total no. of resistant bacterial isolates (n)	Percentage of resistant bacterial isolates (%)
1	Colistin	26	45.61
2	Cephalexin	47	82.46
3	Cefepime	50	87.72
4	Cefixime	55	96.49
5	Amox clav	38	66.67
6	Aztreonam	49	85.96
7	Tetracycline	35	61.40
8	Azithromycin	49	85.96

Overall, Cefixime was the least effective antibiotic used, then followed by Cefepime, with 96.49% and 87.7% of the isolates resistant to these antibiotics respectively. On the other hand, Colistin was most effective of all the antibiotics with 45.61% of the isolates resistant to it. From the results of this study, the order of effectiveness was, Colistin > Tetracycline > Amox clav >Cephalexin > Aztreonam and Azithromycin > Cefepime > Cefixime.

3.4 Multidrug-Resistant (MDR) bacterial isolates

Most of the bacterial isolates in this study were resistant to at least two antibiotics from different classes. Organisms that are susceptible to at least one agent in three or more antimicrobial categories are regarded as Multi-Drug Resistant (Magiorakos et al., 2011).

Table 7: Distribution of MDR isolates

S. No	Pathogens	No. (%) of Bacteria	No. (%) of MDR
1	*K. Pneumonia*	28 (49.12)	27 (96.43)
2	*P. Aeruginosa*	25 (43.86)	24 (96)
3	*E. coli*	4 (7.02)	0 (100)

As shown above, in **Table 6**, almost all the isolates of the pathogens in this study were considered MDR. All isolates of *E. coli*, 96.43% *of K. pneumonia* isolates and 96% isolates of *P. aeruginosa* were MDR.

CHAPTER 4

DISCUSSION

Chapter 4

Discussion

The principal pathogens that usually infect the lower respiratory tract worldwide are *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Klebsiella ssp*, *Haemophilus influenza*, *E. coli*, and *Staphylococcus aureus* (Acar, 1985). These pathogens have caused great concern to the public and are responsible for many deaths around the globe.

In the present study antimicrobial activity of commonly used antibiotics i.e. (cefixime, cefepime, cephalexin, azithromycin, amox clav, colistin, tetracycline, and aztreonam) against the pathogens (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *E. coli*) were examined.

Of the 57 samples that were collected, 49.12% were *Klebsiella pneumonia*, 43.86% were *Pseudomonas aeruginosa* and 7.02% were *E. coli*. A study conducted in India showed that *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *E. coli* were among the most common gram-negative bacilli pathogens from patients suffering from Lower Respiratory Tract Infections (LRTIs) in the ICU. In the study, the samples were collected from trans-tracheal or bronchial aspirates of the patients and they included; *Pseudomonas aeruginosa* (35%), *Acinetobacter baumannii* (23.6%), *Klebsiella pneumonia* (13.6%), *E. coli* (7.4%), *C. freundii* (4.9%), *S. aureus* (1.8%), Coagulase-negative *S. aureus* (0.6%) and *Candida spp* (1.8%) (Bala et al., 2009).

Navaneeth & Belwadi (2002), found similar results with among the gram-negative pathogens from the lower respiratory tract being Klebsiella pneumonia (29.8%), *Pseudomonas ssp* (17.2%), *E. coli* (6.3%), *Enterobacter ssp* (4.2%) *Proteus Mirabilis* (3.3%), *Citrobacter ssp* (2.9%), *P. Aeruginosa* (2.1%).

Even though the percentages vary depending on the studies, the presence of these pathogens i.e. *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumonia* nonetheless contribute to the infections to most of the patients suffering from respiratory diseases.

Antibiotics have been used since the 1940s to treat bacterial infections. However, recently their effectiveness has been reduced since more resistant strains of bacteria have emerged towards them worldwide. The most prime cause of resistance is the excessive use of these antibiotics, which has compelled the bacteria to modify or acquire genes of resistance towards them (Sengupta et al., 2013).

After carrying out the disc diffusion tests, it was observed that most samples were resistant to almost all the antibiotics used. The highest resistance was observed against cefixime, with 96.49% of the samples resistant to this antibiotic. Interestingly, the highest susceptibility was observed with colistin, with 45.61% of the samples were resistant to it. For the rest of the antibiotics, resistance against them by the bacteria was beyond 60%.

Nepal et al (2018) showed that these pathogens were highly resistant to cefixime. From the study, 63.3% of *Klebsiella pneumonia* and 72.7% of *E. coli* were resistant to this antibiotic. Another study conducted by Bhuiya et al (2018), showed that 100% of isolates of *Pseudomonas aeruginosa* from the clinical samples were resistant to cefixime.

According to a study conducted on antimicrobial resistance in Bangladesh, it was revealed that cefixime is among the most frequently used antibiotics to treat bacterial infections. Moreover, due to inappropriate prescription procedures by local pharmacies, the increase in resistance by these pathogens has been intensified (Faiz & Basher, 2011). Therefore, this could be the reason for the very high percentages of resistance against cefixime in this study.

This study also showed that most samples of *Pseudomonas aeruginosa* (72%) and *Klebsiella pneumonia* (46.43%) were more susceptible to colistin compared to any other antibiotics. However, this was not the case for *E. coli* since most of the isolates were resistant to colistin. Nonetheless, 75% of the *E. coli* samples were susceptible to tetracycline.

Colistin is one of the old antibiotics used for the treatment of bacterial infections. It is the last line treatment antibiotic especially for MDR bacteria like *Pseudomonas aeruginosa*, *K. pneumonia*, and *Acinetobacter ssp*. However, it is less frequently used due to its significant toxicity levels (Lim et al., 2010).

Tetracycline is also another old antibiotic used to treat bacterial infections. However, it's not recommendable for children and pregnant women as it causes depression of skeletal growth in premature infants and also discoloration of teeth in children hence it is rarely used (Speer et al., 1992).

However, Habib et al (2003) reported that 90% of the *E. coli* isolates were resistant to tetracycline antibiotics, which strongly contradicts findings from this study. Therefore more research about the tetracycline effect on *E. coli* is needed.

After examining the antibiotic resistance patterns of the bacterial isolates, it was found that most of them were resistant to one or more antibiotics. Magiorakos et al (2011) suggests that organisms that are susceptible to at least one agent in three or more antimicrobial categories are regarded as Multi-Drug Resistant (MDR).

In this study, all the *E. coli* isolates were considered multidrug-resistant. 96% of the *Pseudomonas aeruginosa* and 96.43% of the isolates of *Klebsiella pneumonia* were multidrug-resistant.

Nepal et al (2018) showed that 50% of *Klebsiella pneumonia*, 54.54% *E. coli* and 12.5% of *Pseudomonas aeruginosa* isolates were MDR. This shows that more multidrug-resistant strains are emerging at a very fast rate, a problem that should be tackled immediately.

The threat posed by multidrug-resistant bacteria is very alarming and should be combated immediately or else patients suffering from such bacterial infections like Pneumonia may have minute chances to live. The most promising alternatives currently include; use of probiotics, antibodies, vaccines, and adjunct therapies (Aslam et al., 2018).

Basing on this study, it may be suggested that misuse of drugs should be highly discouraged by all possible means. Besides, a proper plan should be put in place to monitor the distribution as well as the consumption of antibiotic drugs.

Also, using cefixime alone to treat bacterial infections should be discouraged as it may allow more pathogens to acquire resistant genes.

Another suggestion from the findings of this study is that antibiotic drugs such as colistin may be used if the patients are in critical condition, most especially if there is no other option but a lot of care and follow up should be done by a health care professional to monitor the situation of the patient.

Using tetracycline for treatment of infections most especially those caused by *E. coli* may also be considered. However, more research about its antimicrobial effect on *E. coli* should be done since several reports show different results.

Finally, more efforts should also be put in to develop alternative methods to treat bacterial infections other than antibiotics or else more patients are likely to lose their lives.

Conclusion

Susceptibility patterns of resistance to commonly used antibiotics were conducted in this study. *P. aeruginosa*, *E.coli*, and *K. pneumonia* were found to be among the common causes of respiratory infections. These pathogens were also found to be resistant to multiple antibiotics, most especially cefixime. This was probably due to the misuse of drugs by both patients and the clinicians and also the inadequate enforcement in the way they are supplied.

This study confirms the reports on the increase in antibiotic resistance among bacteria, which is a serious threat that requires immediate action.

Therefore, critical monitoring of the distribution and use of antibiotics should be emphasized to prevent the problem from escalating further.

Also, new methods of combatting antibiotic resistance through research should be encouraged such that the mortality rate, especially from patients who suffer from respiratory infections caused by multi-drug resistant pathogens, is reduced.

CHAPTER 5

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