ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CEFTAZIDIME-AVIBACTAM AMONG DIFFERENT CLINICAL SPECIMENS AGAINST *ESCHERICHIA COLI* AND KLEBSIELLA PNEUMONIAE

DR. AAFAQ KHAN

06-114182-001

BAHRIA UNIVERSITY MEDICAL & DENTAL COLLEGE

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CEFTAZIDIME-AVIBACTAM AMONG DIFFERENT CLINICAL SPECIMENS AGAINST ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE

DR. AAFAQ KHAN

06-114182-001

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Philosophy (Pathology)

Department Of Pathology

BAHRIA UNIVERSITY MEDICAL & DENTAL COLLEGE

September 2020

BAHRIA UNIVERSITY ISLAMABAD

APPROVAL SHEET

SUBMISSION OF HIGHER RESEARCH DEGREE THESIS

Candidate's Name: Dr. Aafaq Khan

Discipline: M.Phil. Program Pathology

Faculty/Department: Pathology

I hereby certify that the above candidate's work including the thesis has been completed to my satisfaction and that the thesis is in a format of an editorial standard recognized by the faculty / department as appropriate for examination.

PROF. DR. NAVEED FARAZ
MBBS, MPHIL, MBA, MAS, DPA
Prof of Pathology
Bahria University Medical And Dental College

Signature: _____

Principal Supervisor: Prof. Dr. Naveed Faraz

Date: 24-07-2020

The undersigned signifies that:

1. The candidate presented at a pre-completion seminar, an overview and synthesis of major findings of the thesis, and that the research is of a standard and extent appropriate for submission as a thesis.

2. I have checked the candidate's thesis and its scope, format and editorial standards are recognized by the faculty / department as appropriate.

Signature: _____

Head of Department: Prof. Dr. Ambreen Usmani

Date: 24-07-2020

APPROVAL FOR EXAMINATION

Student's Name: Dr. Aafaq Khan Registration No: 59801

Program of Study: M.Phil.

Thesis Title: Antimicrobial Susceptibility Pattern of Ceftazidime-Avibactam Among Different Clinical Specimens Against *Escherichia Coli* and *Klebsiella Pneumoniae*

It is to certify that the above student's thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index at 9 % that is within the permissible limit set by the HEC for the MPhil degree thesis. I have also found the thesis in a format recognized by the BU for the MPhil thesis.

Principal Supervisor's Seal & Signature:

PROF. DR. NAVEED FARAZ
MBBS, MPHIL, MBA, MAS, DPA
Prof of Pathology
Bahria University Medical And Dental College

Date: 24-07-2020 **Name: Prof. Dr. Naveed Faraz**

AUTHOR'S DECLARATION

I, Dr. Aafaq Khan hereby state that my M.Phil. thesis titled "ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CEFTAZIDIME-AVIBACTAM AMONG DIFFERENT CLINICAL SPECIMENS AGAINST *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*" is my own work and has not been submitted previously by me for taking any degree from this university, **The Bahira University** or anywhere else in the country/world. At any time if my statement is found to be incorrect even after my graduation the university has the right to withdraw/cancel my M.Phil. degree.

Name of student: Dr. Aafaq Khan

Date: 24-07-2020

THESIS COMPLETION CERTIFICATE

Student's Name: Dr. Aafaq Khan Registration No. 59801

Program of Study: M.Phil.

Thesis Title: Antimicrobial Susceptibility Pattern of Ceftazidime-Avibactam Among Different Clinical Specimens Against *Escherichia Coli* and *Klebsiella Pneumoniae*

It is to certify that the above student's thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index at 9 % that is within the permissible limit set by the HEC for the M.Phil. degree thesis. I have also found the thesis in a format recognized by the BU for the M.Phil. thesis.

PROF. DR. NAVEED FARAZ
MBBS, MPHIL, MBA, MAS, DPA
Prof of Pathology
Bahria University Medical And Dental College

Principal Supervisor's Seal & Signature: _____

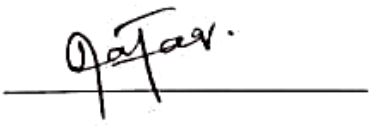
Date: 24-07-2020 **Name:** **Prof. Dr. Naveed Faraz**

PLAGIARISM UNDERTAKING

I, solemnly declare that research work presented in the thesis titled "Antimicrobial Susceptibility Pattern of Ceftazidime-Avibactam among Different Clinical Specimens against *Escherichia Coli* and *Klebsiella Pneumoniae*" is solely my research work with no significant contribution from any other person. Small contribution / help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero-tolerance policy of the HEC and Bahira University towards plagiarism. Therefore, I as an Author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred / cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MPhil degree, the university reserves the right to withdraw / revoke my MPhil degree and that HEC and the University has the right to publish my name on the HEC / University website on which names of students are placed who submitted plagiarized thesis.

Student / Author's Sign: 

Name of the Student: Dr. Aafaq Khan

Date : 24-07-2020

THESIS COMPLETION CERTIFICATE

Student's Name: Dr. Aafaq Khan Registration No. 59801

Program of Study: M.Phil.

Thesis Title: Antimicrobial Susceptibility Pattern of Ceftazidime-Avibactam Among Different Clinical Specimens Against Escherichia Coli and Klebsiella Pneumoniae

It is to certify that the above student's thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index at 09 % that is within the permissible limit set by the HEC for the MPhil degree thesis. I have also found the thesis in a format recognized by the BU for the MPhil thesis.

Co-Supervisor's Seal & Signature: _____

Date: 24-07-2020 **Name:** SURG COMMANDER FAISAL HANIF

DEDICATION

"This dissertation is dedicated to the victims of

Army Public School attack"

May every compassionate heart weeping with me be

Perhaps it may awaken those who may unconscious be

Allama Muhammad Iqbal

ACKNOWLEDGEMENT

I am grateful to Almighty Allah for giving me the patience, strength, knowledge and understanding to go through this project successfully.

My sincere appreciation to my supervisor Professor Dr. Naveed Faraz for his special interest and kind guidance. He has been a continuous source of great encouragement and inspiration for me. I respect and thanks to my co-supervisor Dr. Fasial Hanif for continuously helping me through this project. I am indebted to Dean health sciences, Dr. Ambreen Usmani, for her persistant counseling and mentoring. I am thankful to vice-principal medical college Dr. Khalid Mustafa, his doors were always open for me and he guided me throughout my M.Phil Program.

I owe my deep gratitude to our project guide and chairperson of M.Phil program Dr. Nasim Karim for trusting me and giving me the opportunity to get into MPhil program. Her constant encouragement helped me a lot. I will be forever grateful to her, for her cooperation and kind assistance.

I would like to mention and thank Dr. Luqman Satti for suggesting me this research topic. I heartily thank Dr. Saman Nadeem and Dr. Ashfaq Hussain, who took interest in my research program and gave valuable suggestions during this project work. I am also thankful to statistician Muhammad Irfan for his effort and hard work in analyzing the data

I am thankful to my family, especially my father, who has put special effort for my education and also my mother for being so supportive and kind to me through my MPhil program and research project. I am very thankful to my elder brother Dr. Nasir khan who always helped me in my studies from childhood to till date.

I would not forget to mention my colleague Dr. Kamran and Dr. Sayher Kazmi for their help and guidance in this project. Also, my dear friends Dr. Ayaz Mehmood Qaisarani, Dr. Shizma Junejo, Miss Nimra Naz Siddiqui and Dr. Farhan Farooq for helping me in this project from time to time.

ABSTRACT

As defined by WHO, "Antibiotic resistance is a phenomenon that occurs when bacteria is not able to produce response to the use of particular medicine or medicines". Resistance to antibiotics leads to increased cost of health care, increased morbidity and mortality. There is a need to address the issue of conventional antibiotic resistance by focusing on the pattern of prescription and use of antibiotics. Bacteria achieve antibiotic resistance in various ways; one of the patterns followed by bacteria is by producing enzymes that protect the bacterium. Antimicrobial resistance among members of Enterobacteriaceae is increasing and therapeutic options remain insufficient to treat these infections. The bacteria is resistant to drugs by limiting their uptake, modified its target, inactivate the drug, and stimulate active efflux of a drug. These pathways may be native to the bacteria or acquire from other pathogens. Ceftazidime-avibactam is the combination of drugs effective against all of the resistant isolate of Enterobacteriaceae. Ceftazidime-avibactam is prescribed for the treatment of complicated intra-abdominal infections complicated urinary tract infections, hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). South-Asia is known as a hub for multidrug-resistant (MDR) bacteria. Unfortunately, proper surveillance and documentation of MDR pathogens are lacking in Pakistan. The objectives of the study were to identify the susceptibility of Ceftazidime-avibactam against Escherichia coli and Klebsiella pneumonia and their frequency in different clinical specimens**.** The study design was cross-sectional study. The samples were collected from patients at PNS Shifa Hospital Karachi. The sample size was 150. The age group of individuals ranged from 10-50 years. Ethical permission was taken from hospital review committee. Informed consent for the study was taken from the patients. The data collection form was designed to record the demographic data of patients. The specimens received in lab were inoculated on Blood and MacConkey's agar culture plates. The culture plate was inoculated at 37 ℃ in incubator for 24 to 48 hours. Identification of Enterobacteriaceae was done by colony morphology, gram staining, biochemical tests, and API 20E. After identification, the susceptibility profile of conventional antibiotics was identified against both pathogens. Next, Mueller Hinton agar was used to check the antibiotic

susceptibility of Ceftazidime-avibactam by disk diffusion method and confirmed by E-Test. Ceftazidime-avibactam shows sensitivity against 82.7% of the isolates while 17.3 % isolates were resistant. The minimum inhibitory concentration of microorganisms is measured by E-test method that revealed most of the isolate show sensitivity less than 1 µg/mL concentration whereas few of them showed susceptibility on 2-8 µg/mL concentration, while very limited organisms showed resistance against Ceftazidime-avibactam. We compared our results with other classes of antibiotics used commonly on the samples of Klebsiella pneumonia and Escherichia coli in microbiology laboratory PNS Shifa hospital Karachi. This gives an insight for improved treatment methodologies for future prospects particularly diseases caused by members of Enterobacteriaceae.

It was concluded that Ceftazidime-avibactam is a novel drug combination that shows high sensitivity against Klebsiella pneumoniae and Escherichia coli. Our study results revealed that Ceftazidime-avibactam is more effective on Escherichia coli as compare to Klebsiella pneumoniae. Ceftazidime/avibactam, therefore, presents as an additional treatment option against the multidrug-resistant gram-negative bacteria. It is worth mentioning, no other antibiotic tested has come up with better overall coverage than Ceftazidime-avibactam against resistant *Escherichia coli* and *Klebsiella pneumonia*.

Keywords: Antibiotics resistance, Antibiotics sensitivity, Ceftazidime-Avibactam, Enterobacteriaceae.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

S No	Abbreviations	Stand For
1	CFZ	Ceftazidime avibactam
2	CRE	Carbapenamase resistant Enterobacteriaceae
3	E-TEST	Epsilon meter Test
4	CLSI	Clinical and laboratory standards institute
5	MIC	Minimal inhibitory concentration
6	TSI	Triple sugar iron
7	MDR	Multi drug-resistant
8	MH	Mueller Hinton
9	AST	Antibiotic susceptibility test
10	ESBL	Extended-spectrum beta-lactamase
11	KPC	Klebsiella pneumonia carbapenamase
12	XDR	Xtansive drug resistance
13	VAP	Ventilator-associated pneumoniae
14	CAP	Community-acquired pneumoniae
15	CDC	Center of disease control
16	CPO	Carbapenem producing organism
17	CDDT	Combine disk diffusion test
18	OXA	Oxacillinase
19	VABP	Ventilator-associated bacterial pneumoniae
20	NBL	Naso broncial lavage
21	API	Analytic profile index
22	DD	Disk diffusion
23	PBP	Penicillin-binding protein
24	MBL	Mettalo beta-lactam

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Infections account for a major cause of death throughout the developing world. This is mainly due to the emergence of newer infectious agents and more specifically due to the appearance of antimicrobial resistance. With time, the bacteria have become smarter and along with it, massive imprudent usage of antibiotics in clinical practice has resulted in resistance of bacteria to antimicrobial agents. The antimicrobial resistance is recognized as a major problem in the treatment of microbial infections. The biochemical resistance mechanisms used by bacteria include the following: antibiotic inactivation, target modification, altered permeability, and "bypass" of metabolic pathway. Determination of bacterial resistance to antibiotics of all classes (phenotypes) and mutations that are responsible for bacterial resistance to antibiotics (genetic analysis) are helpful (Kapoor, Saigal, & Elongavan, 2017).

The inadequate and inappropriate prescription practices of antibiotic drugs are responsible to develop resistance (Ayukekbong, Ntemgwa, & Atabe, 2017). The underdose of drugs develops the new bacterial strain which is resistant against conventional antibiotics (Parsonage et al., 2017). The high treatment rate and illiteracy are the two important factors that lead to antibiotic resistance (Chokshi et al., 2019). To overcome this scenario, the focus of attention of microbiologists is to develop the drug or introduce the combination of drugs against multi-drug resistance organisms (Ayukekbong et al., 2017).

1.1.1 Enterobacteriaceae

The Enterobacteriaceae is the normal flora of human colon and frequently found in large intestine. They are the member of gram-negative rods family with some special distinctive characteristics. These organisms are responsible for most of the clinical as well as subclinical infections including urinary tract infection, bloodstream infection, respiratory tract infection, and found frequently in hospital-acquired infections. Escherichia coli, Klebsiella, Proteus, Enterobacter, Serratia, Citrobacter and Salmonella are the member of Enterobacteriaceae mostly found in clinical specimens of urine, blood, pus, cerebral spinal fluid (CSF) and respiratory specimens of infected person (Levison, 2014).

Enterobacteriaceae family is a gram-negative rods (I. Khan et al., 2015), which was earlier reported by scientist Rahn in 1937 (Oren & Garrity, 2017). This large family includes both pathogenic bacteria and harmless symbionts species (Sumathy, 2018). The more commonly found pathogens include *Klebsiella Pneumoniae*, *Escherichia coli*, *Salmonella*, *Shigella*, (Kaushik et al., 2018), *Citrobacter,* and *Enterobacter* (Dos Santos et al., 2015). Members of the Enterobacteriaceae family are commonly termed as "enteric bacteria" or “enterobacteria (Liu et al., 2016), as many of the members live in small- and large-intestines of living organisms.

Enterobacteriaceae are Gram-negative, facultative anaerobes, that produce lactate and many other ends by-products by fermenting sugar. With exceptions, they undergo nitrate reduction to produce nitrite. The variation in catalase reactions is also found among them. In bacteria, numerous cellular pathways are involved to regulate the sugar metabolism in which cAMP is one of the regulators of xylose, lactose, arabinose and lactose utilization (Ammar, Wang, & Rao, 2018)

The enteric bacteria induce infections includes urinary tract infections (prostatitis, pyelonephritis, cystitis) (Ahmed et al., 2015), intra-abdominal infections (cholangitis, peritonitis, diverticulitis, cholecystitis, and pancreatitis) (Scott, 2019), septic arthritis (Ross, 2017), central nervous system infections (brain abscess, ventriculitis, cerebral infarctions, and cyst formation) (Brouwer & Van De Beek,

2017), ophthalmic infections (intraocular infection, conjunctiva, and eyelids purulent infections) (Shiferaw et al., 2015), osteomyelitis (Bhowmik et al., 2018), and soft-tissue infections (necrotizing fasciitis) (Devaney et al., 2015), lower respiratory tract infections (pneumonia) (Clegg & Murphy, 2017). The diagnosis of these infections requires multiple and different laboratory tests, powerful and expensive antibacterial drugs, and prolonged hospitalization of patients.

The existing databases of mortality in hospital settings adults reveals higher association of carbapenem-resistant *Enterobacteriaceae* (CRE) comparative to carbapenem-sensitive *Enterobacteriaceae* (CSE) induce infections. The identification of mortality rate among bloodstream infections caused by *Enterobacteriaceae, K. pneumoniae*, was found at a 2- to 3-fold higher rate in CRE relative to CSE in previous study. Whereas, the studies are conducting to identify the exact reason for increase mortality rate in CRE patients. The delays to take therapy and inappropriate medical treatment is supposed to be possible cause behind CRE-induced mortality (Martin et al., 2018). *Escherichia coli* is one of the major causes that lead to community-acquired UTI and nosocomial UTI. Around 50% of females experienced at least one episode of UTI (Madappa & Go, 2017).

Enterotoxigenic *E. coli*, was found to be the eight-leading reason for diarrheal mortality in 2016, and the frequency of deaths was approximately 3.2%, while it was accounted for 4.2% deaths in less than 5-year children (Khalil et al., 2018).

In the previously reported study, antibiotic susceptibility trends and incidence of *Klebsiella pneumoniae* neonatal sepsis were identified for 6-year (i.e. 2006–2011) in a neonatal ICU of Karachi, Pakistan. The results were showed that 104 of total 2768 neonates developed late-onset *K. pneumoniae* sepsis with 3.7% overall incidence and the highest annual incidence was reported in the year 2010. Approximately 62% of cases were reported in males and 65% were reported in premature and very low birth weight children (Saleem et al., 2013).

Klebsiella. Pneumoniae is found in abundance in mammals and ecological environment. It can produce pathogenic effects globally. The large pieces of evidence of community-acquired and hospital-acquired infections are present in South Africa

and Taiwan. Community-acquired *Klebsiella. Pneumoniae* is commonly found in places that are associated with alcoholism. Many infections are acquired when the organism affects different body organs such as urinary tract, liver, lungs etc. (Magana et al., 2018).

1.1.2 *Escherichia coli*; pathogen of gastrointestinal tract and urinary tract

The Gram-negative *Escherichia coli* (*E. coli)* is a rod-shaped, facultatively anaerobic, coliform bacterium. This is the member of genus *Escherichia* and found in the intestine of warm-blooded living organisms (endotherms) (Tenaillon et al., 2010).

The strains of *E. coli* are responsible to develop disease, however, not all are associated to produce infection and naturally live in the human or animals' gastrointestinal tract. The virulent strains lead to urinary tract infection (Fasugba et al., 2015), neonatal meningitis (Wijetunge et al., 2015), Crohn's disease (Chalopin et al., 2016), hemorrhagic colitis (Amani et al., 2015), gastroenteritis (O'Ryan et al., 2015). Numerous *E. coli* strains, for instance, O157:H7, can produce Shiga toxin which is used for bioterrorism (Kintz et al., 2017). These toxins produce inflammatory cascade in gut cells to produce lesions and lead to bloody diarrhea (common manifestation of a Shiga toxin-producing *E. coli* infection). Whereas, Uropathogenic *E.coli* (UPEC) and Enterotoxigenic *E. coli* (ETEC) are the causative pathogens of urinary tract infections (Ward et al., 2016) and traveler's diarrhea (Madhavan & Sakellaris, 2015), respectively.

The contamination of invasive devices such as urinary catheters (Kirmusaoglu et al., 2017), respiratory support equipment (Jadhav et al., 2013), neonatal ward devices (Chmielarczyk et al., 2014) in hospitalized patients is at pronounced risk to infect patients.

Countries in the World Health Organization (WHO) European Region have reported significantly increase verocytotoxin-induced Escherichia coli (O104: H4) infection rate, which causes hemolytic urinemic syndrome (HUS) and bloody diarrhea in large number of Europeans, Germans, and North Americans. More recently, this is

again found in increase rate in France (Bordeaux region), whereas one case is reported in Sweden (Yang et al., 2017).

The pathotype of each *E. coli* is categorized on the bases of type of virulence factors. These pathogenic factors include enterotoxins, lipopolysaccharide, fimbrial adhesins, cytotoxins, and capsule. The Pathogenic *E. coli* is further categorized by serotype, which is according to antigenic variation in the fimbrial or F antigens, O antigen of the liposaccharide, and flagellar H antigens (Croxen et al., 2013).

The fimbrial adhesins possess pathogenic E. coli enters in animal gastrointestinal tract, binds with intestinal epithelial cellular receptors to form colonization in jejunal mucosa and / or ileal mucosa which subsequently produces toxins in gut that instigate loss of water (H_2O) and electrolyte from the lumen of intestine and results in weight loss or severe dehydration that possibly leads to death (Clements, Young, Constantinou, & Frankel, 2012).

The pathogenic *E. coli* with fimbrial adhesins enters in gastrointestinal tract to binds with intestinal epithelial cell surface receptors and form colonization in jejunal and ileal mucosa of intestine to initiate the production of toxins. These toxins activate inflammatory endothelial cells of organism blood vessels, that leads to progress edema in multiple tissues, and possibly cause ataxia or death (Bhunia, 2018).

E. coli produces focal to extensive colonization in intestine of gut. E. coli is injected their specific receptor into the host epithelial cell with the help of syringe-like apparatus. This adhesion is facilitated strong bacteria- receptors attachment and initiate intracellular signals for microvilli or brush border effacement, and cell cytoskeleton reorganization, epithelial cell degeneration, and polymorphonuclear cells infiltration in lamina propria, all these events lead to cause diarrhea (Roxas et al., 2018).

The virulent *E.coli* colonizes through fimbrial adhesins in extraintestinal tract, respiratory tract, or other mucosal surfaces. During viral infection or insufficient colostrum, the bacteria pass from mucosa to the bloodstream and produce resistance against complement and phagocytes and persistently grow in the organism and

produce aerobactin. These proteins play vital role to impair tissue and activate cytokines that resulting in serositis, shock, urinary tract infection, pneumonia, mastitis, or may lead to death (Manges et al., 2019).

The E. coli. infected individuals experience severe hemorrhagic colitis, abdominal cramps, vomiting, fever, and diarrhea. Rarely, perforation and bowel necrosis (tissue death) were also observed lacking peritonitis, Gram-negative pneumonia, hemolytic-uremic syndrome, mastitis, and sepsis (Wasey & Salen, 2019).

Plenty of data on Shiga toxin-producing *E. coli* (STEC) is specifically related to O157:H7 serotype, as biochemically differentiation of this strain is easier relative to other E. coli strains. The considerably significant reservoirs that cause STEC include goats, cattle, cats, deer, sheep rabbits, horses, pigs, dogs, chickens, and turkeys (Persad, 2016).

STEC is further isolated from wells, streams, ponds, and water troughs, and found to easily survive for many days to months in water-trough sediments and fertilizer. Waterborne mode of STEC transmission is well reported, this includes recreational waters and contaminated drinking-water (Castro-Ibáñez, Gil, & Allende, 2017).

The asymptomatic infected patients have been observed, where carriers found no manifestations but significantly considerable to infect other individuals (Castro-Ibáñez et al., 2017). Visiting farms and public gathering places probably directly associated with farm animals is also recognized as a vulnerable aspect to acquire STEC infection.

1.1.3 *Klebsiella pneumoniae*; pathogen of respiratory system

Klebsiella pneumoniae is a Gram-negative, encapsulated, facultative, non-motile anaerobic, rod-shaped bacteria, that ables to ferments lactose. The infection is manifested by coughing, Fever, shortness of breath, yellow or bloody mucus, chills,

and chest pain. The infections that are occur by *Klebsiella Pneumoniae* includes urinary tract infection, thrombophlebitis, cholecystitis, bacteremia, diarrhea, osteomyelitis, pneumonia, upper respiratory tract infection, wound infection, meningitis, and sepsis. In an old age individual, Klebsiella is second on ranking followed by E. coli to develop urinary tract infections (UTI).

A study has shown results that the most prevalent infection occurring in hospitals is hospital-acquired pneumonia (HAP), in ICU ventilator-associated pneumonia (VAP) is most common (Cunha & Brusch, 2018). *Klebsiella pneumoniae* is among the causative organisms of Nosocomial pneumonia along with other gram-negative rods (Bassetti, Taramasso, Giacobbe, & Pelosi, 2012). Leading cause of most disease fatality throughout the population in different parts of world is due to ESBLs and carbapenemases generating *Enterobacteriaceae* (Tacconelli et al., 2014). In USA the major causative organisms of Health Care Associated Infections are *Klebsiella pneumoniae, Escherichia coli and Klebsiella oxytoca* isolates. Unfortunately, three or more classes of antibiotics have developed resistance to these isolates therefore classified into Multidrug resistance strains. Different types of infections are caused by these strains i.e. bloodstream infections linked with central line, urinary tract infections due to catheter, VAP and other surgical site associated infections. Furthermore, among reports from different hospitals reporting severe cases of Hospital care associated infections, 20% found carbapenem-resistant Klebsiella isolates are causative agents which are most of time also resistant to multiple drugs (Weiner et al., 2016).

The virulent *K. pneumoniae* comes into contact with respiratory tract and blood circulation to produce pneumonia and bloodstream infection, respectively. Under healthcare facilities, pathogenic *K. pneumoniae* possible transmit through one person to another by contaminated hands, less frequently, by contaminated environment; the mode of spreading straightaway from the environmental settings to patients is still on debated and further elucidation is needed. Though, the bacteria transmission through air is not reported. Non-healthy persons on healthcare facilities are vulnerable to exposure to typical *K. pneumoniae* if they needed oxygen ventilators, have internal- or external-wounds or using intravenous catheters. All these

medical equipment settings are allowed pathogenic *K. pneumoniae* to contact with the body and leads to infection (Paczosa & Mecsas, 2016).

The *Klebsiella pneumonia* enters in bloodstream when patient passes from sepsis or septic shock (Falcone et al., 2016). The previously conducted study at King's College, London has observed HLA-B27 and Klebsiella surface molecules accounted to progress ankylosing spondylitis (Rashid & Ebringer, 2007). The risk for Klebsiella is increased in patients with enteric chronic pulmonary disease (Rashid & Ebringer, 2007), nasal mucosa atrophy (Gonzales Zamora & Murali, 2016), and rhinoscleroma. Therefore, the development of new antibiotic-resistant therapy for K. pneumonia is focused on attention.

Typical *Klebsiella pneumoniae* acts as an opportunistic bacterium producing nosocomial infections (hospital-acquired infection), but a subcategory of hypervirulent serotypes that are capable of enhanced capsule polysaccharide formation, mainly K1 and K2, influence healthy individuals to cause potentially fatal invasive infections. Capsule polysaccharide is a vital virulence factor of K. pneumoniae, which associates key contribution to resisting phagocytosis, suppresses early inflammatory defense mechanism, resist antimicrobial peptides molecules, and inhibit dendritic cell maturation. Further K. pneumonia associated virulence factors are; outer membrane proteins, lipopolysaccharide, fimbriae, iron-regulated surface determinants, and nitrogen metabolism (Li et al., 2014).

1.1.4 Conventional antibiotics resistance and ceftazidime-avibactam potential

Amoxicillin is an FDA approved β-lactam antibiotic that is generally used for the treatment of infections that are caused by Gram-negative or positive microorganisms (Nabipour, Hosaini Sadr, & Thomas, 2015). Mode of action involves bactericidal activity by acting on penicillin-binding receptors which stimulate autolytic enzymatic activity leading to lysis of the cell wall of bacteria (Akhavan & Vijhani, 2019).

Literature has suggested the resistance of Amoxicillin has been developed

against Enterobacteriaceae to an alarming level. A study conducted by Periasamy Hariharan et al. suggested that penicillin was 82.8% resistance against *E.coli*. Similarity Periasamy Hariharan et al. also mentioned in his study that Klebsiella had more resistance than E.coli. Furthermore, 93.1% of Klebsiella species were observed non-susceptible to Penicillin. About 30% of Klebsiella species were resistant to ampicillin in the clinical isolates (Hariharan et al., 2015).

Escherichia coli isolates were found 82.8% of resistance to ampicillin, 77.6% to ciprofloxacin (Hariharan et al., 2015), and 72.4% to tetracycline. Furthermore, there was a considerable resistance observed with piperacillin (50%), gentamicin (48.3%), and ceftazidime (34.5%). Ceftazidime-tazobactam, piperacillin-tazobactam, and meropenem were found susceptible to Escherichia coli (Hariharan et al., 2015). In Klebsiella species, the resistance was found with ampicillin (93.1%) gentamicin (50%), piperacillin (50%), tetracycline, ciprofloxacin and ceftazidime (36-41%). The moderate level of resistance was found to ceftazidime-tazobactam (5.2%) and meropenem (1.7%) and piperacillin-tazobactam (1.7%). The very similar level of trend was found in Enterobacter species, but there was a slightly higher resistance to ceftazidime-tazobactam and piperacillin-tazobactam (11.5%) (Hariharan et al., 2015).

The study was conducted in Pediatrics department of Holy Family Hospital, Rawalpindi by Afzal to identify the spectrum of pathogens and their sensitivities in children with UTI. Total of 150 children between the age of 1 to 12 years was recruited having ≥ 101 °F fever from more or less than 10-days. In the results, pathogens found maximum non-susceptibility to amoxicillin (Afzal, 2008).

Clavulanic acid increases the spectrum of amoxicillin by rendering of β-lactamase-produced pathogenic microorganisms that are sensitive to the antibiotic (Bush & Bradford, 2016). The reported trial on amoxicillin-clavulanic acid has shown that this combination is clinically and bacteriologically more than monotherapy of amoxicillin (Mhmoud, Fahal, & van de Sande, 2014). Furthermore, this shows at least as biologically active as shown by various other relative anti-pathogenic agents, included orally administered doxycycline, cotrimoxazole, bacampicillin, and cephalosporins against soft tissue infections, skin infections, otorhinolaryngological infections, urinary tract infections and respiratory tract infections. The combination of

amoxicillin/clavulanic acid is also medically used treatment for gynecological infections (Lachiewicz, Moulton, & Jaiyeoba, 2015), chancroid (Ronald, 2015), gonorrhea (Organization, 2017), and against surgical infection (Menon, Gopinath, Li, Leung, & Botelho, 2019) as prophylactic drugs.

Clavulanic acid irreversible prevent both intracellular- and extracellular-β-lactamases (Garber, 2015), potentially active for broad spectrum of enzymes such as Sykes classes (class II to V), staphylococcal *β*- lactamase (Armin et al., 2017), while ineffective for class I cephalosporinases and *Bacteroides fragilis* -producing β-lactamase, and cephalosporinases (class I). Clavulanic acid prevents β-lactamases by increases inactivation of amoxicillin, which in turn restores antimicrobial activity of amoxicillin once the resistance is increased because of bacterial β-lactamase synthesis threatened their medical potential (Todd & Benfield, 1990).

Yaseen et al conducted the study in 564 pregnant females between the age of 17 to 44 years in antenatal healthcare center Jinnah Medical College Hospital (JMCH) Karachi, Pakistan during 2017. In total, approximately 8.50% had observed occasionally UTI during the period of their pregnancy. 54.2% of patients were found infected with *Escherichia coli*, and 16.77% were found *Klebsiella pneumonia*. Overall, Amoxicillin/ clavulanic was shown 50% sensitivity (Yaseen, Rashid, & Naqvi, 2020).

A third-generation cephalosporin, ceftriaxone was tested against activity of Enterobacteriaceae in most studies done by different researchers over the period. In broad-spectrum drugs, ceftriaxone is exhibited antibacterial activity for Gram-positive and negative strains (Donnelly et al., 2017). Mode of action involves selective and irreversible bonds of drug with penicillin-binding proteins (PBPs), leading to inhibition of the cross-links of peptidoglycan polymers and thus preventing bacterial cell wall synthesis (Raju, Reddy, & Vasu, 2017).

Research shows that *E. coli* strains that were responsible for bovine calf scours were observed non-susceptible to 3rd-generation cephalosporin in Dakota, U.S.A (Bradfor et al. 2011) with the prevalence of Enterobacteriaceae on rise. Research in South America, which studied sample of a group of ESBLs – cefotaximases (CTX-

M), has implicated the microorganism for massive outbreaks of cefotaxime-resistant enterobacteria with the exception ceftazidime which did not have much effect on its activity (Meyer et al., 2010).

It was alarming for microbiologists and pharmacologist to know that since 2004 the frequency of third-generation cephalosporins non-susceptibility has been gradually increased in 19th countries in Europe along with a gradual rise in cases of invasive E.coli and K. pneumonia. It is unfortunate to know that a novel type of ESBL resistant to cefotaxime but not significantly affecting ceftazidime (Meyer et al., 2010). Shah has studied the study in department of Medicine, Shifa College of Medicine, Islamabad, Pakistan to identify the E. coil susceptibility of MDR pathogens and possible role of ESBL in E. coli resistance. Therefore, 378 *E. coli* pathogens from multiple sources were identified in the period of 6-month. Overall, they have found 34% resistance of ceftriaxone (Shah, 2002).

Cefepime is a fourth-generation of cephalosporin used against organisms which are multi drug resistant as an empirical monotherapy to treat different body infections (Arsalan, Naqvi, Ali, Ahmed, & Shakeel, 2015). Cefepime is inhibited the bacterial cell wall synthesis (O'Connor & Eranki, 2019). Resistance against Cefepime by E. coli and Klebsiella isolates is high (Peerayeh, Rostami, Eslami, & Rezaee, 2016). E. coli isolated showed 75.5% resistance to Cefepime whereas, Klebsiella strains were 79.5% resistant (Zandi et al., 2017).

To study the *in -vitro* antibacterial effect of Cefpirome (Cephalosporin), a multi-center study was carried out by Hafeez *et al* in 13 health care centers. They collected 1300 and identified by the API identification systems. Kirby-Bauer method was used to test the sensitivity. The results in their study were suggested that Cefpirome has a potential against gram-positive bacteria and gram-negative bacteria that were resistant to other 3rd- generation cephalosporins (Hafeez et al., 2000).

Meropenem administration as a extended infusion has proven to the treatment of choice against infection caused by E. coli and K. pneumonia (Paczosa & Mecsas, 2016). Carbapenemase-producing Enterobacteriaceae (CPE) is continuously spreading worldwide and is a source of massive discomfort for health care

professionals (Doi Y et al 2015). Past studies have proven that meropenem is very effective therapy against Enterobacteriaceae, Meropenem without combination of any other antimicrobial agent has shown effectiveness against broad-spectrum which are Gram-negative bacteria, including against those organisms which were multi drug-resistant and left health care professionals with very limited options. It has been observed that meropenem is stable in the presence of numerous beta-lactamases such as AmpC beta-lactamases and ESBLs (Lomoyskaya et al. 2017).

Ceftazidime-tazobactam, piperacillin-tazobactam and meropenem were found non-susceptibility against Escherichia coli isolates. Concerning Klebsiella species, the low to moderate level of resistance was observed from piperacillin-tazobactam, meropenem (1.7%) and ceftazidime-tazobactam (5.2%). Alike pattern was shown in Enterobacter species, but, piperacillin-tazobactam and ceftazidime-tazobactam were shown 11.5% of resistance (Hariharan et al., 2015).

To analyze the frequency of MBL producing *E. coli* and *Klebsiella pneumoniae*, phenotypic techniques for MBL detection and choices of treatment available, the study was conducted by Javed *et al* in Children's Hospital, Lahore, Pakistan, from March, 2013 to February 2014. They processed a total, 17,651 samples which include urine, pus, catheter tips, blood, and CSF of microbial infections, and pathogens were tested by using microbiological techniques. They were observed 11.47% carbapenem resistance in strains which comprised of 32.6% in *E.coli* and 67.4% in *Klebsiella pneumoniae* (Javed et al., 2016).

One of the most frequently prescribed aminoglycosides is Amikacin. Amikacin is effective against microbial agents, bacterial pathogens, and nephrotoxins. The adjunctive therapy of this drug is observed active against Gram-negative pneumonia to treat pneumonia in intubated patients and patients placed in mechanical ventilators. The administration of AMK inhaler in in-vitro pharmacodynamic models was shown promising results with achievable epithelial lining fluid concentrations against pathogens of Gram-negative strain. Moreover, the AMK in this model as monotherapy and adjunct therapy with meropenem AMK (when MICs were $\leq$ 256 mg/L) demonstrated rapid and sustainable bactericidal killing (Sutherland, Verastegui, & Nicolau, 2016).

The surveillance study was presented by Sutherland et al which observed many US healthcare facilities that suggested the excellent potential of amikacin agents in contemporary strains of E. coli, and K. pneumoniae, which was collected from respiratory tract or human blood. Whereas, another study suggested that E. coli has exhibited 4.9% resistance to Amikacin (Shatalov, 2015). Khorshed has observed highly susceptibility of amikacin against samples of E. coli isolates (Khorshed, 2018). For Pneumonia, Khorshed observed that high resistance rate was present in urine samples towards Amikacin (Khorshed, 2018). Also, Shatalov suggested as high as 100% resistance of AMK towards K. pneumonia (Shatalov, 2015).

Doxycycline (DOX), member of tetracyclines, is second-generation antibiotics. The antibacterial spectrum of DOX includes frequently isolated streptococci, certain Enterobacteriaceae and staphylococci pathogens. Furthermore, antibacterial activity also observed towards atypical pathogenic agents associated with pulmonary infections, Rickettsia, sexually transmitted infections and the infectious pathogens of leptospirosis, anthrax, plague, brucellosis, melioidosis, and Q fever (Carris et al., 2015). Susceptibility of Enterobacteriaceae rods to selected tetracyclines was studied by (Alicja Sekowska et al. 2009) which showed among ESBL-positive strains 52.0% were susceptible to tetracycline and doxycycline (Hariharan et al., 2015).

Periasamy Hariharan et al. carried out the study into *Escherichia coli* isolates showed resistance to tetracycline by 72.4%. Besides, there was a significant resistance observed against gentamicin 48.3%. In research related to susceptibility of Klebsiella sp., level of resistance to gentamicin was 50 and resistance towards tetracycline was 36-41% (Hariharan et al., 2015).

The spectrum of pathogens and their sensitivities were identified by Afzal in children who were infected with UTI in Pediatrics department, Holy Family Hospital, Rawalpindi, Pakistan. They were found that *Escherichia. coli* and *Klebsiella* were the most common uropathogens in them. Aminoglycosides were found to sensitive in both species (Afzal, 2008).

Co-trimoxazole (Trimethoprim/sulfamethoxazole) antibiotic is medically used

to treat and prevent multiple pathogenic infections. The composition included 1:5 ratio of trimethoprim and sulfamethoxazole administered orally or direct intravenously. The most potentially active in skin infections, methicillin-resistant Staphylococcus aureus

(MRSA), cholera, respiratory tract infections, UTI, and travelers' diarrhea. they are further used in pneumocystis toxoplasmosis and pneumonia and HIV (human immune deficiency syndrome) or AIDS (acquired immune deficiency syndrome) people (Kemnic & Coleman, 2019).

Ciprofloxacin antibiotic is medically used against range of bacteria-induced infections, for instance, typhoid fever (García-Fernández et al., 2015), skin infections (Suhaeri et al., 2018), bone infections (Landersdorfer et al., 2020), joint infections (Naqvi et al., 2018), intra-abdominal infections (G. J. Khan, Khan, Majeed, Siddiqui, & Khan, 2015), respiratory tract infections (Connett et al., 2015), urinary tract infections (Fasugba et al., 2015), and diarrhea (Pop et al., 2016). Sometimes, it is administered as adjunct therapy (Tauzin et al., 2019). The mode of administration may orally, ocular, otic route, or intravenously. Ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid seems as faintly yellowish to light yellow crystalline substance with 331.4 g/mol molecular weight (Trevino et al., 2019).

Tazobactam- piperacillin combination impedes mostly class A and C of β-lactamases and some plasmid-mediated AmpC cephalosporinases, therefore prevent hydrolysis of and cefotaxime broadens spectrum against ESBL-produced Enterobacteriaceae (Sutherland & Nicolau, 2015).

Ceftazidime-avibactam combination is approved by US-FDA (Food and Drug Administration) against gram-negative bacterial activity and for the treatment of urinary tract infections (UTI) and intra-abdominal infections. (van Duin & Bonomo, 2016).

Ceftazidime is structurally like cephalosporins and composed of pyrazole substitution at 3rd position of ceftolozane skeletal side chain. The heavier side chain in

molecule is recovered the steric hindrance to inhibit the process of hydrolysis that is induced by AmpC β-lactamases enzyme (van Duin & Bonomo, 2016)

Avibactam, antibiotic, is a manufactured non-β-lactam-β-lactamase inhibitor which includes [3.2.1]-diazabicyclooctane scaffolding. Another third-generation cephalosporin as well as beta-lactamase inhibitor amalgamation is Ceftazidime–avibactam (Levasseur et al., 2012). It was approved on February 25, 2015 on account of similar clinical indications (Goodlet, Nicolau, & Nailor, 2016)., 2016; Liscio et al., 2015).

Ceftazidime-avibactam is combination which includes ceftazidime, which is a well conclusively proved antipseudomonal cephalosporin along with avibactam, an earliest in order amongst class non ß-lactam ß-lactamase inhibitor having action counter to Ambler class A [inclusive of ESBLs like SHV, TEM plus CTX-M as well as), class C (AmpC), *Klebsiella pneumoniae* carbapenemase (KPC) as well as few of array D (OXA-48) enzymes (Aktaş, Kayacan, & Oncul, 2012).

The drug can effectively restore the antimicrobial action of ceftazidime when tested in vitro. Ceftazidime-avibactam showing definitively lowered minimum inhibitory concentrations for opposing numerous aggregations in ceftazidime-resistant gram-negative bacteria. These include a few carbapenem-resistant *Enterobacteriaceae*. This combination therapy is considered as a potential drug of choice for complicated intra-abdominal infections complicated UTI inclusive of pyelonephritis, *Citrobacter koseri, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis, Klebsiella oxytoca, Escherichia coli, Pseudomonas aeruginosa, and Citrobacter freundii* (Crandon et al., 2012).

Ceftazidime-avibactam drug is recognized as a novel cephalosporin that does not have β-lactam ring but having ability to inhibit β-lactamase. Tazobactam with avibactam is targeted serine β-lactamases (active site of enzyme). Avibactam, diazabicyclooctane non-β-lactam antibiotics, that binds covalently and reversibly to β-lactamases to inactivate another β-lactamase enzyme. The medical benefit of avibactam has potential to prevent enzymes of narrow-spectrum β-lactamases,

ESBLs, AmpC β-lactamases (as found in Enterobacteriaceae family and pathogenic *P. aeruginosa*), and carbapenemases of the *Klebsiella pneumoniae* carbapenemase family (class A) (van Duin & Bonomo, 2016).

The foremost antimicrobial activity of ceftazidime-avibactam is against gram-negative bacteria. For gram-positive pathogenic strains, this combination has been shown no anti-enterococcal, minimal anti-staphylococcal, and limited anti-streptococcal activity. The drug was used in adjunct therapy and shown anti-bacterial activity against specific anaerobic bacteria, such as *Propionibacterium* and *Fusobacterium* species. But, anti-Bacteroides activity was not as much likely, and anti- *Clostridium* species are non-susceptible (van Duin & Bonomo, 2016).

It has been a matter of serious concern since antimicrobial resistance of Enterobacteriaceae isolates has gradually and constantly increased to an alarming level leading to very limited therapeutic options and antimicrobial agents which are vulnerable mainly due to challenges posed at their safety profile and efficacy level are very often the last treatment option for patients with infection due to these microbes. Meta-Analysis showed that patients suffering from bacteremia because of ESBL producing microorganisms had a considerably enhanced the rate of mortality according to data and were also, unfortunately, getting delayed treatment against the antimicrobials. Therefore, it is very much important to have reliable treatment options to cure infections occurring due to multiple drug resistance and pan drug resistance isolates (Schwaber & Carmeli, 2007).

The criteria for selection of most appropriate antimicrobial's agent is based on epidemiology of the local microbial, risk factors of patients for MDR pathogens, and specific characteristics of the patient that can affect the selection of antimicrobial agent for the treatment of infection. While the resistance levels between pathogen and drug and the microbial epidemiology vary significantly from one hospital to another hospital.

One of most serious concerns in terms of developing resistance against antimicrobials is beta-lactam drugs, which are effective to counter most pathogens. Additionally, these antibiotics have low toxicity to humans, but the gradual and

constant increase in resistance of these antibiotics is very alarming (Livermore et al., 2012). β-lactamases enzyme is found in resistant strain of gram-negative organisms, these enzymes act on the β-lactam ring to destroy it and to develop resistance (Tooke et al., 2019). Based on sequences of amino acid molecules, β-lactamases are categorized into A, B, C, and D classes (Philippon et al., 2016). Among these classes, class A, C, and D are similar as their beta-lactamases have serine residue in their catalytic site, whereas B is different as its enzymes have one or zinc atom in its structure (Bradford, 2001).

The National Healthcare Safety Network (NHSN) acknowledged that around 20% frequency of Enterobacteriaceae were MDR that was analyzed from all acquired infections of hospital. For many years, carbapenems have been quite successfully used to deal with infections caused by MDR Gram-negative bacilli, which includes pathogens like *P. aeruginosa* and Enterobacteriaceae (Tacconelli et al., 2014).

It's a matter of grave concern that new types of b-lactamases have emerged which are capable to abolish carbapenems, together with *Klebsiella pneumoniae* carbapenemases enzymes (KPCs) or different classes of metallobeta-lactamases enzymes (MBLs), resulting in very complex and less effective treatment option to cure Gram-negative infections, which have posed a major problem to physicians. It is stated in a current meta-analysis that the infection caused by CRE has significantly increased the rate of mortality as related to carbapenem-susceptible strain (Falagas et al., 2014).

Most of the front-line antibiotics develop resistant to Carbapenem-resistant Enterobacteriaceae (CRE), appeared as reasons for infections throughout the world. There is more than 70 % rate of mortality was observed in serious CRE-infected patients. In the United States KPCs, chiefly KPC-2 as well as KPC-3 are most commonly present due to carbapenem resistance among Enterobacteriaceae. Treatment of CRE infections has strongly depended on different antibiotics like tigecycline, colistin and aminoglycosides which are restricted to sub-optimal pharmacokinetics, toxicity, and resistance emergence (Chiotos, Han, & Tamma, 2016).

According to the results of the study recruited in U.S. hospitals, the avibactam

reinstates the activity of ceftazidime against *Enterobacteriaceae*. And suggested that this therapy could become a significant addition to the treatment of serious infections caused by Gram-negative bacteria (Castanheira et al., 2018). Furthermore, the study was conducted against most of ceftazidime susceptible as well as non-susceptible bacterial strains. They added avibactam and observed the considerable enhance action of ceftazidime by showing MICs 256-times decreased for ESBL isolates, 8 times to 32-times for CTX-M isolates and more than 128-times for KPC isolates. Total, ceftazidime-avibactam MICs were less than from cefepime, piperacillin-tazobactam, ceftriaxone, and cefotaxime and parallel or higher from imipenem (Karlowsky et al., 2016).

A collection of approximately 34,000 different strains of Enterobacteriaceae, together with ESBL- and Amp C-generating isolates, taken from medical institutes in 39 countries showed that ceftazidime-avibactam has potent in-vitro antimicrobial activity. Avibactam is the first recognized drug that is clinically used as a beta-lactamase inhibitor with action against AmpC-mediated resistance whereas other β-lactamase inhibitors for instance tazobactam, clavulanic acid, and sulbactam only shows inhibition in class A b-lactamase enzymes (Karlowsky et al., 2016).

The 2-years study carried out in USA from 2011 to 2013 demonstrated that ceftazidime-avibactam combination is proven to be very efficient against resistant subsets which also included ceftazidime-non susceptible Enterobacter cloacae and ESBLs phenotypes isolates. Few of the most common enzymes which were detected among ESBLs resistant phenotypes that are affected by this drug are CTX-M-14 and 15 like, KPC as well as SHV (Castanheira et al., 2018). The study on murine model was carried out in France in which septicemia was introduced by Enterobacteriaceae species to generate AmpC or ESBL. The result of study proved the efficacy of synergistic effect of ceftazidime-avibactam as compared to usage of ceftazidime alone counter to a broad range of *Enterobacteriaceae* pathogens in model ceftazidime proved to be useless when used alone because of presence of b-lactamases formed by these bacteria. Result of this showed that *ceftazidime-avibactam* was 4 times more effective where ceftazidime alone was ineffective. Avibactam drug was also observed with different combinations such as different β-lactams, but potency level *ceftazidime-avibactam* was the highest.

A study was conducted in Canada, result of the study led to the conclusion that synergistic effect achieved as a result of combination of avibactam with ceftazidime expands its effectiveness toward *Enterobacteriaceae and P. aeruginosa*. Mechanism of action of avibactam mainly involved inhibition of isolates containing serine ß-lactamases, having ESBL, AmpC, and KPC enzymes. Further, it was noted that avibactam did not increase the ceftazidime effectiveness to counter *Burkholderia species, Acinetobacter species,* or majority of anaerobic Gram-negative rods. Statistics analysis showed that ceftazidime-avibactam combination is a potent bactericidal activity in human serum. When data were conducted in animals, result of the studies indicated that *ceftazidime-avibactam* is effective against microbes that had developed ceftazidime resistance and were effective against infections such as gram-negative meningitis, septicemia, pyelonephritis, and pneumonia. Ceftazidime-avibactam combination when compared the carbapenem showed that the level of activity of both the antimicrobials is similar in complicated urinary tract infection and complicated intra-abdominal infection, together with infection caused through cephalosporin-non-susceptible gram-negative pathogens. One of the major advantages observed in clinical trials of *ceftazidime-avibactam* is that its safety profile and tolerability level have found excellent but it has also minimal serious adverse effects (Philippe Lagacé-Wiens et al., 2014b).

Research proves the effectiveness of *Ceftazidime-avibactam* in empiric treatment of UTI, the same research which was based on randomized control trial, conducted in Germany also suggested that *Ceftazidime-avibactam* offers an alternative option to therapeutic usage carbapenems (Wagenlehner et al., 2016). A research conducted in USA in 2012 also proved that Ceftazidime-avibactam is a potentially useful addition to antimicrobials for clinicians deal with challenge of multidrug resistance Gram-negative bacteria, which were isolated in vitro surveillance program from blood which was causative organisms for different infections mainly pneumonia, abdominal infections and UTI (R. K. Flamm et al., 2014).

1.2 Hypothesis

A) Null Hypothesis

Escherichia coli and *Klebsiella pneumonia* are not susceptible to ceftazidime-avibactam.

B) Alternative Hypothesis

Escherichia coli and *Klebsiella pneumonia* are susceptible to ceftazidime-avibactam.

1.3 Objectives of study

i. To evaluate the susceptibility pattern of ceftazidime-avibactam against *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical specimens.

ii. To determine frequency of *Escherichia coli* and *Klebsiella pneumoniae* in different clinical specimens.

iii. To correlate the susceptibility pattern of ceftazidime-avibactam against *Escherichia coli* and *Klebsiella pneumoniae* in different age groups and gender.

iv. To evaluate superiority of drugs among *Escherichia coli* and *Klebsiella pneumonia* as compared to traditional antibiotics.

1.4 Rationale of the study

The increased prevalence of conventional antibiotics resistance against Enterobacteriaceae was leading the poor treatment outcomes. Therefore, it was necessary to introduce the new combination of antibiotics that act against Enterobacteriaceae like *Escherichia coli* and *Klebsiella pneumonia*.

1.5 Significance of study

The study is throwing light on the patterns of susceptibility exhibited by ceftazidime avibactam towards Escherichia coli and Klebsiella pneumonia when the infections due to Enterobacteriaceae are extremely common. As emerging drug resistance towards various bacteria species is a global problem therefore a study like this is immensely important. This study is build foundation for detecting a significant solution towards large number of infections caused by Enterobacteriaceae.

1.6 Operational definitions

Antimicrobial resistance, Antimicrobial Susceptibility, Antimicrobial Activity, Enterobacteriaceae, Avibactam, Disk Diffusion, Zone of Inhibition

1. Antimicrobial resistance

Enterobacteriaceae isolates having zone diameter of inhibition of ≤21mm against ceftazidime-avibactam disk 30/20 µg after 24-48 hours is considered as antimicrobial resistance strain (CLSI, 2016).

2. Susceptibility

Susceptibility pattern of ceftazidime-avibactam was determined against *Escherichia coli* and *Klebsiella pneumoniae* through disk diffusion test and E-test (Liofilchem disk). The zone diameters in disk diffusion test greater than 21mm were taken as sensitive for ceftazidime-avibactam with 30/20 µg disc after 24-48 hours of incubation (CLSI, 2016). Whereas, the zone diameters in E-Test greater than 8mm was taken as sensitive for ceftazidime-avibactam (CLSI, 2016).

3. Enterobacteriaceae

The Enterobacteriaceae are glucose-fermenting, gram-negative rods which are facultative anaerobes and reduce nitrate to nitrite. Their identification will be

confirmed through routine biochemical tests and API 20E as Escherichia coli and Klebsiella pneumonia (Levison, 2014).

4. Avibactam

Avibactam is one of the novels non-β-lactam β-lactamase inhibitors. This tends to act against class A, C, and some class D β-lactamase enzymes. Avibactam is currently being analyzed in combination with ceftazidime (ceftazidime-avibactam) for its efficiency against bacterial-induced infections (Winkler et al., 2015).

5. Antimicrobial activity

The process of killing and reducing the growth of disease-causing microbe by different modes of action is called antimicrobial activity (Levison, 2014).

6.Disk diffusion

The Kirby-Bauer method is usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by clinical laboratory standards institute. The disk-diffusion agar method assesses the efficiency of Ceftazidime Avibactam on Mueller Hinton agar against different Enterobacteriaceae isolate. For the disk diffusion method, ceftazidime-avibactam disks were obtained from Liofilchem. The content of ceftazidime-avibactam in each disk was KPC 30 μg/20 μg (Wang et al., 2020).

7. Epsilometer -test

For the E-test gradient diffusion method, ceftazidime-avibactam Etest strips were obtained from Liofilchem. The tests were performed in strict accordance with the manufacturer's instructions. The ceftazidime concentration gradient ranged from 0.016 to 256 μg/mL with avibactam at a constant concentration of 4 μg/mL. When the Etest MIC value was between the standard value and twice the standard value (0.016, 0.032, 0.064, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256), the high standard value was considered as the MIC (Wang et al., 2020).

8.Zone of inhibition

The Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. After 24 to 48 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate (CLSI, 2016)

CHAPTER 2

LITERATURE REVIEW

Antibiotics act on bacteria in several pathways inside the human body to treat and prevent infections. The importance of antibiotics cannot be denied, however their overuse, lack of new drugs and uncontrolled infections lead to development of antimicrobial resistance. Several times, antibiotics are not adequately recommended or prescribed for the patients, or inappropriate dose is prescribed for a longer or shorter time duration. As per World Health Organization, antibacterial resistance is the resistance of a pathogen to an antibacterial drug to which it was sensitive earlier. Consequently, standard treatment strategies become ineffectual; infections continue and transmission to other individuals may occur (Shaikh, 2017). Antibacterial resistance represents detrimental human health effects and an increase in global environmental and economic risk. This association between bacterial resistance and misuse of antibiotics is reflected as a serious public health problem (Broom et al., 2015).

During the year 2000-2010, a significant increase in the use of antibiotics was observed globally. The increase in population, increased access of individuals to antibiotics, and the socioeconomic status were found to be the primary reasons in the development of antibiotic resistance (Morrill & LaPlante, 2015).

Gram-negative pathogen resistance has been the leading cause of antibiotic resistance. The major pathogens include multidrug-resistant (MDR) ESBL-producing Enterobacteriaceae and CRE. These micro-organisms often tend to form numerous b-

lactamase which leads to acquired resistance to cornerstone drugs such as b-lactams enzymes including ESBL-producing Enterobacteriaceae (SHV-2, CTX-M-15, TEM-3), Ambler class C cephalosporinases (CMY-2, AmpC) which are resistance to most b-lactams antibiotics. Currently, the focus of attention is to develop a therapy against b-lactams resistant organisms and carbapenemases- producing organisms (Klebsiella pneumoniae carbapenemase-2, New Delhi metallo–b-lactamase-1, OXA-48, VIM and IMP (Zasowski, Rybak, & Rybak, 2015).

In USA, carbapenem-resistant Enterobacteriaceae (CRE), and extended-spectrum b-lactamase (ESBL-producing) accounts for approximately 35,000 healthcare-associated infections and 2,300 deaths reported in a single year. From 2000 to 2009, the percentage of UTI that was caused by *Escherichia coli* (3.3 % to 8.0 % for *E. coli*) and *Klebsiella pneumoniae* exhibiting ESBL production (from 9.1 %-18.6 % for *K. pneumoniae*) has increased more than two-fold. Altogether in USA, the hospitalized patients with urinary tract infection, infected by resistant gram-negative strain micro-organisms have enhanced frequency of ESBL-producing organisms which was approximately 300%. Furthermore, the frequency of Enterobacteriaceae that is isolated from the academic institutions of U.S. exhibited carbapenem resistance which increased from 1.2 % to 4.2% in 2001 and 2011, respectively, with *Klebsiella* species accounting for maximum increase. Regardless of the less rate of infections caused by carbapenem-resistant *Klebsiella pneumoniae*, they are associated with increase in the rate of mortality and morbidity and utilize a considerably high cost of healthcare resources. (Golan, 2015).

One of the mechanisms of antimicrobial resistance in Gram-negative strain is the synthesis of β-lactamase enzyme that hydrolyzes the β-lactam ring and turns β-lactams inactive. Based on Ambler division, β-lactamases are classified into four classes i.e A to D. Enzymes of A, C, and D classes hydrolyzes the β-lactam ring structure by an acyl intermediate of serine moiety, while class B involves a zinc element as a co-factor to retain it's inhibitory activity; consequently, these enzymes (typically carbapenemases) are often termed as metallo-β-lactamases (MBLs) and includes IMP-1, NDM-1, and VIM-1. Enzymes of class A include serine carbapenemases, narrow- and ESBLs, these categories of antibiotics are commonly used for Enterobacteriaceae family. Class A β-lactamases are staphylococcal

penicillinase, KPC, SHV-1 and 2, IMI, CTX-M-15 and TEM-1. Class C enzymes are mainly cephalosporinases, ACT-1, MIR-1, FOX-1, P99, and CMY-2. The micro-organisms that produce class C enzymes are *Serratia*, *Morganella morganii, Enterobacter Acinetobacter*, *Citrobacter and Pseudomonas,* and Providencia species. Class D β-lactamases are included carbapenemases, *Pseudomonas aeruginosa, Acinetobacter baumannii*, and ESBL (Liscio, Mahoney, & Hirsch, 2015).

Meropenem has antibacterial potential against carbapenem, and retains it's stability for beta-lactamases produced-hydrolysis by bacteria, such as cephalosporinases and penicillinases. This antibiotic exerts it's bactericidal activity by binding with penicillin-binding proteins (PBP) in the cell-wall of bacteria to prevent cross-linking of peptidoglycan. This inhibits bacterial cell-wall formation and causes cell death (Dhillon, 2018).

The *Enterobacteriaceae* is causing infections that possessing the carbapenemase gene representing the paradoxical phenotype of antibiotic resistance against almost all ß-lactams that also includes meropenem. Kayama et al. have reported firstly *Klebsiella pneumoniae* that possess $bla_{\text{OXA-181}}$ displaying similar resistance phenotype in stealth-type (Kayama et al., 2015).

The resistance of meropenem antibiotics is related to significantly increased total health care cost, but this is only reported among out-patients. The ICU (intensive care unit) patients are independently related to causing an increase in the total cost of hospital stay instead of resistance to the phenotype of micro-organism. Direct medical expenses are often increased in the intensive care unit facility; consequently, comparative influence of resistance of carbapenem monotherapy is probably less in the critically ill-affected population. The total cost of hospital on average is about $22,000 increase in meropenem-resistant *P aeruginosa* patients (Judd et al., 2016)

Enterobacteriaceae-producing carbapenemases are also termed as carbapenem-resistant *Enterobacteriaceae* (CRE), which initiate resistance to numerous ß-lactam agents that include "last-line" carbapenem antibiotics. The resistance of carbapenem agent probably initiates during the deficiencies of porin, which permits less ß-lactam entrance into the bacterial cell membrane, therefore, it is combined with extended-spectrum b-lactamase. The prevalence of carbapenem-

resistant *Enterobacteriaceae* infections has increased over the previous few decades, particularly in healthcare facilities, and per se CRE has been identified by the US Centers for Disease Control and Prevention as a more lethal human health risk. The Centers for Disease Control and Prevention have estimated that greater than 9000 infections are due to CRE, such as *Escherichia* species and carbapenem-resistant *Klebsiella* species, every year in the USA. CRE can produce several critical infections, including UTI, device-associated infections pneumonia, and intra-abdominal infections, or asymptomatic colonization. Carbapenem-resistant *Enterobacteriaceae* infections result in about 600 deaths every year. Infections caused by Carbapenem-resistant *Enterobacteriaceae* are a high cause of concern, as mortality rates of Carbapenem-resistant *Enterobacteriaceae* are higher and were from 18% - 48% when given medical therapy. These outcomes are probably due to a delay in giving active medical treatment to patients, pharmacologic restrictions of accessible treatment possibilities, and the fact that patients with Carbapenem-resistant *Enterobacteriaceae* bacterial infections tend to be severely ill (Morrill, Pogue, Kaye, & LaPlante, 2015). OXA enzymes expression, specifically OXA-23 (Zowawi et al., 2015).

The CRE hypervirulent *Klebsiella pneumoniae* pathogenic strains are observed as a significant human health threat, as they are multidrug-resistant (MDR), highly transmissible pathogens, and simultaneously hypervirulent. Proper preventive measures have to be quickly made effective to control increase dissemination of such typical micro-organisms in the healthcare facilities and the associated communities (Gu et al., 2018).

Some of the non-fermenting, gram-negative *Enterobacteriaceae* infections exhibit a remarkably increased range of intrinsic resistance for antimicrobials because of a wide range of expression found for resistance. They cross the bacterial cell membrane more slowly due to porins, that cause a conformational change and possess a highly restricted entry channel on their surfaces. Moreover antibiotic exposure down regulates porin levels. Antibiotic molecules that have traversed successfully in the porin channels of bacteria are probably eliminated by the activity of efflux pumps of organism (Rise 2016)

Similarly, some of the *Enterobacteriaceae species*, is expressed as a wide range of antimicrobial-deactivating beta-lactamases. The beta-lactam exposure to pathogen produces an inducible broad-spectrum AmpC and beta-lactamase. Poor membrane permeability, inducible AmpC expression, efflux pumps, together produce resistance to various beta-lactams antibiotics. The clinical efficacy of carbapenems against infections increases partly due to the presence of AmpC as strong inducers and poor substrates. (Morita, Tomida, & Kawamura, 2014)

The CRE hypervirulent *Klebsiella pneumoniae* pathogenic strains are observed as a significant human health threat, as they are multidrug-resistant (MDR), highly transmissible pathogens, and simultaneously hypervirulent. Proper preventive measures have to be quickly undertaken to control increased dissemination of such typical micro-organisms in the healthcare facilities and the associated communities (Gu et al., 2018).

The expenses for Carbapenem-resistant *Enterobacteriaceae* induced infection is comparatively higher than various other chronic/acute impairments. Expenditure rises proportionally as Carbapenem-resistant *Enterobacteriaceae*- induced infection increases, such as the cost is increased by 2.0 times, 3.4 times and 5.1 times for the inflectional frequency of 6 per 100 000 persons, 10 per 100 000 persons, and 15 per 100 000 persons, respectively. According to the dramatically increasing frequency in the infection of CRE prevalence, proper control and preventive therapeutic approaches should be taken immediately, before it turns to an endemic. (Bartsch et al., 2017)

In the combination of ceftazidime-avibactam, ceftazidime is a 3rd -generation cephalosporin antibiotic that is administered through the intravenous route and the other drug, i.e. avibactam does not have a β-lactam ring but act as a β-lactamase inhibitor. European Union considers this adjunct combination of ceftazidime-avibactam as the treatment of pneumonia acquired in hospitals such as ventilator-mediated pneumonia infection in patients, complicated UTI such as complicated intra-abdominal infections, pyelonephritis and other aerobic Gram-negative micro-organisms -induced infections in and out-patients (Shirley, 2018).

The in-vitro study of ceftazidime-avibactam combination has shown a potentially excellent activity in most important Gram-negative strain's microorganisms, such as most of the AmpC- Enterobacteriaceae, drug-resistant Pseudomonas aeruginosa isolates, Klebsiella pneumoniae carbapenemase-Enterobacteriaceae, ESBL- Enterobacteriaceae and OXA-48-producing Enterobacteriaceae; whereas no activity has been found against metallo-β-lactamase-producing pathogens (Shirley, 2018)

Previous studies have reported that ceftazidime-avibactam in combination therapy is well tolerated and has a safety profile similar to the use of ceftazidime as monotherapy. This observation is supported by the lack of interaction in pharmacokinetics that was observed in ceftazidime-avibactam combination, along with this, it is further supported by published clinical analysis during drug development phase-II in patients. In these clinical trials on patients, the frequency of treatment-mediated side effects and serious adverse events was observed approximately similar between ceftazidime-avibactam treated along with non-treated patients. Critical side effects due to adjunct ceftazidime-avibactam therapy included diarrhea, increased liver enzyme concentration, and acute kidney failure. Even though no patients were found to have CDAD (Clostridium difficile–associated diarrhea), it is one of the key labeled warnings and consideration for ceftazidime-avibactam, as with all systemic antimicrobial agents. Whereas no case was reported with Clostridium difficile–mediated diarrhea, this is a significant threat for ceftazidime-avibactam tolerance identification, similar to all systemic antimicrobial agents (Zasowski et al., 2015).

The drug development of phase I clinical data for safety analysis was designed to estimate the effect on heart QTc interval by administering ceftazidime-avibactam in healthy volunteers. This study has indicated very little adverse effects in electrocardiogram abnormalities. The point to be noted is that no dose-related side events have been observed in the previous and present studies. (Zasowski et al., 2015). Therefore, ceftazidime-avibactam combination is represented as a new therapeutic option against critical and difficult-to-treat pathogenic infections.

The ceftazidime-avibactam efficacy was partially identified by administering ceftazidime agent alone to treat various bacterial-mediated infections, such as complicated urinary tract infections and intra-abdominal tract infections.

Another study reported that ceftazidime-avibactam combination therapy was observed in phase II clinical trials of drug development to evaluate both efficacy level and safety profile for the treatment of complicated intra-abdominal and urinary tract infections. Therefore, volunteers 18 years of age or older with confirmed diagnoses of complicated intra-abdominal tract infections were recruited, and the pathogenic bacteria was identified as non-resistant against ceftazidime-avibactam therapy. The infection criteria were based on following diagnosis: appendiceal perforation, diverticulitis with intraabdominal abscesses, abscess /perforation, cholecystitis with gangrenous perforation/ rupture or secondary peritonitis (excluding spontaneous pathogenic bacterial peritonitis-mediated chronic ascites).

A previous study on avibactam has shown linear relationship which remains unaffected by the ceftazidime coadministration with avibactam. The combination parallel increases the peak plasma concentration and the AUC (area under curve) with increased range of doses (50 to 2,000 mg). There are insignificant results for accumulation of ceftazidime or avibactam in healthy participants after multiple intravenous infusions of ceftazidime-avibactam (2 g /0.5 g) at 8-hourly interval consecutively for 11 days.

Ceftazidime- avibactam is primarily eliminated by kidneys and approximately 80%-90% is excreted unchanged by the kidneys. The avibactam clearance is reduced with impaired kidney function and requires dose adjustments in patients with renal impairment. Ceftazidime- avibactam does not require dose adjustment based on age or gender. Ceftazidime drug less than 10% is protein-bound and does not dependent on concentration. Avibactam-human-plasma-protein binding is also low (5.7%-8.2%) (Zasowski et al., 2015). Ceftazidime- avibactam displays time-sensitive bactericidal activity in most of the Gram-negative strain's pathogens as dose range increases from 2-8 mg/L.

In human participants, the blood serum bactericidal killing of Ceftazidime-avibactam co-therapy has been seen against ceftazidime-resistant *Klebsiella*

pneumoniae isolates (32 mcg/mL of minimum inhibitory concentration) for 8-hours followed by 1g of ceftazidime and 250mg of avibactam intravenous infusion, and for 12-hours followed by 2g of ceftazidime and 500mg of avibactam intravenous infusion. The continuous intravenous infusion of avibactam with greater than 0.1 mcg/mL and simultaneously ceftazidime serum concentrations develop a static effect on the growth of a Klebsiella pneumoniae Carbapenems-producing isolates of K. pneumoniae (Zasowski et al., 2015).

Nosocomial pneumonia increases the mortality rate and healthcare-related expenses. Gram-negative bacteria, specifically Enterobacteriaceae is likely to produce hospital-acquired pneumonia (Peleg & Hooper, 2010). Enterobacter often produces numerous antibacterial resistance mechanisms, such as carbapenemases and ESBL. Limited therapeutic options are present against infections-induced by bacteria with carbapenemases and ESBL. An increased frequency of mortality and treatment expenses are observed in patients who received inappropriate or delayed empiric medication (Torres et al., 2018). The previous study suggested that ceftazidime-avibactam combination as a possible alternative therapy against carbapenems in ventilator-associated pneumonia or nosocomial pneumonia patients, in patients previously infected by Gram-negative strain of bacteria (Torres et al., 2018). Ceftazidime–avibactam administration has shown positive outcomes in hospitalized-nosocomial pneumonia patients. (Tuon et al., 2018)

The avibactam effect on the susceptibility of Enterobacteriaceae was studied and there was found to be a reduction in MIC90 against *Pseudomonas aeruginosa* (Enterobacter). Ceftazidime-avibactam has shown variable susceptibility patterns for P. aeruginosa, as this depends on the characteristics of bacteria and the resistance phenotype of micro-organism. Generally, ceftazidime-avibactam combination susceptibility for P. aeruginosa ranges between 80–90%, and this is improved as compared to monotherapy of ceftazidime according to data reported in other studies. The susceptibility effect between meropenem-NS and ceftazidime-NS in urinary isolates of *Pseudomonas. aeruginosa* as mentioned in a global surveillance program was reported above 50% (Sharma, Park, & Moy, 2016). Regardless of MIC_{90} reductions in some previously reported studies, Acinetobacter pathogenic species are highly non-susceptible to ceftazidime-avibactam combination. This might be due to

the variable susceptibility of avibactam drug against the OXA b-lactamases, Ambler class D enzyme, and numerous other antibiotic resistance pathways prescribed to treat *Acinetobacter species*. (Zasowski et al., 2015).

The susceptibility of ceftazidime-avibactam combination demonstrates activity in bacteria that produce Ambler class beta-lactamases, particularly A and C (Guedes, Duro, Fonseca, Abreu, & Rocha-Pereira, 2020; Lagacé-Wiens et al., 2014b). The study was conducted in 72 U.S. healthcare centers in 2012 on 701 extended-spectrum β-lactamase- Enterobacteriaceae phenotype pathogens for genes of various b-lactamase enzyme. The ceftazidime-avibactam susceptibility outcome from this study revealed that MIC90 of 118 in KPC-producing organisms was 2 mg/L, and for Ambler class C (particularly CMY-2–like producers) and ESBL (SHV-ESBLs, CTX-M-14–like producers and CTX-M-15– like producers) bacteria was 0.25– 0.5 mg/L. Another study similarly identifies potency (1 mg/L MIC90) through the *in-vitro* model of ceftazidime-avibactam combination against 42 different strains of KPC-producing *Klebsiella pneumonia*. The co-administration of avibactam agents leads to a huge decline in the MIC folds (4- to 1204-) against Enterobacteriaceae strains-produced Ambler class b-lactamases, A and C, such as KPC, EBSL, AmpC enzymes. Whereas, avibactam-ceftazidime had less impact overall, and likely to affect a little more on Ambler class B and D b-lactamases -producing bacterial strains. The *in-vitro* study on Ambler class D (OXA-48 carbapenemase), ceftazidime-avibactam administration in OXA-48–producing *E. coli* and in 25 strains of *K. pneumoniae* has found MIC $\leq$ 0.008 mg/L and MIC range $\leq$ 0.008–1 mg/L, respectively (Zasowski et al., 2015).

The activity of ceftazidime-avibactam combination found in anaerobic pathogens is limited. The published study pointed out that avibactam-ceftazidime combination results in a 2-fold to 4-fold decline in the MIC90 for most of the anaerobic pathogens. However, it was retained above the cutoff point of CLSI susceptibility (16 mg/L set) for the remaining cephalosporins drugs in many of the examined bacteria; therefore ceftazidime-avibactam does not appear to possess clinically proven anaerobic coverage by itself. Furthermore, the addition of metronidazole with ceftazidime-avibactam produced more decrease in the MIC90 level for various micro-organisms. This outcome suggests that ceftazidime-avibactam co-administration regimens with metronidazole agents are beneficial options against

progression of polymicrobial-induced infections that involve anaerobic pathogens. Like ceftazidime the drug, ceftazidime-avibactam combination has a limited spectrum of antibiotic effect in gram-positive microorganisms. The data elucidated poor activity in pathogenic *Staphylococcus aureus* strains but similar activity in beta-hemolytic streptococci by showing MIC90 greater than 32 mg/L and 0.5 mg/L, respectively (Zasowski et al., 2015)

The study was performed on thigh infection model of murine to evaluate the ceftazidime-avibactam combination in 27-isolates of *P. aeruginosa* ceftazidime-avibactam, this was shown 4–32 mg/l MICs. Bactericidal activity was 0.7 to 3-log decline in bacterial cell counts, this has been found in 16 out of 17 bacterial pathogens with ceftazidime-avibactam combination treatment with MICs of £8 mg/L and 5 out of 8 bacterial isolates with ceftazidime-avibactam with MICs of 16 mg/L. Followed by the 24-hour incubation with ceftazidime-avibactam treatment, the colonies of bacteria were not found from thigh homogenates that were previously seeded with drug in culture petri dishes, therefore, they concluded that non-susceptibility was not developed following ceftazidime-avibactam combination treatment. (Mawal, Critchley, Riccobene, & Talley, 2015)

The detailed molecular epidemiology and genomic studies pointed out that many drug-non-susceptible bacterial strains have the potential to transfer and produce epidemic outbreak. The beneficial therapeutic option of susceptible drugs for resistant strains provides strong motivation to renewed drugs and vaccines. Growing pieces of evidence also draw attention towards control measures in health care facilities for dramatically enhanced ratio of infections. The investigation of preventive therapeutic strategies is required to break the infectious transmission cycle that develops by drug-resistant patients. The mobility and large-scale migration of people across cross-border indicate the persistent increase of drug-resistant infections. This shows that drug-resistant infections are the global concern that present a serious life threat to both endemic and non-endemic settings and the non-beneficial outcome to identify new therapeutic strategies that could probably compromise traditional management for infection spreading and cause a decrease in momentum to achieve infection-free environment in the future (Marais, 2016).

The ceftazidime-avibactam has the property of penetration into the epithelial lining of lungs, this makes it as a potent antibiotic for the treatment for pneumonias (Marais, 2016). Ceftazidime-avibactam, which was previously prescribed to patients with multidrug-resistant infections, was used in the tuberculosis model in one of the previously reported studies, and this showed markedly dead fast-growing, semi-dormant and intracellular Mycobacterium tuberculosis from the model. Furthermore, multidrug-resistant clinical pathogens have potent susceptibility profiles to ceftazidime-avibactam regime and clinically optimum concentrations were able to inhibit these pathogens. The resistance mechanism is initiated due to mutations in the transpeptidase domain (enzyme that cross-links peptidoglycan for bacterial wall synthesis) of the penicillin-binding protein of bacteria, suggested that the therapeutic agent results in dead pathogenic Mycobacterium tuberculosis bacilli by modulating synthesis of cell wall. D. Deshpande *et al* have recognized exposure targets (concentrations) to get maximum positive effects against tuberculosis (Deshpande et al., 2017).

Urinary tract infections (UTIs) are globally encountered problems having high morbidity, mortality, and healthcare expenses. This becomes more advanced if associated with chronic urinary retention in men, acute pyelonephritis recent urinary instrumentation, urologic abnormalities, or obstruction, or urinary catheters. The gram-negative bacteria, such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis* are often associated to produce urinary tract infections, and these organisms frequently introduce MDR mechanisms, specifically with ESBL to prevent potential of antibacterial agents that earlier suggested as first-line treatment option. The global health threat of carbapenem-resistant *P. aeruginosa* and Enterobacteriaceae has declined the antibiotic activity of carbapenems and emphasized the need to introduce new antibacterial options urgently (Wagenlehner et al., 2016)

In a previous study, approximately 20% of pathogenic microorganisms were ceftazidime resistant. The potential clinical value of ceftazidime-avibactam against ceftazidime-resistant pathogenic bacteria has marked its effectiveness as a carbapenem-sparing treatment. The study in *in-vitro* and *in-vivo* model has suggested

ceftazidime-avibactam combination is effective against infections of carbapenemase-producers (Wagenlehner et al., 2016).

Avibactam-ceftazidime restores antibacterial activity in beta-lactamase-producing Gram-negative infectious microorganisms (Goodlet et al., 2016; Philippe Lagacé-Wiens, Andrew Walkty, & James A. Karlowsky, 2014a). The ceftazidime-avibactam combination (94 mg/L) has found the decrease MIC90 level of ceftazidime agent (by >128-fold to £8 mg/L) in KPC-producing Enterobacteriaceae. The observed MIC90 level was according to FDA suggested susceptibility cutoff range for ceftazidime-avibactam combination regime. The antibacterial activity of ceftazidime-avibactam combination as adjunct therapy in CTX-M producers in Enterobacteriaceae was raised by 8-fold to 32-fold relative to ceftazidime monotherapy. Furthermore, the MICs level declined to <1 mg/L against SHV or TEM b-lactamase–producing E. coli and K. pneumoniae pathogens, the combination with avibactam agent decreased the MIC (256-fold) relative to ceftazidime as monotherapy (Wagenlehner et al., 2016).

Enterobacteriaceae resistance to AmpC-mediated ceftazidime was overcome by using ceftazidime–avibactam regime, decreasing the MIC value for fully restored mutants and isolates according to suggested FDA susceptibility cutoff point of ceftazidime-avibactam regime. (Mawal et al., 2015)

CHAPTER 3

METHODOLOGY

3.1 Research Design

The observational cross-sectional *in-vitro* study was carried out to investigate the response and frequency of ceftazidime-avibactam drug against isolated clinical specimens of gram-negative Enterobacteriaceae i.e. *Escherichia coli* and *Klebsiella pneumonia* which were resistant to the conventional antibiotics.

Ethical approval

Since the current study involved human participants, therefore ethical approval that is primary consideration of the research study was taken from Faculty of research committee, Ethical review committee (ERC) of Bahira University Medical and Dental college and PNS Shifa hospital to protect subjects' rights, welfare and dignity. Next to this, informed consent was taken from patients.

3.2 Subjects

To carry out the study, the samples were collected from amongst 150 indoor and outdoor patients at PNS Shifa Hospital Karachi. The age group of individuals in this study includes 10-50 years. Permission from hospital review committee to conduct the research was taken. The subject evaluation form was designed to record demographic data of patients.

3.3 Setting

Different samples of *Enterobacteriaceae such as Escherichia coli* and *Klebsiella pneumonia* were collected from different wards (Surgery, Medicine, Gynecology, ENT and Pediatrics) and ICU (intensive care unit) at PNS Shifa hospital. Next to this, the specimens were inoculated on blood and MacConkey's agar culture plates. Culture plate was inoculated at 37 ℃ in incubator for 24 to 48 hours. Identification of *Escherichia coli* and *Klebsiella pneumoniae* was done by colony morphology method, gram staining technique, biochemical analysis, and API 20E test. After identification, susceptibility of antibiotics was evaluated by using Mueller Hinton agar.

3.4 Inclusion criteria

Escherichia coli and *Klebsiella pneumonia* was collected from different clinical specimens of patient. Clinical specimens include urine, blood, pus, CSF and respiratory specimens. The specimens of age group from 10 to 50 years of age and both genders were received from different wards at PNS Shifa hospital.

3.5 Exclusion criteria

Repeated samples from same patient. The same isolate infects same patient again.All the other members of Enterobacteriaceae were excluded except *Escherichia coli* and *Klebsiella pneumoniae*.

3.6 Duration of study

September 2019-May 2020

3.7 Sample size estimation

The approximated/estimated sample size was determined by using the method

of sample size (WHO World Health Organization) by taking 95 % confidence interval, 5% margin of error and prevalence of susceptibility is 90 % (Zhang et al., 2018).

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Here $Z_{0.05}$=1.96, P=90% or 0.9 and d=5% or 0.05 then

$N=[(1.96)^2 (0.9)(1-0.9)]/(0.05)^2$

n=[3.8416(0.9)(0.1)]/(0.0025)

n=0.34574/0.0025

n=139

Calculated sample size is 139 patients.

Zhang, W., Guo, Y., Li, J., Zhang, Y., Yang, Y., Dong, D., ... & Hu, F. (2018). In vitro and in vivo bactericidal activity of ceftazidime-avibactam against Carbapenemase-producing Klebsiella pneumoniae. *Antimicrobial Resistance & Infection Control*, *7*(1), 142.

3.8 Sampling technique

Non-probability convenient sampling

3.9 Human subjects and consent

Samples of outdoor and hospitalized patients in PNS Shifa hospital Karachi were included. Data was acquired about demographic characteristics like age groups and genders.

Consent was taken in form of thumb impression and signature after verbally explaining the project. In case of children, consent was taken from parents (as given in appendices C).

3.10 Materials

a. Clinical samples

The clinical samples of patients included urine, blood, CSF, pus and respiratory specimens of patients admitted in PNS Shifa hospital.

b. Culture media

MacConkey Agar, Blood agar, and Mueller Hinton agar

c. Antibiotic Disc

Ceftazidime-avibactam disc and e-test strips (Liofilchem disk).

d. Equipment

Autoclave, Hot air oven, Incubator and Automated blood culture system.

e. Performa

as mentioned in Appendices (C and D)

3.11 Parameter of study

This study evaluated the antibiotic susceptibility of ceftazidime-avibactam on Mueller Hinton agar against *Escherichia coli* and *Klebsiella pneumonia*. Clinical profile consisted of age, gender, and clinical diagnosis.

3.12 Protocol of study

The data was entered into a specially designed Subject Evaluation Form. Permission was taken from Hospital Ethical Committee. Informed consent from all the 150 patients was taken for this study. Age, gender and hospital identity number of patients were recorded on specially designed proforma. The samples include urine, blood, pus and respiratory specimen that was collected in Microbiology department,

PNS Shifa hospital.

The Gram stain of the samples was performed to identify the gram-negative rods by the pink color of the colonies. All specimens like urine, blood, pus, and respiratory specimen were inoculated on Blood agar and MacConkey's agar. Culture plates were incubated at 37 ºC in ambient air for 24 to 48 hours. On blood agar, circular, grey and moist colonies were observed, while on MacConkey's agar circular, pink and lactose fermenting colonies were found. Further identification of organisms was done for biochemical tests. API 20E system and Triple sugar iron (TSI) was used to biochemical identification of *E. coli* and *Klebsiella. pneumoniae*.

A suspension was equivalent to 0.5 McFarland of all isolated *E. coli* and *Klebsiella. pneumoniae* were inoculated by using cotton swab on a Müller-Hinton agar plate. Antimicrobial susceptibility tests (AST) against Ceftazidime-avibactam were done following Kirby-Bauer disc diffusion method using Ceftazidime-avibactam disc (30/20 µg) and Epsilon meter test (E-test) method. Susceptibility of all the conventional antibiotics was interpreted by combine disk diffusion (CDD) method according to CLSI guidelines. The interpretation of zone diameter was carried out as per CLSI guidelines, 2019 as mentioned in operational definition.

Sample collection protocol

- **Urine**

1. The sterile container was given to the patient with brief instructions about mid-stream urine collection.

2. Boric acid powder (0.1 g/10 ml of urine) was added in the container to preserve the sample.

3. The sample container was labeled and forwarded to the Microbiology Laboratory of PNS Shifa within 48-hours.

4. The sample (10 ml) was transferred aseptically into conical tube and

centrifuged at the speed of 500–1000 g for approximately 5-minutes. The supernatant was separated and used for biochemical analysis.

5. The sediment in the conical tube was remixed by tapping at the bottom of the tube and transferred single drop to a sterile glass slide and spread it to make a thin smear and put coverslip on it.

6. The smear was allowed to airdry and protected from dust and insects and stained it through the Gram technique.

7. The smear was examined through microscope.

- **Blood**

1. The vein was located in the upper arm and disinfected the site about 50 mm in diameter by 70% ethanol.

2. The top of the bottle was wiped by ethanol-ether swab.

3. About 20 ml of blood was withdrawn and dispensed about into the culture medium bottle that contained about 25 ml of broth.

4. The blood was mixed with the broth in the EDTA container.

5. The media was incubated and the culture was protected from sunlight until it was in incubation.

- **Pus**

1. The pus specimen was collected by using a sterile swab and inserted the swab into container that had amines transport medium. Next, the stick of the swab was broken to cap the bottle tightly.

2. The smear was made on a sterile slide for Gram staining and allowed it to air-

dry by heat-fix the smear.

- **Sputum**

1. A sterile container was given to the patient and requested to cough deeply to produce a sputum specimen.

2. The container was labelled.

Isolation and Preservation of *Escherichia coli* and *Klebsiella pneumoniae* from clinical samples

Different clinical samples of subjects were taken and isolation of *Escherichia. coli* and *Klebsiella pneumoniae* colonies were done, these organisms were kept preserved in nutrient broth with glycerol in a volume of 2ml in screw-capped bottles at -20℃ as shown in figure 3.1.

Identification of E. coli and Klebsiella pneumoniae

Clinical samples of pus, urine, nasal swab, throat swab, blood and respiratory aspirate were taken, and identification of gram-negative rod was done by conventional phenotypical methods which include gram staining, culture media, triple sugar Iron, and API20E.

Gram staining

Procedure

The Gram stain was performed by applying the sample from infected site onto a glass slide. To fixed smear of bacterium, the slide is then treated with crystal violet (primary stain). Iodine was added to form a crystal violet complex as per figure 3.1. Decolorizer was added like ethanol, followed by safranin. Pink rod-shaped colonies were observed on microscope.

Figure 3.1 Collection and storage of sample

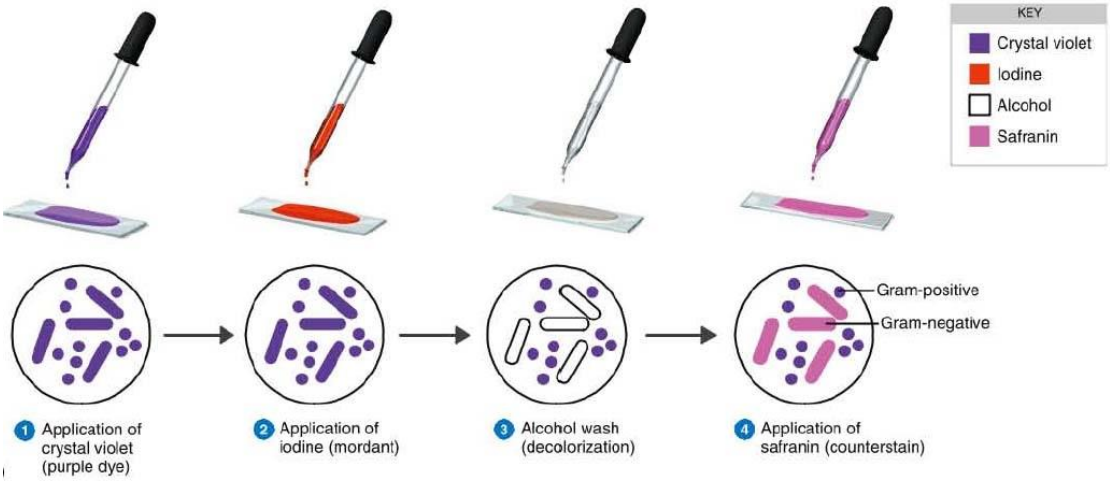


Figure 3.2: Procedure of gram staining (Becerra, S.C et al., 2016)

Culture media

Blood agar

E coli is a gram-negative bacillus. This grows well on commonly used medium. Colonies are big, circular, gray, moist and beta (β) hemolytic. Most E coli strains are nonpigmented. Large, mucoid, and grayish colonies of *Klebsiella pneumoniae* without hemolysis were observed when cultivated on blood agar in anaerobic conditions at the temperature of 37°C and duration of 24-hours.

MacConkey agar

MacConkey agar contains bile salts as inhibitor of the Gram-positive flora and neutral red as acid production from lactose indicator. on MacConkey agar plate the colonies of E. coli and *Klebsiella pneumoniae* are pink as both ferment lactose but the colonies of E. coli are smaller in size as compare to *Klebsiella pneumoniae*.

Biochemical test

Triple sugar iron test (TSI)

In the context with positive test for organism, the triple sugar iron test (TSI) was assessed to evaluate ability of *Escherichia coli* and *klebsiella pneumonia* to ferment the sugar molecules and produce gas (hydrogen sulfide). Principally, the phenol red reagent and ferrous sulfate solution indicate the acidification of agar medium and hydrogen sulfide production by organism, respectively. Initially, the fermentative bacteria utilize glucose which turns the medium acidic in 8-12 hours by showing yellow appearance, whereas, butt of the tube, in anaerobic condition, sustain the acidic condition due to presence of organic acids that are led by glucose fermentation. The slants turn the acidic medium into alkaline in aerobic state by oxidizing the fermentation products into CO_2 and H_2O and peptone, the slant subsequently oxidized and produced alkaline amines.

In the present study, the 24-hour bacterial culture in tryptic soy broth tube was

used to proceed TSI assay. Initially, the inoculation needle was sterile by heated in blue flame in vertical direction till it became red hot. Then allowed the needle to cool for 20-sec. The culture was taken in the needle, stabbed into medium of triple sugar iron tube and streaked slant-surface in back and forth direction. Followed by flaming the tube mouth, capped it and incubated the tubes for duration of 24-hours at the temperature of 37°C. Followed by incubation period, the fermented dextrose was observed by red slant or yellow butt due to alkaline-acid reaction, and fermented dextrose, lactose, and sucrose were identified by formation of yellow slant or butt due to acid-acid reaction, whereas, red slant or butt (alkaline-alkaline reaction) was shown non-fermented carbohydrate. The formation of gas molecules (CO_2 and H_2) was showed by bubbles or cracks that formed in the agar, while, presence of H2 turns the media black. Figure 3.5

The 24-hours incubation time was strictly followed to prevent false-positive outcomes because of oxidation of fermented products in aerobic conditions by sugars, especially lactose and sucrose and the turning of acidic medium to alkaline state by slant.

Figure 3.3: Growth of E.coli on blood's agar

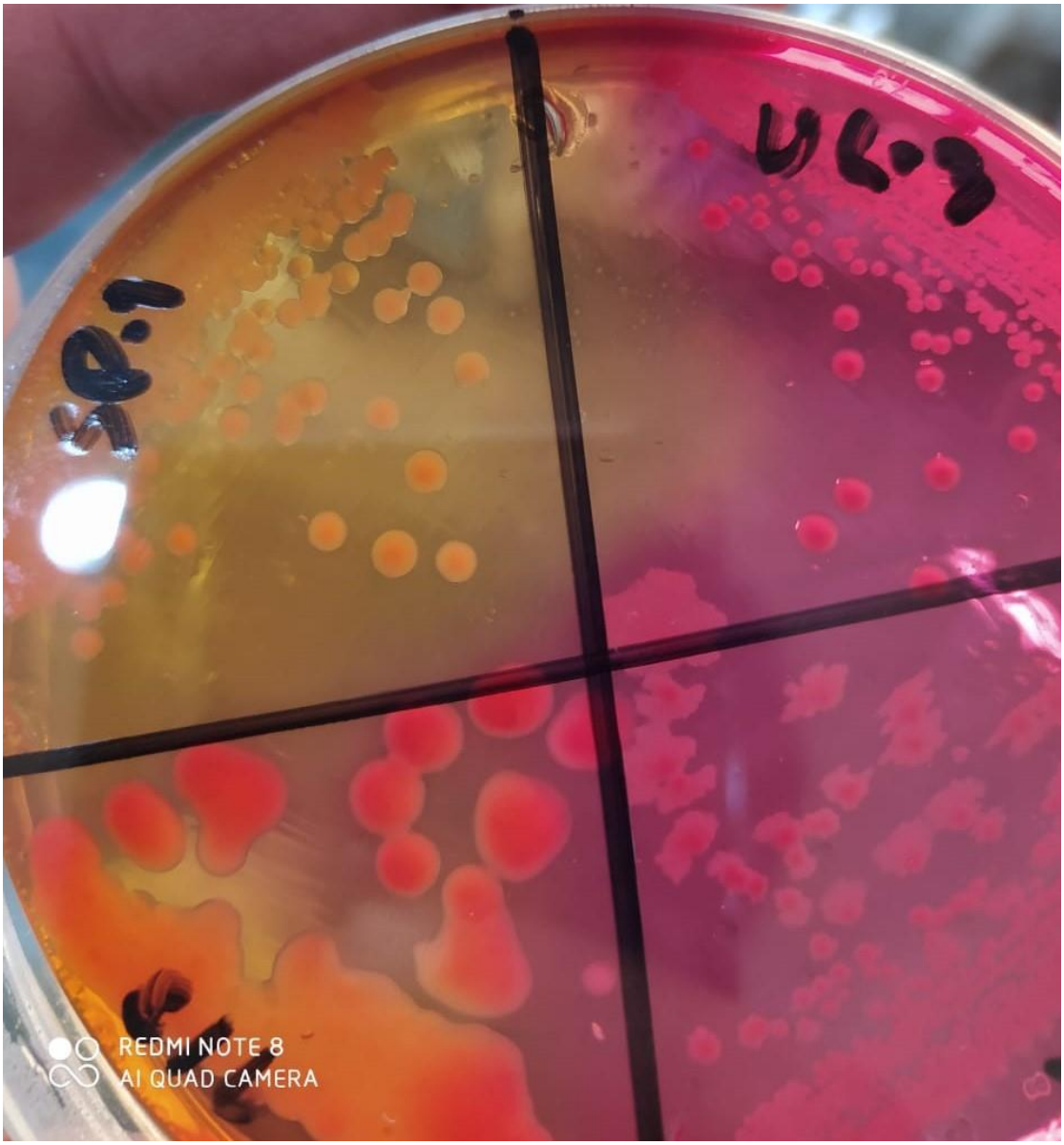


Figure 3.4: MacConkey's agar shows lactose fermenting organism

] Figure3.5: Triple sugar iron test

API20E Biochemical Test Strip

Consistently the biochemical analysis of *Escherichia coli* and *Klebsiella pneumoniae*, the Analytical Profile Index 20E biochemical test was conducted to analyzed and differentiate the strain of organisms. The commercially available API20E Biochemical test Strip was taken which was divided into 20 dehydrated compartments. The well-isolated Escherichia coli and Klebsiella pneumonia pure colony were suspended in sterile distilled water (10mL) for emulsification before the experiment preceded. The loop of the needle was flamed till it turns to red hot. Then allowed the needle to cool for the duration of 20-seconds. Take the bacterial suspension in sterile pipette (up to brim) and fill all the tubes and cupule of strips. Two drops of parafilm oil were added only in ODC (ornithine decarboxylase), ADH (arginine dehydratase test), H2S (hydrogen sulfide), LDC (decarboxylase) and URE (urease enzyme) tubes and the strip was kept at 37℃ for 24-hours. Followed by incubation, Voges-Proskauer -1 (40 % KOH) and Voges-Proskauer-2 (2α-Naphthol) were added in Voges-Proskauer tubes, Kovacs was added in IND (Indole) tube, TDA reagent (ferric chloride) was added in TDA (Tryptophan deaminase) tube for 10-minutes. The colors were compared against the chart and record the results to further analyze. figure 3.6

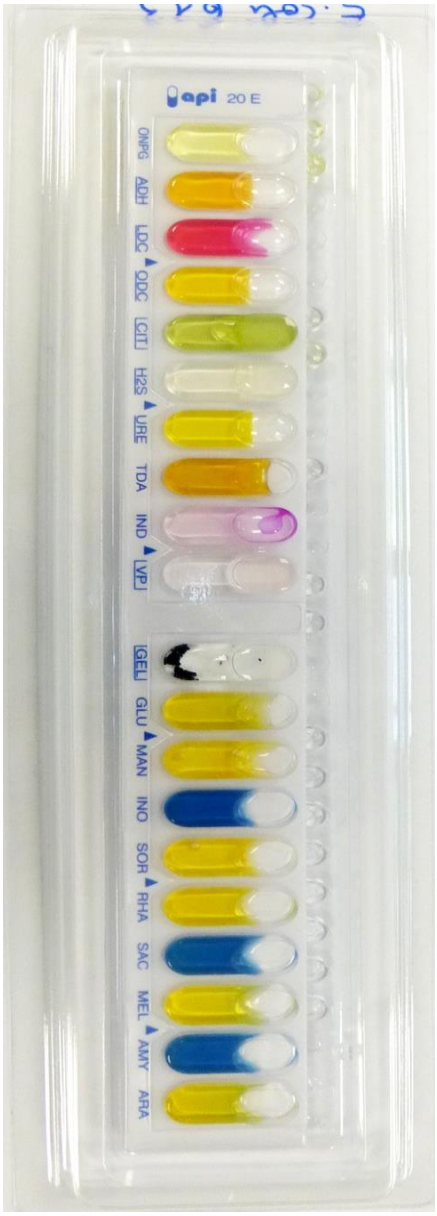


Figure 3.6: API 20E used for diagnosis of microorganism

Citrate

The citrate test has detected the ability of pathogen to get carbon and energy. Bacteria are produced citrate-permease enzyme, which is capable to convert the citrate to pyruvate. This pyruvate is metabolized to produce energy. Growth is the prominent marker for the citrate (intermediate metabolite of Krebs cycle) utilization. The bacteria are metabolized the citrate, the ammonium is converted to ammonia, which ultimately enhanced alkalinity. The change in pH level is turned the bromothymol blue indicator that turns green color to blue color above pH 7.6. The indication of E. coli is citrate positive while the indication of Klebsiella pneumoniae is citrate negative (Moini et al., 2015; Thompson et al., 2015).

Indole

The indole test is identified the capability of specific pathogens to undergoes the decomposition of tryptophan into indole. Bacteria that express tryptophanase enzyme, undergoes in the deamination and hydrolysis process of tryptophan amino acid. Indole generates by tryptophan deamination. In indole Kovac's Reagent combination, the solution is turned from pale-yellow coloration to cherry-red coloration on oily-layer tube's top. E. coli is indole positive while Klebsiella pneumoniae is indole negative.

Urease

Klebsiella pneumoniae is analyzed by urease test, as it is urease positive. Urea is produced by the amino acid's decarboxylation. Urea hydrolysis is produced the ammonia and release carbon dioxide gas. The previously formed ammonia is alkalinized and shift the PH level that is analyzed by phenol-red coloration to light-orange coloration at pH 6.8 to pH 8.1. Within 24 hours, the rapid urease-positives bacteria are turned the media into pink.

Antibiotic susceptibility disc diffusion method

Based on biochemical analysis of *Escherichia coli* and *Klebsiella pneumonia*, the ceftazidime-avibactam susceptibility potential against organism was detected by disk diffusion susceptibility assay on Muller-Hinton agar, as consistent with clinical laboratory standard international- (CLSI) 2019 recommendation. The disk diffusion method uses the principle of an antibiotic-impregnated disk, that places over the surface of an inoculated-agar medium. They absorb moisture diffuses antibiotic outward by agar and ultimately increases antibiotic concentration. The high concentration gradient is found at the antibiotic-disk edge, whereas, the increased distance with disk gradually diminishes the inhibitory activity against organisms and them able to grow easily. Followed by incubation, the visible zone forms in case of growth inhibition.

In the present study, the petri dish outside rim was labeled with the name of the pathogen, i.e. *Escherichia coli* and *klebsiella pneumonia*, and patient ID. Next, the *isolated* colony of organism was picked up aseptically from pure Muller-Hinton agar isolation *plate and* suspended in 10mL sterile distilled water for emulsification. The test tube containing organism was gently flamed to sterile, and the cotton swab dipped in *Escherichia coli* and *Klebsiella pneumonia* broth to collect the organism. Then, the bacteria-labeled cotton swab was used gently in Muller-Hinton agar plate side-to-side direction with rotation to maintain the uniform layer of bacteria. The plate was divided into quadrants and carefully place the ceftazidime-avibactam disk (20/30µg) in each quadrant one-by-one with forceps that were initially sterilized with flame and then ethanol. Followed by, the plate was sealed tightly and incubated at 24-hours. The Zone diameters greater than 21mm was taken as sensitive, whereas, equal to 21 and less than 21 diameters were considered as intermediate and resistant, respectively, for 30/20 µg ceftazidime-avibactam disc at 24-48 hours of incubation. The illustrations of the applied method have illustrated in figure 3.7.

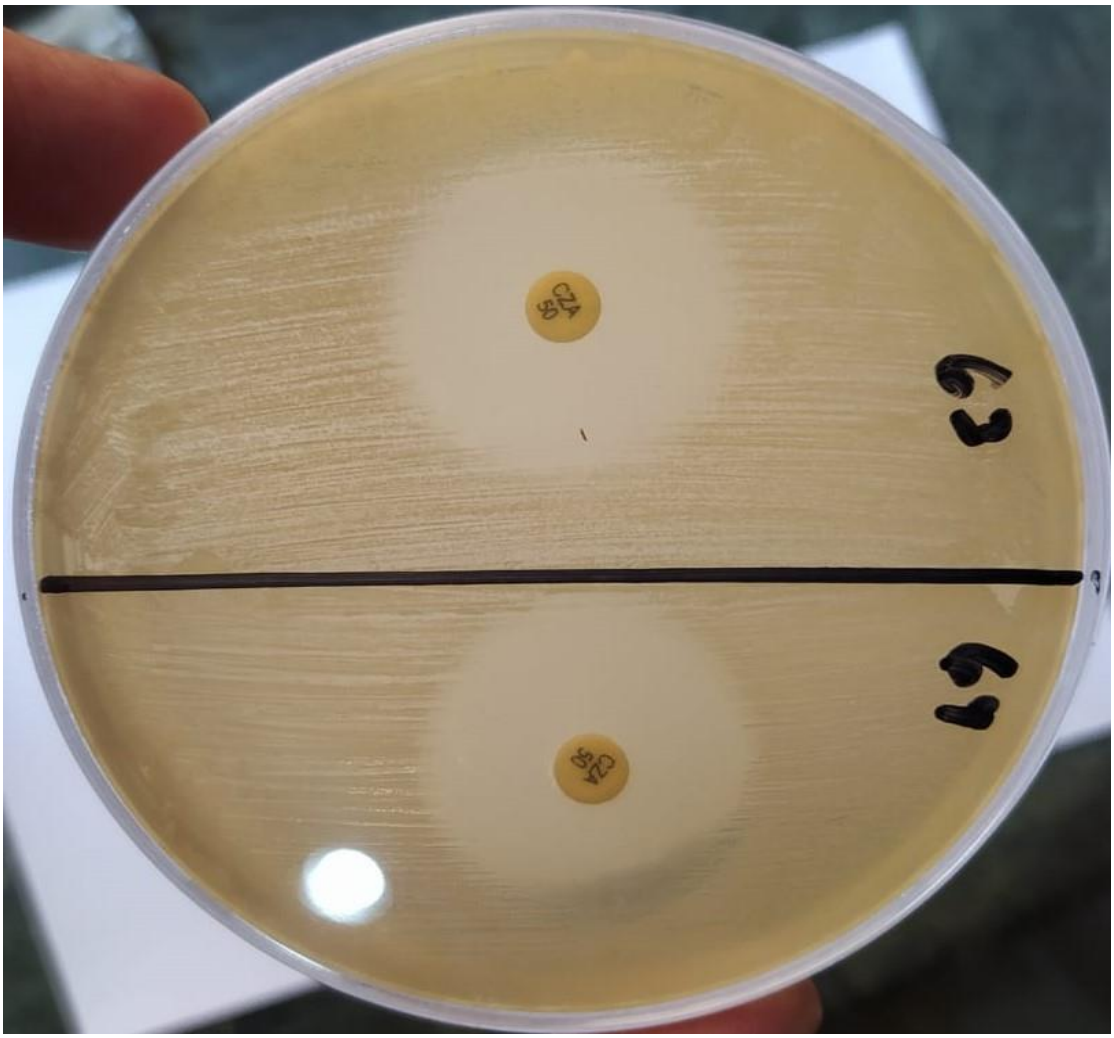


Figure 3.7: Antibiotics susceptibility testing by disk diffusion method

Epsilon meter test (E test)

The Epsilon meter test was carried out only on ceftriaxone and meropenem, as found as resistant against *Escherichia coli* and *klebsiella pneumonia* in disk diffusion assay. The quantitative E test assay detects susceptibility of ceftazidime-avibactam acting on the principle of diffusion and dilution. Principally, the E-test MIC strip in inoculated Muller-Hinton agar plate acts on plastic carrier surface of the strip to instantly release antibiotics in the agar culture medium. Followed by incubation, bacterial growth in the form of colonies and the symmetrical inhibition is visible.

In the present study, the isolated test strain bacterial colonies were emulsified by suspending into distilled saline water and then turbidity was compared with 0.5-McFarland latex standards scale of turbidity. Then, the cotton-swab was dipped into bacterial inoculum and pulled it out straightly. To remove the excess tube liquid, rotated the swab multiple times against inside of the test tube and above the level of fluid. The streaking was done directly on petri dish of Muller-Hinton agar by continuedly rotating the plate at 60^{o} angle and then left the plate (5-minutes) for moisture absorbance. The E end' of the strip was placed at the edge of petri dish with facing the scale upward. One strip was used onto a 90 mm agar plate and incubated for the duration of 24-hours at the temperature of 37°C. The diameter for zone of inhibition was less than 9, equal to 8, and more than 8 was considered as sensitive, intermediate and resistant, respectively, as recommended by clinical laboratory standard international- (CLSI) 2019, as shown in figure 3.8. The proper precaution was used to save the E-test package by stored at freezer (-20°C) and keep it at room temperature before use.

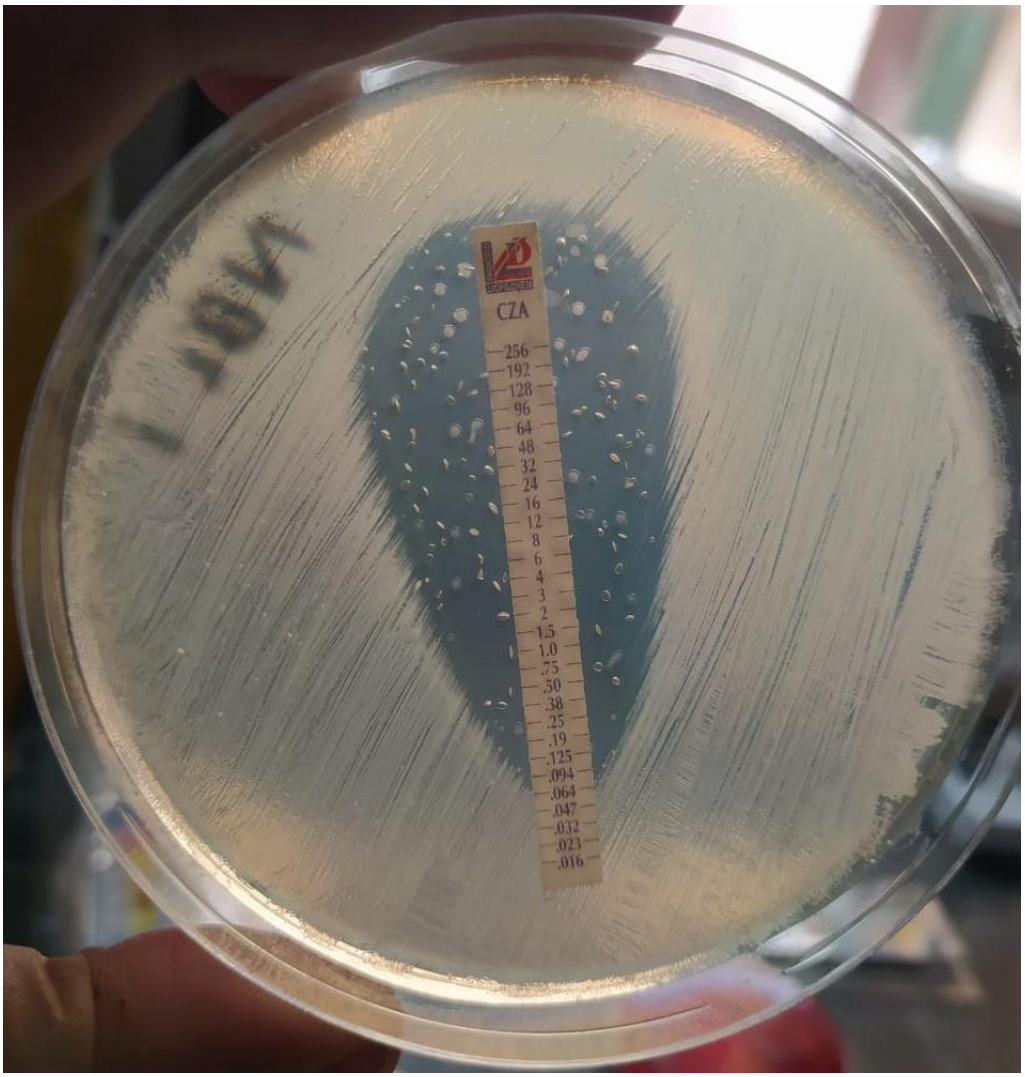
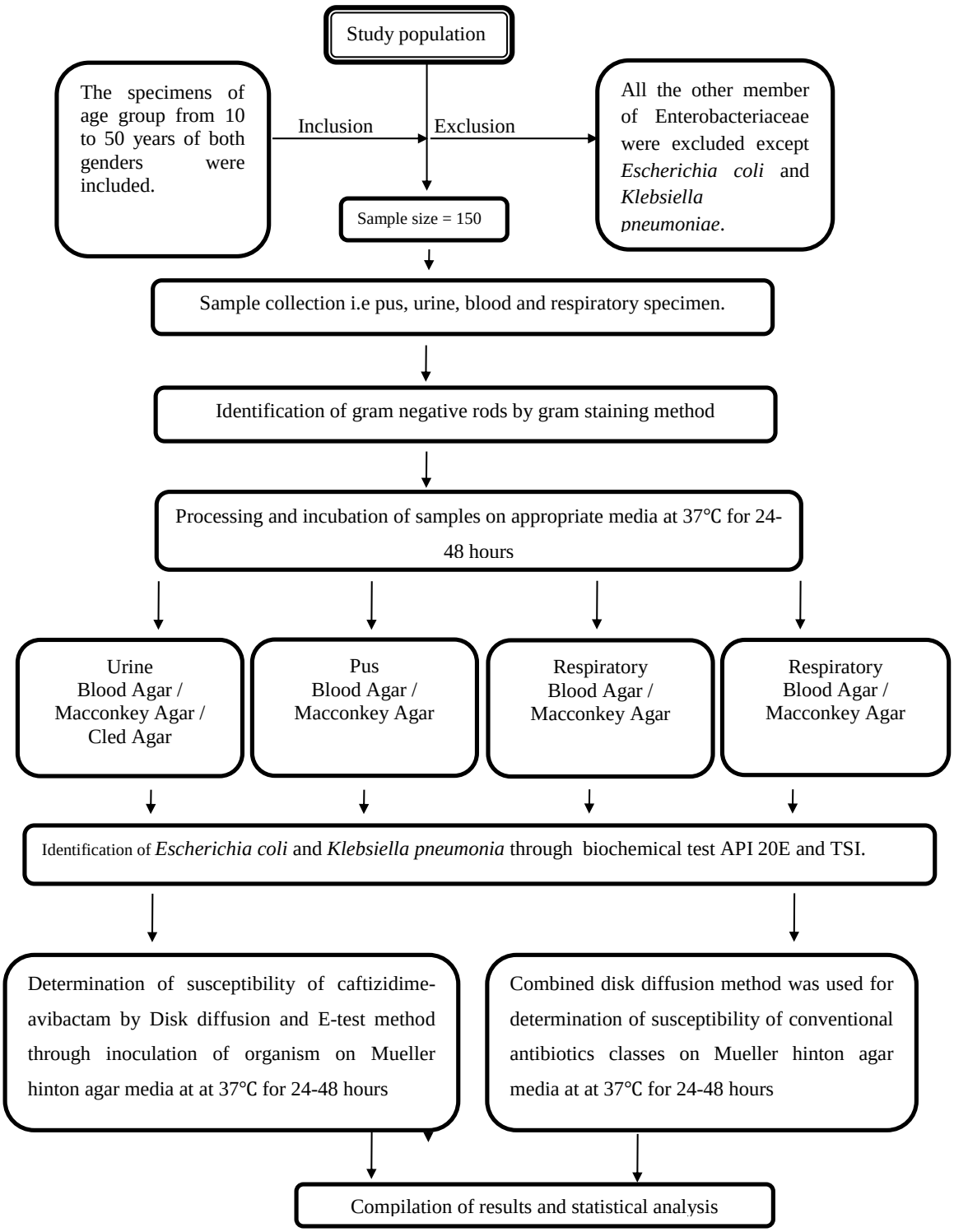


Figure 3.8: Antimicrobial susceptibility test by E-test method

3.13 Flow chart / Algorithm of study



3.14 Statistical Analysis

The collected data were analyzed by using IBM Social Package of Statistical Sciences (version 23). Mean and Standard deviation (SD) were calculated for age in years. Frequencies and percentages (%) were calculated for males and females. Outcome variable i.e. specimen (urine, pus, wound swab, sputum and blood), sensitivity pattern of Ceftazidime-avibactam was measured and recorded following the interpretive criteria. Effect modifier was controlled through stratification of gender, specimen (urine, blood, pus, pus, sputum and respiratory specimens), age to analyze the outcome of these variables. For qualitative data analysis, post-stratification chi-square test was applied. P-value of less than 0.05 was considered as statistically significant.

CHAPTER 4

RESULTS

The present study was conducted to identify the susceptibility of ceftazidime-avibactam against Enterobacteriaceae i.e. Escherichia coli and Klebsiella pneumoniae isolates. The data for susceptibility was analyzed in specimens of 150 patients of either gender among 10 years to 50 years that meet the inclusion criteria.

For identifying the descriptive statistics, SPSS version 23 was used. The qualitative data were shown in terms of frequency and percentages. The quantitative parameters were shown interims of mean ± standard deviations. To analyses the effect of modifiers on findings, stratification was done. For qualitative data analysis, post-stratification chi-square (χ^2) statistic test was used. The analysis was considered p-value less than 0.05 as significant.

Out of 150 patients there was 67(44.7%) male and 83 (55.3%) female. (Table 4.1). 68 (45%) were found with Klebsiella pneumoniae (KP) and 82 (55%) with Escherichia coli (EC) as presented in Figure 4.1. In KP group there were 24(35.3%) male and 44 (64.7%) female while in EC group, there was 43(52.4%) were male and 39(47.6%) were female (Table 4.1).

The overall mean age was 33.10±11.39 years. The age (years) of patients in KP group was 36.16±12.32 years and EC group was 30.56±9.93. The descriptive statistics of patients age are summarized in Table 4.2. The age was further stratified in groups. Frequencies of patients in age groups are presented in Figure 4.2.

Among 150 patients, most 97(64.7%) of samples were taken from urine. The detailed frequency distribution of samples is shown in Table 4.3 and figure 4.3.

In our study, E test was done for 150 patients, out of which 82.7% were sensitive and 17.3% resistant. 73.5% of patients were found sensitive and 26.5% were resistant in KP group while in EC group, 90.2% were sensitive and 9.8% were resistant as presented in Table 4.4 and figure 4.5.

In KP group, most of patients (44.1%) were found with ≤1 μg/ml MIC of CZF-AVI while in EC group, 54.9% were found with ≤1 μg/ml MIC of CZF-AVI while carbapenem-resistant organism findings show 75% sensitive in KP group and 83.3% sensitive in EC group as presented in Table 4.5 and Table 4.6 respectively.

In KP group, most common sensitive antibiotics were polymyxin B 300ug (100%), Fosfomycin 200ug (73.9%) and doxycycline 30ug (63.6%) respectively while most common sensitive antibiotics in E coli group was polymyxin B 300ug (100%), Fosfomycin 200ug (90.2%) and amikacin 30ug (63.4%) respectively as presented in Table 4.7 and Table 4.8 respectively.

The two groups were compared by using chi-square test for findings. P-value of less than 0.05 was considered as significant. Stratification was also done for gender and age group to compare two groups for findings.

The Stratification was also done for gender and age groups. Detailed frequencies and results of stratified categories are presented from Table 4.9 to Table 4.10 (Figure 4.5 to Graph 4.9.

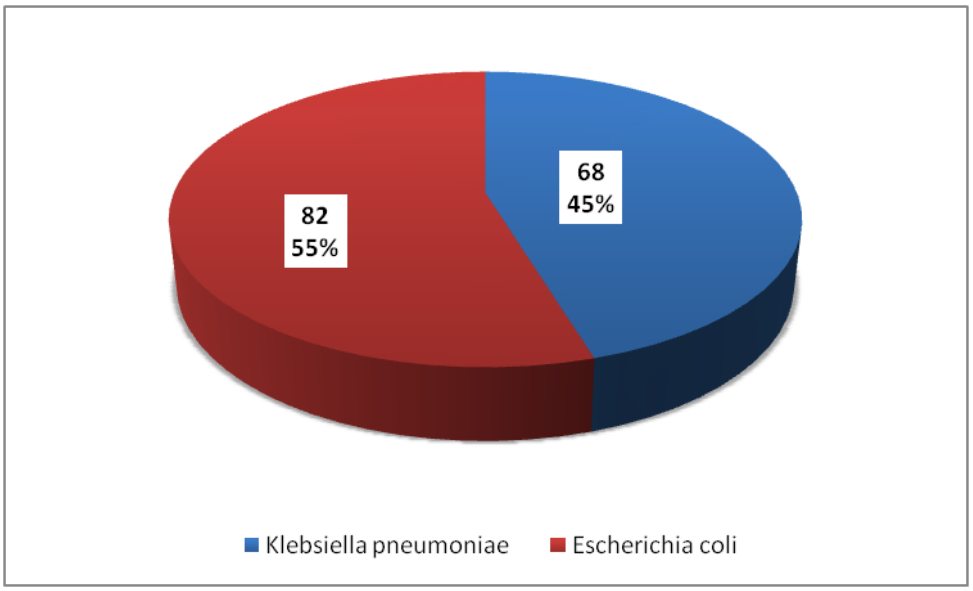


Figure 4.1: Frequency of microorganisms in clinical specimens (n=150)

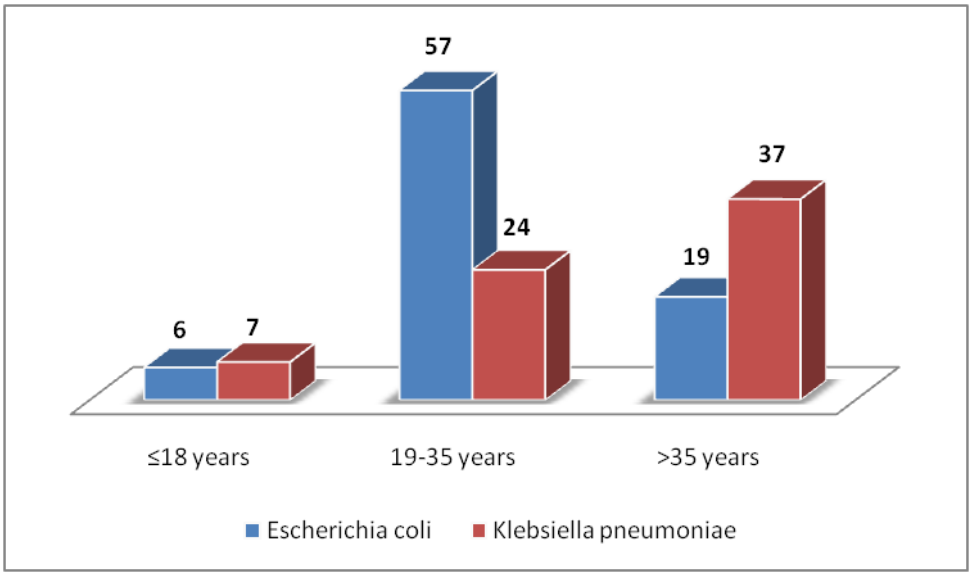


Figure 4.2: Frequency of patients according to age groups (n=150)

Table 4.1: Frequency distribution of gender (n=150)

Gender	Klebsiella pneumonia (%)e	Escherichia coli (%)	Total (%)
Male	24 (35.3%)	43 (52.4%)	67 (44.7%)
Female	44 (64.7%)	39 (47.6%)	83 (55.3%)
TOTAL	68	82	150

Table 4.2: Descriptive statistics of samples by age (years) (n=150)

Descriptive statistics	**Klebsiella pneumoniae** (n=68)	**Escherichia coli** (n=82)	**Overall** (n-150)
Mean	36.16	30.56	33.10
SD	12.32	9.93	11.39
Median	38.50	28.50	32.00
Range	40	37	40
Minimum	10	13	10
Maximum	50	50	50

Table 4.3: Frequency distribution of samples (n=150)

	Klebsiella pneumonia (%)	**Escherichia coli (%)**	**Total (%)**
Blood	8 (11.8%)	9 (11%)	17 (11.3%)
Naso Bronchial Lavage	7 (10.3%)	0 (0%)	7 (4.7%)
PUS	7 (10.3%)	22 (26.8%)	29 (19.3%)
Urine	46 (67.6%)	51 (62.2%)	97 (64.7%)
TOTAL	68	82	150

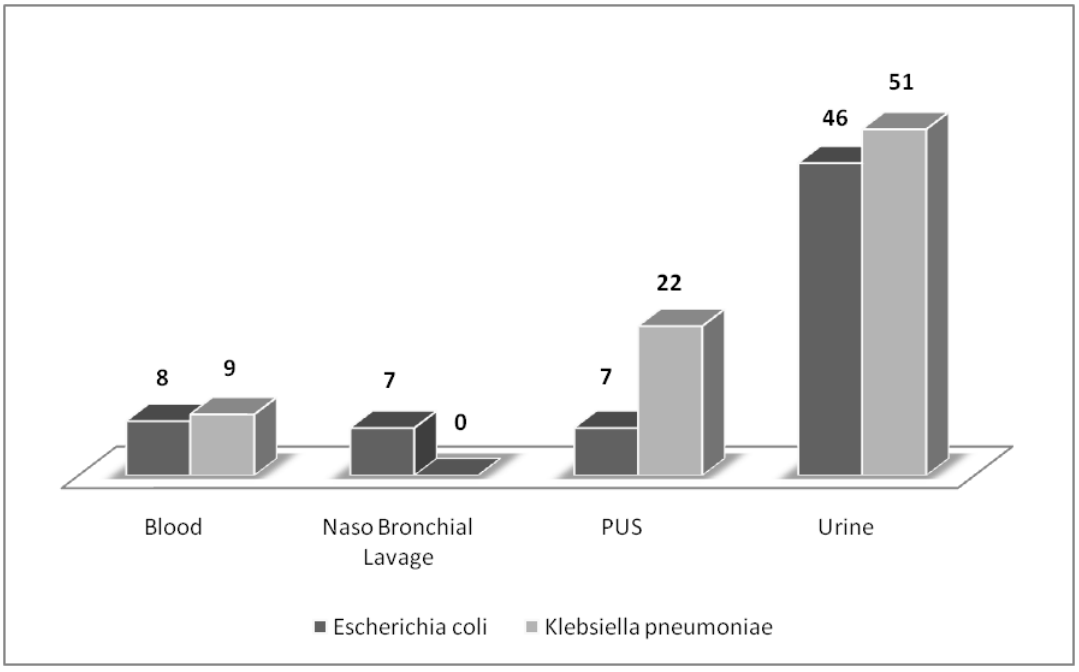


Figure 4.3: Frequency distribution of samples

Table 4.4: Antimicrobial susceptibility pattern of ceftazidime-avibactam by disk diffusion method (n=150)

Antimicrobial susceptibility	Klebsiella pneumonia (%)	Escherichia coli (%)	Total (%)
Sensitive	50 (73.5%)	74 (90.2%)	124 (82.7%)
Resistant	18 (26.5%)	8 (9.8%)	26 (17.3%)
TOTAL	**68**	**82**	**150**

Table 4.5: Antimicrobial susceptibility pattern of ceftazidime-avibactam by e-test method (n=150)

Antimicrobial susceptibility	Klebsiella pneumonia (%)	Escherichia coli (%)	Total (%)
Sensitive	50 (73.5%)	74 (90.2%)	124 (82.7%)
Resistant	18 (26.5%)	8 (9.8%)	26 (17.3%)
TOTAL	**68**	**82**	**150**

Table 4.6: MIC distribution of ceftazidime-avibactam (n=150)

: MIC distribution	**Klebsiella pneumonia (%)**	**Escherichia coli (%)**	**Total (%)**
≤1 μg/ml	30 (44.1%)	45 (54.9%)	75 (50%)
2-8 μg/ml	20 (29.4%)	29 (35.4%)	49 (32.6%)
16 μg/ml	17 (25%)	8 (9.7%)	25 (16.7%)
> 32 μg/ml	1 (1.5%)	0 (2.4%)	1 (.7%)
TOTAL	68	82	150

Table 4.7: Antimicrobial susceptibility pattern of Klebsiella pneumoniae (n=68)

Antibiotic	Sensitive	Resistant
Amoxicillin-clavulanate	11(16.2%)	57(83.8%)
Ceftazidime	6(8.8%)	62(91.2%)
Ceftriaxone	8(11.8%)	60(88.2%)
Cefepime	12(17.6%)	56(82.4%)
Cefotaxime	6(8.8%)	62(91.2%)
Ciprofloxacin	8(11.8%)	60(88.2%)
Nitrofurantoin (urine only n=46)	20(43.5%)	26(56.5%)
Imipenem	38(55.9%)	30(44.1%)
Meropenem	36(52.9%)	32(47.1%)
Piperacillin tazobactam	4(5.9%)	64(94.1%)
Fosfomycin (urine only n=46)	34(73.9%)	12(26.1%)
Gentamicin	14(20.6%)	54(79.4%)
Amikacin	12(17.6%)	56(82.4%)
Polymyxin B	68(100%)	0(0%)
Doxycycline(n=22)	14(63.6%)	08(36.3%)
Levofloxacin	8(11.8%)	60(88.2%)
Cotrimoxazole	7(10.3%)	61(89.7%)

Table 4.8: Antimicrobial Susceptibility Pattern of *Escherichia Coli* (n=82)

Antibiotic	Sensitive	Resistant
Amoxicillin-clavulanate	14(17%)	68(83%)
Ceftazidime	12(14.6%)	70(85.4%)
Ceftriaxone	11(13.4%)	71(86.6%)
Cefepime	18(21.9%)	64(78.1%)
Cefotaxime	17(20.7%)	65(79.3%)
Ciprofloxacin	39(47.6%)	43((52.4%)
Nitrofurantoin (urine only n=51)	18(35.3%)	33(64.70%)
Imipenem	53(64.6%)	29(35.4%)
Meropenem(10ug)	57(69.6%)	25(30.4%)
Piperacillin-tazobactam	16(19.5%)	66(80.5%)
Fosfomycin(200ug) (urine only n=51)	46(90.2%)	05(9.8%)
Gentamicin (10ug)	24(29.3%)	58(70.7%)
Amikacin(30ug)	52(63.4%)	30(36.6%)
Polymyxin B	82(100%)	0(0%)
Doxycycline(n=31)	13(41.9%)	18(58.1%)
Levofloxacin	28(34.1%)	54(65.9%)
Cotrimoxazole	14(17.1%)	68(82.9%)

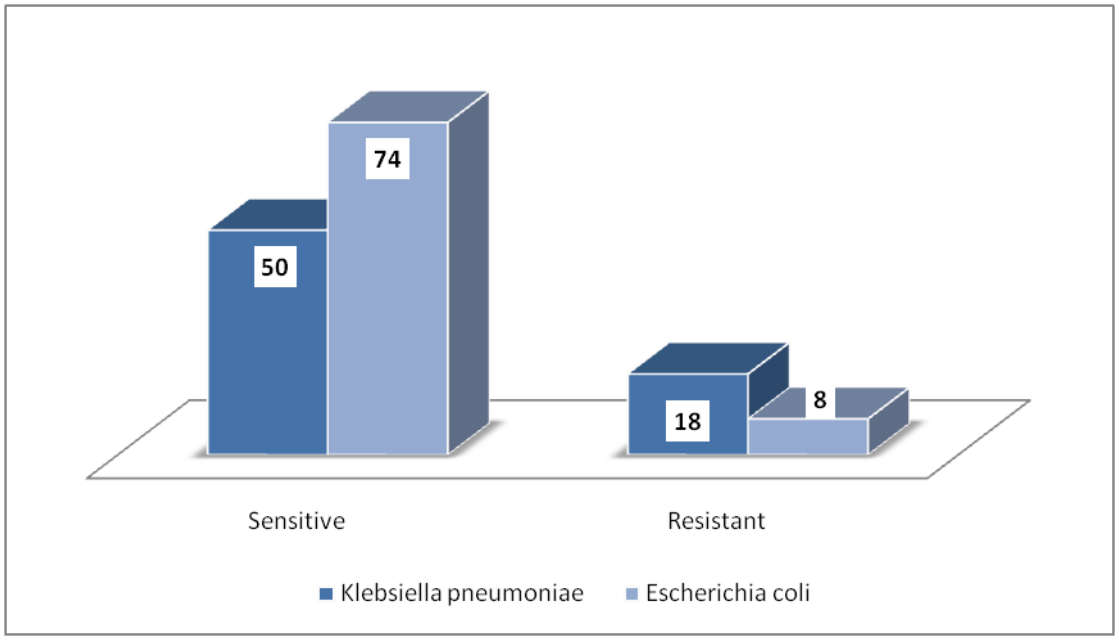


Figure 4.4: Antimicrobial susceptibility pattern of ceftazidime-avibactam

Table 4.9: Antimicrobial susceptibility pattern of microorganisms for male patients (n=67)

Antimicrobial susceptibility	Klebsiella pneumoniae	Escherichia coli	Total	P-value
Sensitive	15 (62.5%)	37 (86%)	52	
Resistant	9 (37.5%)	6 (14%)	15	S 0.027
TOTAL	24	43	67	

For analysis Chi-Square Test was applied. P-value of less than 0.05 was considered as statistically significant.S is represented as statistically significant at less than 0.05.

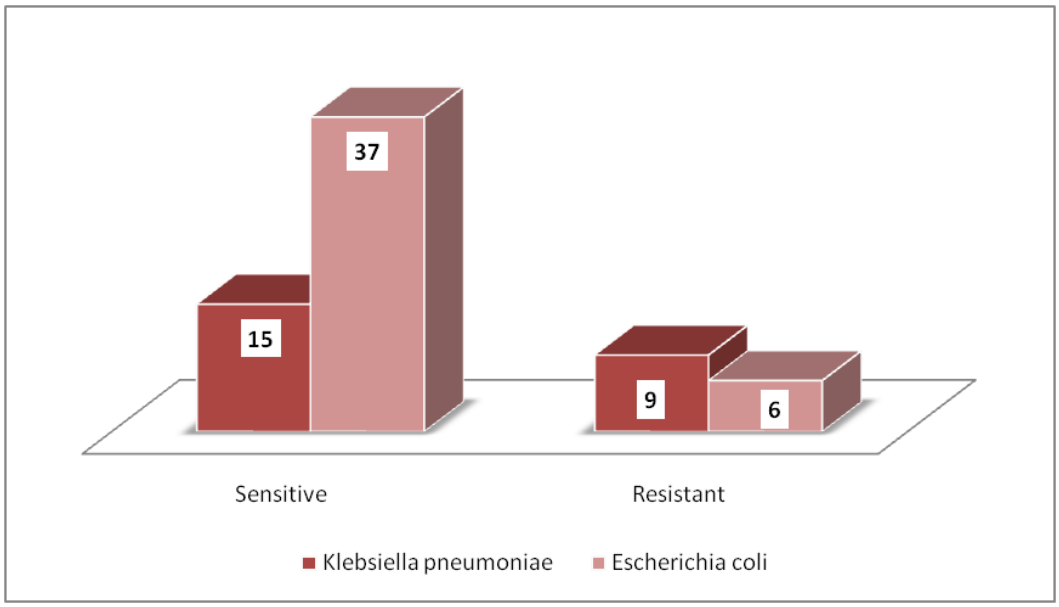


Figure 4.5 Antimicrobial susceptibility pattern of microorganisms for male patients (n=67)

Table 4.10: Antimicrobial susceptibility pattern of microorganisms for female patients

Antimicrobial susceptibility	Klebsiella pneumoniae	Escherichia coli	Total	P-value
Sensitive	35 (79.5%)	37 (94.9%)	**72**	
Resistant	9 (20.5%)	2 (5.1%)	**11**	S 0.040
TOTAL	**44**	**39**	**83**	

For analysis Chi-Square Test was applied. P-value of less than 0.05 was considered as statistically significant. S is represented as statistically significant at less than 0.05.

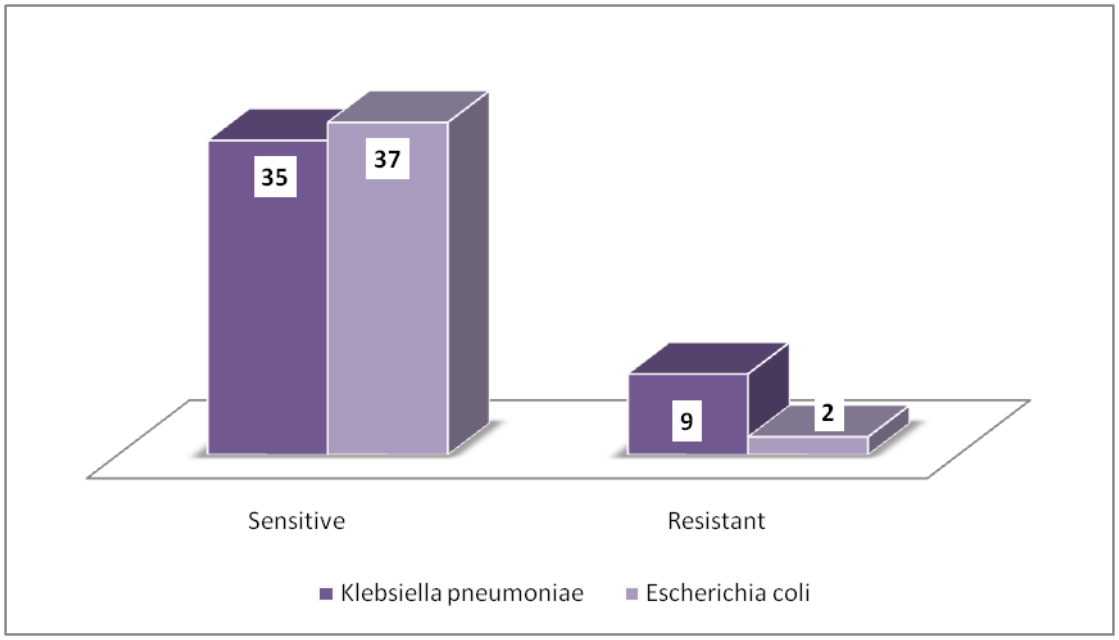


Figure 4.6: Antimicrobial susceptibility pattern of microorganisms for female patients (n=83)

Table 4.11: Antimicrobial susceptibility pattern of microorganisms for patient with age ≤18 years (n=13)

Antimicrobial susceptibility	Klebsiella pneumoniae	Escherichia coli	Total	P-value
Sensitive	6 (85.7)	6 (100)	**12**	
Resistant	1 (14.3)	0 (0)	**1**	NS 0.335
TOTAL	**7**	**6**	**13**	

For analysis, Chi-Square Test was applied. P-value less than 0.05 was considered as statistically significant. NS Is represented not statistically significant.

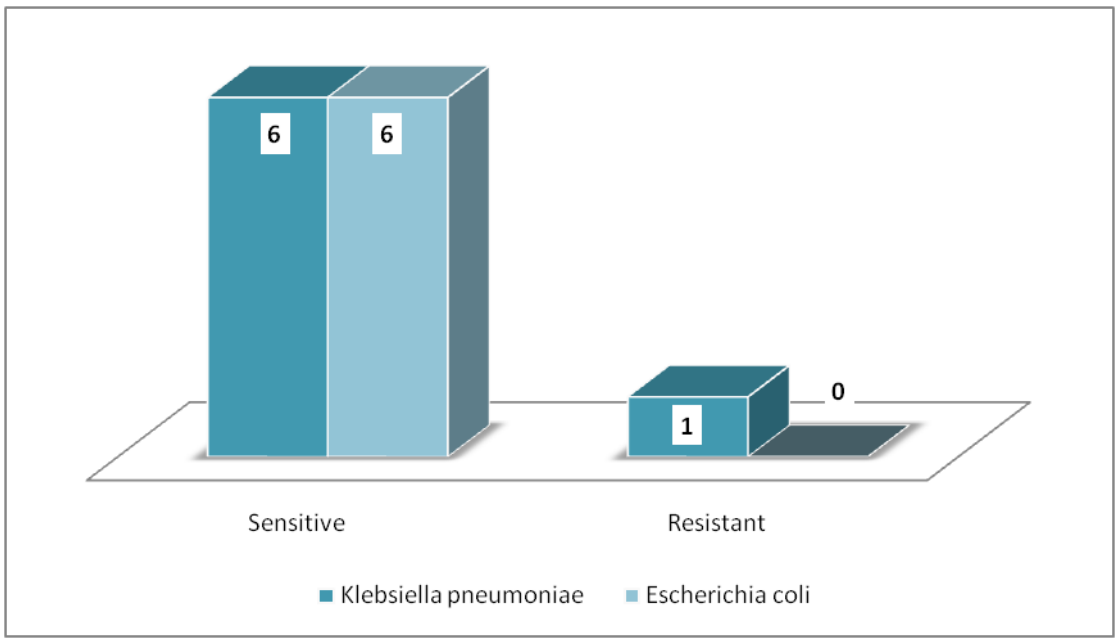


Figure 4.7: Antimicrobial susceptibility pattern of microorganisms for patient with age ≤18 years (n=13)

Table 4.12: Antimicrobial susceptibility pattern of microorganisms for patient with age 19-35 years (n=81)

Antimicrobial susceptibility	Klebsiella pneumoniae	Escherichia coli	Total	P-value
Sensitive	17 (70.8%)	50 (87.7%)	**67**	
Resistant	7 (29.2%)	7 (12.3%)	**14**	NS 0.066
TOTAL	**24**	**57**	**81**	

For analysis, Chi-Square Test was applied. P-value of less than 0.05 was considered as statistically significant.NS is represented not statistically significant.

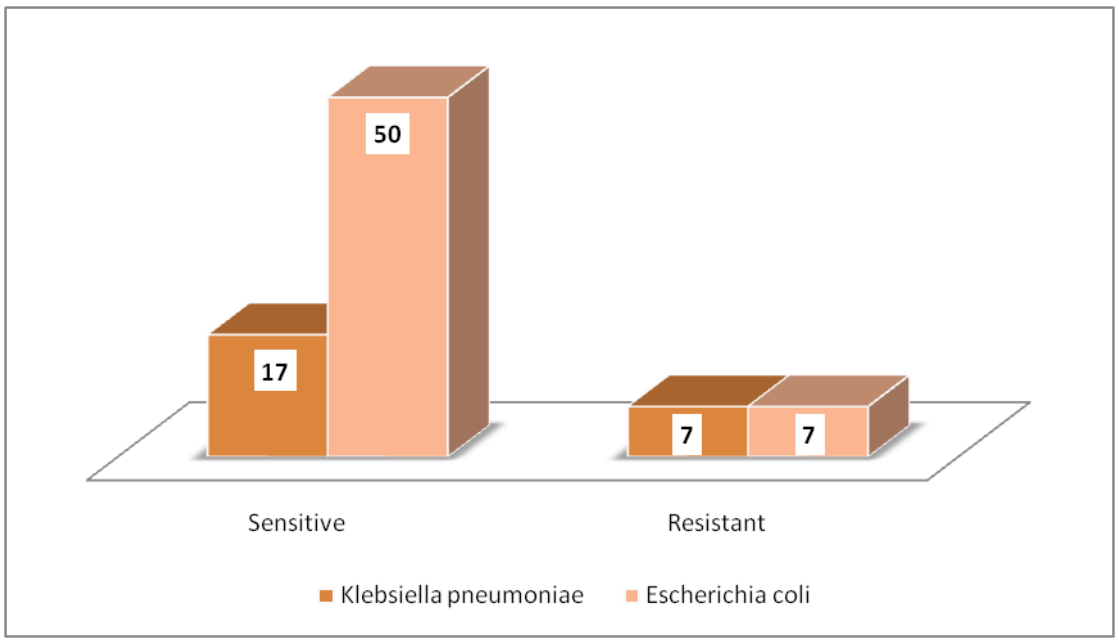


Figure 4.8: Antimicrobial susceptibility pattern of microorganisms for patient with age 19-35 years (n=81)

Table 4.13: Antimicrobial susceptibility pattern of microorganisms for patient with age >35 years (n=56)

Antimicrobial susceptibility	Klebsiella pneumoniae	Escherichia coli	Total	P-value
Sensitive	27 (73)	18 (94.7)	**45**	
Resistant	10 (27)	1 (5.3)	**11**	S 0.05
TOTAL	**37**	**19**	**56**	

For analysis, Chi-Square Test was applied. P-value of less than 0.05 was considered as statistically significant. S is represented as statistically significant.

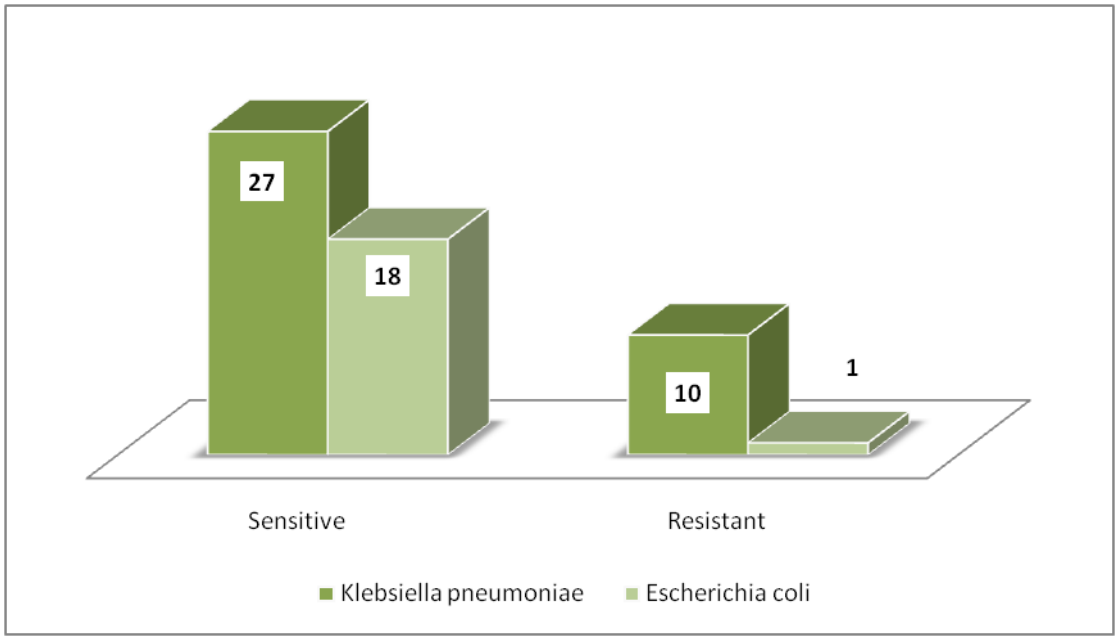


Figure 4.9: Antimicrobial susceptibility pattern of microorganisms for patient with age >35 years (n=56)

CHAPTER 5

DISCUSSION

An ever increasing problem around the world is antimicrobial resistance to gram-negative bacilli to an alarming level, thus making it difficult to obtain treatment options for various serious hospital-acquired infections (Bhardwaj et al., 2017; Ruppé, Woerther, & Barbier, 2015). Choices for various reliable and effective therapeutic options are seriously limited due to infections caused by organisms that have developed resistance mechanisms, which unfortunately in turn lead to poor prognosis of treatment given to patients in this regard and also burden the health care system which gross expenses (Arendrup & Patterson, 2017; Gandra et al., 2019; Lim et al., 2016). Evidence suggests that an alarming proportion of all bacteria isolated throughout the world shows gram-negative bacteria having ESBL enzymes (Eichenberger & Thaden, 2019; Kaye & Pogue, 2015) Therefore, the current study was designed to address the susceptibility and frequency of ceftazidime-avibactam antimicrobial activity in commonly prevalent Enterobacteriaceae like *E. coli* and *K. pneumoniae*.

In our study, the mean age of patients infected with *Klebsiella Pneumonia* was 36 years and mean age of patients infected with E. coli was 30 years. Total sample size was 55% *E.coli* and 45% *K. pneumoniae* which were isolated from the specimens of the patients at PNS Shifa Hospital in 6 months, represented in Figure 4.1.

Our primary objective was to assess the frequency of the pathogens in various samples. The present study conducted in Karachi, Pakistan has found most frequent

microbial content in urine samples, accounting to 67% of *Klebsiella pneumoniae* and 62% of *E. coli*, pus sample was found to have the second most frequent microbial content with 10.3 % of *Klebsiella pneumonia* e and 26.8% of *E. coli*. In blood sample, 11% of both pathogens have been found. *Klebsiella pneumoniae* was found in 10.3% samples of naso-bronchial lavage, however, E. coli have not been observed in any such sample (Table 4.3). In terms of gender our sample size included 44.6 % males and 55.4% females as shown in Table 4.1. Similarly, Liao et al have conducted the trial in Taiwan to assess the ceftazidime-avibactam antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae*. In their study, the E.coli frequency in the sample of sputum, urine, pus, blood were 36%, 34%, 13% and 8%, respectively, while in *Klebsiella pneumoniae* were 68%, 15%, 10% and 3%, respectively (Liao et al., 2019).

Followed by the identification of pathogens, ceftazidime-avibactam was used to identify its antimicrobial activity. We identified susceptibility of ceftazidime-avibactam (30/20 µg/mL) combination by disk-diffusion test, which was further confirmed by performing E-test. Results of these tests have shown 73.5% susceptibility for *Klebsiella pneumoniae* and 90% susceptibility for E. coli. The brake points were categorized according to CLSI guidelines of 2019 by using the disk diffusion method that showed values ≤ 20 were found to be resistant and values ≥ 21 represented sensitivity. These results were further confirmed by using E-Test, with ≤ 8 µg/mL ~~were~~ considered as sensitive and 8 µg/mL considered as resistant per CSLI guidelines. Overall, 82.7% samples of both the pathogens showed susceptibility towards ceftazidime-avibactam (Table 4.4).

Similarly, with our findings of drugs against *Escherichia coli, Klebsiella pneumoniae*, Woodford et al reported in their study that ceftazidime-avibactam was effective for bacterial isolates with OXA-48-like carbapenems or KPC. Moreover, MICs of ceftazidime-avibactam was proven to have excellent categorical similarities when compared to those investigated centrally through micro broth dilution (Woodford et al., 2018).

A study was done by Alatoom et al at Cleveland Clinic, United Arab Emirates to determine the antimicrobial activity of ceftazidime-avibactam combination for

ESBL producers and CRE. The method used to identify the in-vitro susceptibility was E-test strip MIC. In their study it was observed that all samples of ESBL pathogens were susceptible to ceftazidime-avibactam combination by showing 0.125 μg/ml MIC_{50}, while, 45% CRE microorganisms were observed to be susceptible to ceftazidime-avibactam combination by showing ≥256 μg/ml MIC_{50}. (Alatoom et al., 2017). In our study, we found 50% of isolates sensitive against ceftazidime-avibactam (Table 4.7 and Table 4.8).

García-Castillo et al conducted a study in 76 United States healthcare centers in 2011. They collected bacterial isolates from patients that were PHP (hospitalized with pneumonia), including VAP (ventilator-associated pneumonia). In 99.8% isolates of the Enterobacter species, 99.4% of ceftazidime resistant isolates, were found susceptible by giving ceftazidime-avibactam. (García-Castillo et al., 2018). The present study has also shown positive findings by giving ceftazidime-avibactam against the samples of Enterobacteriaceae (E.coli and *Klebsiella Pneumoniae*) that were isolated from urine, pus, blood, and naso-bronchial lavage of indoor and out-patients of PNS Shifa hospital, Karachi, Pakistan (Table 4.7 and Table 4.8).

Similar results were obtained using ceftazidime-avibactam as an alternate option to treat MDR Gram-negative infections. The outcomes of study in United States healthcare centers indicated that ceftazidime-avibactam combination itself is a promising regime for the antibiotic therapy in infections caused by strains of MDR and XDR (King et al., 2017; Krapp et al., 2017; Tuon et al., 2018).

In another study, ceftazidime-avibactam combination was also found to be the most effective drug that was tested against Enterobacteriaceae by showing MIC_{50}/MIC_{90}, 0.12/0.25 g/ml, with 99.8% inhibition at 4 g/ml and 99.3% inhibition at 1 g/ml, respectively (Sader, Castanheira, & Flamm, 2017).

After confirmation of the efficacy of antibiotic therapy against both the pathogens, analysis of MIC of the combination was carried out in our study and results showed that ≤1 ug/mL is the minimum concentration which has shown maximum activity with sensitivity of 44.1% against *Klebsiella pneumonia* and 54% sensitivity against E.coli. 29.4% *Klebsiella pneumoniae* and 35.4%. E. coli were

sensitive at the concentration of 2-8 µg/ml. At 16 µg/ml concentration, 25% *Klebsiella pneumoniae* and 9.7 % E. coli were found resistant to Ceftazidime-avibactam. On the concentration of > 32 µg/ml, 1.5% *Klebsiella pneumonia* and 0% E. coli were found to be resistant. (Table 4.5)

Flamm *et al.*, 2016 performed the in-vitro anti-microbial activity of ceftazidime-avibactam combination for Gram-negative bacteria, which were from hospitalized pneumonia patients (PHP) and patients with ventilator-associated bacterial pneumonia (VABP). Among the sample of Enterobacteriaceae obtained from PHP, ceftazidime-avibactam MIC_{90} for E. coli was observed between 0.25–0.5 mg/L and *Klebsiella* species recorded at 0.5 mg/L. From the sample isolated from patients with VABP, ceftazidime-avibactam (0.25 mg/L) was observed effective for *E. coli* and *Klebsiella* spp. Though, ceftazidime-avibactam combination was inhibited 79.2 to 95.4% of VABP pathogens at ≤ 8 mg/L of MIC (R. Flamm, Nichols, Sader, Farrell, & Jones, 2016). Similar results were obtained in our study; we have found that at ≤1 µg/ml, ceftazidime-avibactam was found sensitive.

A study performed by Berkhout et al. in 2015 showed good results of ceftazidime-avibactam combination on the MICs and checkerboard assays *in-vitro* for sample 19 E. coli and 32 Klebsiella pneumonia isolates. According to Berkhout et al the *in-vitro* findings concluded that avibactam inhibits β-lactamases, such as ESBLs and carbapenems (Berkhout et al., 2015). These results are consistent with our findings.

Our next focus was to identify the antimicrobial activity of the commercially available antimicrobial drugs (Table 4.7 and Table 4.8). From the analysis of the data followed by exposing both of these microorganisms with the drugs, we have found that combination of Piperacillin-tazobactam- (100/10ug), Amoxicillin-clavulanate (20/10ug) (b-lactam b-lactamase inhibitors) has 5.2 % and 16.2 % sensitivity against *K. pneumoniae* and 17.5 % and 19.5 % sensitivity against E. coli. Amoxicillin-clavulanate and Piperacillin-tazobactam were resistant same as the previously reported findings (Kim et al., 2015; Najjuka et al., 2016; Rizwan, Akhtar, Najmi, & Singh, 2018).

A similar susceptibility pattern of Amoxicillin-clavulanate has been found in our study as reported by Yaseen et al, in a study in 564 pregnant females between the age of 17 to 44 years in antenatal healthcare center Jinnah Medical College Hospital (JMCH) Karachi, Pakistan during 2017. On an average, 8.50% had occasionally experienced UTI during the period of their pregnancy. 54.2% of patients were found infected with *Escherichia coli*, and 16.77% with Klebsiella pneumonia. Overall, Amoxicillin/ clavulanic showed 50% resistance (Yaseen et al., 2020).

Another study was conducted in the Pediatrics department of Holy Family Hospital, Rawalpindi by Afzal to identify the spectrum of pathogens and their sensitivities in children with UTI. A total of 150 children between the age of 1 to 12 years were recruited having ≥ 101 °F fever for nearly 10-days. Similar to our findings, their results revealed maximum non-susceptibility of pathogens to amoxicillin (Afzal, 2008).

Piperacillin-tazobactam was found to have the lowest rate of resistance in the study of (Nepal et al., 2017). Furthermore, Sader et al collected the Gram-negative isolates (n=11,185) from hospitalized patients with pneumonia in United States medical centers to identify susceptibilities of antimicrobial drugs, and their results indicated that very few antimicrobial agents showed activity against the most frequently found isolated pathogens (Sader, Farrell, Flamm, & Jones, 2014). With an ESBL positive phenotype, the Piperacillin-tazobactam showed very low coverage against Klebsiella spp. These results are similar to our findings.

In the present study cephalosporin response against E. coli and *Klebsiella Pneumoniae* was studied as well. Our results have showed more ceftazidime resistance against E. coli and *Klebsiella Pneumoniae* (91.2% and 85.4%, respectively) as compared to the previous study (66% and 55%, respectively) (Liao et al., 2019).

Other third-generation cephalosporins have shown antimicrobial resistance to drug therapy. In case of ceftriaxone, cefepime and cefotaxime, the resistance found in *Klebsiella Pneumoniae* was 88.2%, 82.4% and 91.2% respectively (table 4.7). Ceftriaxone, cefepime and cefotaxime has found only 13.4%, 21.9% and 20.7% sensitivity, respectively, against E. coli. This has showed reduced effectiveness of

third generations cephalosporins against E. coli as compared to *Klebsilla pneumoniae* (table 4.8). Literature suggests similar findings of increased resistance to third-generation cephalosporins among gram-negative bacteria (GNB) (Liao et al., 2019).

We have found that ceftriaxone was non-susceptible with both the pathogens, by showing low level of sensitivity and high level of resistance, similar to the previous findings (Harris et al., 2018; Hayden & Won, 2018; Heng et al., 2018).

In the previous study, cefepime (cephalosporins) was ineffective against both pathogens. They suggested that the addition of AAI101 with cefepime could restore the antimicrobial activity of the cefepime (cephalosporin). They found gradually increased MIC_{50} by showing >64 mg/L for cefepime to 0.13 mg/L for cefepime-AAI101 (Crandon & Nicolau, 2015).

Similar to our findings of cephalosporins, a study was conducted by Javed *et al* in Children's Hospital, Lahore, Pakistan, from March 2013 to February 2014 to identify the frequency of MBL producing *E. coli* and *Klebsiella pneumoniae*, phenotypic techniques for MBL detection and choices of treatment available. They processed a total of 17,651 samples which included urine, pus, catheter tips, blood, and CSF of microbial infections, and pathogens were tested by using microbiological techniques. They observed that all of the organisms were 100% resistant to cefotaxime, ceftriaxone, ceftazidime, and cefixime (Javed et al., 2016)

Shah conducted a study in the department of Medicine, Shifa College of Medicine, Islamabad, Pakistan to identify the E. coil susceptibility of MDR pathogens and possible role of ESBL in E. coli resistance. Total of 378 *E. coli* pathogens from multiple sources ~~was~~ were identified over a period of 6 months. Overall, they have found 34% resistance to ceftriaxone (cephalosporin) (Shah, 2002).

In the present study the response of quinolones against *Klebsiella Pneumoniae* and *E. Coli* was studied as well. Ciprofloxacin and levofloxacin antibacterial agents exhibited only moderate level of activity against Enterobacteriaceae, as shown in the Table 4.7 and 4.8. Our results of ciprofloxacin and levofloxacin have found 11.8% sensitivity against *K. pneumonia*, while 47% sensitivity of ciprofloxacin and 34% sensitivity of levofloxacin has been found against E.coli. The decrease in the

frequency of sensitivity is may be due to improper diagnosis or inappropriate dosage prescription to the patients, which subsequently increased their resistance gradually.

A study performed by Shakti Rath in India in 2015, reported an increase in the resistance of fluoroquinolones in E. coli in a hospital of India. Ciprofloxacin and levofloxacin were found to have a resistance of around 85% and 27 % respectively in their study. The result of the activity of ciprofloxacin is ~~like~~ similar to our study but results for the activity of levofloxacin ~~is~~ are very different, one reason could be ~~being~~ the time elapsed, another reason could be the difference in sample size, with our sample size being very small as compared to the sample size of more than 1600 in their study.

Furthermore, our results have shown consistency with the results of Reis et al for this drug against bacteria. They suggested that bacterial resistance can be prevented by undertaking proper control measures that decrease the spread of resistant pathogens (Reis et al., 2016). Similarly with our findings of levofloxacin, all AmpC beta-lactamase-producing *Klebsiella pneumoniae* strains were resistant (77.8%) to levofloxacin in the study of Younas et al that was conducted in the Institute of Child Health and the Children's Hospital Lahore, Pakistan (Younas et al., 2018). Moreover, levofloxacin resistance was found in many other recent studies (Higashino et al., 2017; Maina et al., 2013).

Furthermore as reported in previous studies, nitrofurantoin was found resistant to *Escherichia* coli and *Klebsiella pneumoniae* in our findings (Erdem et al., 2018; Xu et al., 2019). Similar to our findings, Shah conducted a study in the department of Medicine, Shifa College of Medicine, Islamabad, Pakistan to identify the E. coil susceptibility of MDR pathogens and the possible role of ESBL in E. coli resistance. A total of 378 *E. coli* pathogens from multiple sources were identified in the 6-months period. In their results, nitrofurantoin showed low-level resistance to multidrug-resistant organisms in urine samples (Shah, 2002).

In the present study imipenem and meropenem response against *Klebsiella Pneumoniae* and E. Coli were studied as well, results showed that imipenem and meropenem, have shown sensitivity in both pathogens. Similarly, with our results, the

study was conducted by Javed *et al* in Children's Hospital, Lahore, Pakistan, from March 2013 to February 2014 to identify the frequency of MBL producing *E. coli* and *Klebsiella pneumoniae*, phenotypic techniques for metallo beta-lactamases (MBL) detection and choices of treatment available. They processed a total of, 17,651 samples which include urine, pus, catheter tips, blood, and CSF of microbial infections, and pathogens were tested by using microbiological techniques. They observed only 11.47% carbapenem resistance in strains which comprised of 32.6% in *E.coli* and 67.4% in *Klebsiella pneumoniae* (Javed et al., 2016).

According to Torres *et al*., 2018 study, ceftazidime-avibactam was as effective as meropenem for nosocomial pneumonia patients. The study showed that ceftazidime-avibactam combination has a potential against carbapenem resistance in hospitalized nosocomial pneumonia patients (Torres et al., 2018). When compared to our study we have found that 10.3% of the patients were admitted in hospital and after some time suffered from pneumonia (Table 4.3 and Figure 4.3).

Consistent with our findings concerning carbapenems, in United States medical centers, Sader et al collected the Gram-negative isolates (n=11,185) from hospitalized patients with pneumonia to identify susceptibilities of antimicrobial antimicrobials. With an ESBL positive phenotype, the carbapenems such as imipenem and meropenem showed very low coverage against Klebsiella spp (Sader et al., 2014).

Furthermore, two aminoglycoside drugs were used in our study namely, gentamicin and amikacin to study the response against E.coli and *Klebsiella Pneumoniae*. The results concluded that 29.3 % of gentamicin and 63.4% of amikacin was sensitive to E. coli. However, sensitivity of gentamicin and amikacin against *Klebsiella Pneumoniae* was much reduced by showing only 20.6% and 17.6% activity respectively.

Similarly, with our findings of aminoglycosides susceptibility, the spectrum of pathogens and their sensitivities were identified by Afzal in children who were infected with UTI in Pediatrics department, Holy Family Hospital, Rawalpindi, Pakistan. They found that *Escherichia coli* and *Klebsiella* were the most common uropathogens and aminoglycosides were found to be resistant to both species (Afzal,

2008).

In our study, 73.9% sensitivity of fosfomycin has been found in *klebsiella Pneumonia* and 90.2% sensitivity has been found against E. coli, which is similar to previous studies. (Chen, Wang, Ding, Zhang, & Li, 2019; Cho et al., 2015; Mashaly, 2016; Tulara, 2018). On the basis of *in-vitro* antimicrobial activity, fosfomycin exhibited an activity against MDR and XDR (Falagas et al., 2016; Sastry & Doi, 2016; Vardakas et al., 2016).

Furthermore, Yaseen et al conducted the study in 564 pregnant females between the age of 17 to 44 years in antenatal healthcare center, Jinnah Medical College Hospital (JMCH) Karachi, Pakistan during 2017. In total, approximately 8.50% had occasionally suffered with UTI during the period of their pregnancy. 54.2% of patients were found infected with *Escherichia coli*, and 16.77% with *Klebsiella pneumonia*. Overall, Fosfomycin showed 81.3% sensitivity (Yaseen et al., 2020). However, fosfomycin has shown vital spectrum deficiencies that ~~is~~ prevented their medical usage for the empirical treatment of serious infections.

While, amikacin has shown 63% and 17% sensitivity against E.coli and *Klebsiella Pneumonia* which is also quite similar to previous findings (Abduzaimovic et al., 2016; Kirac, Keskin, & Karahasanoğlu, 2018; Roldan-Masedo et al., 2019).

Synthetic drug Polymyxin B was found to be very effective against *Klebsiella Pneumonia* and *E. Coli*, Effectiveness of Polymyxin B was 100 % against these microbes in our study, similar outcomes were observed in another study conducted in Pakistan (Abid et al., 2017). However, the susceptibility testing of polymyxin is done by broth microdilution which is difficult to do. Secondly, polymyxin is nephrotoxic and its clinical efficacy remains questionable.

The data was further analyzed according to age of the patients concluding that samples collected from patients aged <18 years of age had shown 85.7% susceptibility against *Klebsiella pneumoniae* and 100% against E. coli (Table 4.11 & figure 4.7). In samples of isolates obtained from patients of age ranging 19-35 years, 70.8% and 87.7% susceptibility has found against *Klebsiella pneumoniae* and 100%

against E. coli, respectively (Table 4.12 & Figure 4.8). Furthermore, the samples of >35 years of patients have shown 73% sensitivity against *Klebsiella Pneumoniae* and 94% against E. coli. (Table 4.13 & Figure 4.9).

Moreover, in our study susceptibility level of isolates was identified, according to gender. In male patients, 62% and 86% susceptibility of our combination has found against *Klebsiella Pneumoniae* and E. coli respectively (Table 4.9 & Figure 4.5). In female patients, 79.5% and 94.9% susceptibility of our combination has found against *Klebsiella Pneumoniae* and E. coli respectively (Table 4.10 & Graph 4.6).

At last, the comparative analysis of the conventional antibiotics with ceftaxidime-avibactam was done, and it has shown the decreased sensitivity or high resistance of Amoxicillin-clavulanate, ceftriaxone, ceftazidime, piperacillin/tazobactam and meropenem with respect to ceftaxidime-avibactam. Our findings are consistent with the previously conducted studies against E.coli and *Klebsiella pneumonia* (Liao et al., 2019; Ramalheira & Stone, 2019; Sader et al., 2015)

Many previous studies are reported that ceftazidime-avibactam is represented a promising treatment regime to recover the infections that are caused by Enterobacteriaceae family as compared to most of the currently available antibacterial drugs that are non-susceptible to organisms (Alatoom et al., 2017; Liscio et al., 2015; Zasowski et al., 2015). Similarly, maximum conventional antibiotics that are shown in Table 4.4 & 4.5 have shown non-susceptibility towards E.coli and *Klebsiella pneumonia*.

In ceftazidime-avibactam, we ~~have~~ found 73.5% sensitivity against *Klebsiella pneumoniae* and 90.2% against E. coli. Piperacillin-tazobactam and Amoxicillin-clavulanate are b-lactam b-lactamase inhibitors, they act on b-ring of bacteria and inhibit the synthesis of bactrim, while ceftazidime-avibactam does not act upon the b-ring, therefore it possesses more sensitivity as compared with them. The b-ring of bacteria tends to make the b-lactam b-lactamase inhibitors resistant towards them. (Table 4.7 and 4.8)

Similarly, an internationally carried out research showed promising results of ceftazidime-avibactam regarding treatment for patients with carbapenems and ceftazidime-resistant microorganisms (Carmeli et al., 2016). We have also found the maximum sensitivity of ceftazidime-avibactam while carbapenems were resistant to both the pathogens.

Moreover, another research aimed at collecting Enterobacteriaceae bacterial isolates, such as ESBL-positive phenotypes and treating these isolates by ceftazidime-avibactam. Their results suggested that ceftazidime-avibactam combination is a promising agent for infections of MDR organisms associated with the Enterobacteriaceae family, when the other antimicrobial therapies showed poor results (Ramalheira & Stone, 2019). Consistently in our study, we have found a similar susceptibility of ceftazidime-avibactam and resistant profile of most of the conventional antimicrobial drugs.

While fosfomycin and polymyxin B has shown increased susceptibility against both pathogens as compared to ceftazidime-avibactam. However, the susceptibility testing of polymyxin is done by broth microdilution which is difficult to perform. Secondly, polymyxin is nephrotoxic and its clinical efficacy remains questionable. Fosfomycin has shown vital spectrum deficiencies that prevents their medical usage for the empirical treatment of serious infections.

American hospitals which used ceftazidime-avibactam combination, concluded that the drug significantly showed antimicrobial activity for a variety of gram-negative bacteria which were isolated from admitted patients, including the pathogens which were non-sensitive to most of the available antibiotics, for example, meropenem-was not susceptible to KPC-producing Enterobacteriaceae (Berkhout et al., 2015). In our study, we found similar antibacterial activity of ceftazidime-avibactam combination against *E. coli* and *K. Pneumoniae* which was promising and would bring positive results for health care system as compared to carbapenem.

In summary it is concluded that ceftazidime-avibactam combination in the present study observed effective in-vitro antimicrobial activity and broad antimicrobial spectrum for variable strains isolated from the patients at PNS Shifa

hospital in 12 months (ranging from mid of May 2019 to mid of May 2020). Our results indicated that a combination of ceftazidime-avibactam presents as an effective and promising treatment option to treat infections that are caused by E.coli and *Klebsiella pneumonia*. The drug also shows promising results against those infections which are resistant to most of the antimicrobial drugs that are currently available for clinical use.

CHAPTER 6

CONCLUSION

6.1 Conclusion of the study

The prevalence of antibiotics resistance among infections caused by Klebsiella pneumoniae against all the classes of antibiotics is relatively high as compared to Escherichia coli. Ceftazidime-avibactam is a novel drug combination that shows high sensitivity against Klebsiella pneumoniae and Escherichia coli. our study shows that Ceftazidime-avibactam is more effective on Escherichia coli as compare to Klebsiella pneumoniae. Recently carbapenems are the last choice for treatment of ESBL producing gram-negative rods. We concluded that ceftazidime-avibactam is effective on most of the carbapenems resistant microorganisms. our study shows that as compare to Amoxicillin-clavulanic acid and Piperacillin-tazobactam (b-lactam b-lactamase inhibitors) avibactam (a non-b-lactam b-lactamase inhibitor) is effective against large number of microorganisms having b-lactamases.

We concluded that ceftazidime-avibactam is highly effective on ceftazidime resistant microorganism. in contrast to Ceftazidime-avibactam. Fluoroquinolones levofloxacin show moderate activity against Escherichia coli but most of the Klebsiella pneumoniae are resistant to levofloxacin. Polymyxin B is high susceptibility same as Ceftazidime-avibactam but its antimicrobial susceptibility is done by broth microdilution which is difficult to do. Some previous research shows that polymyxin B is nephrotoxic and its clinical efficacy is also questionable. In our

study, Nitrofurantoin shows limited activity as compare to Fosfomycin which is relatively high but less then susceptibility of ceftazidime-avibactam. Cefepime and Cefotaxime show limited sensitivity as compare to Ceftazidime-avibactam against both microorganisms.

This study shows that disk diffusion and E-test are reliable methods for the detection of antimicrobial susceptibility pattern of Ceftazidime-avibactam for *Escherichia coli* and *Klebsiella pneumoniae* while each method shows same resistant and sensitive organism. the Minimum inhibitory concentration (MIC) of microorganisms is analyzed by E-test method that reveal1 most of the isolate show susceptibility less than 1 µg/mL concentration as well as few of them are susceptibility on 2-8 µg/mL concentration while very limited organisms show resistance against Ceftazidime-avibactam. Ceftazidime/avibactam will be additional treatment option against the MDR gram-negative bacteria. Thus, no other antibiotic tested to come up with better overall coverage than ceftazidime-avibactam.

6.2 Recommendations

i. Clinical trials should be done to analyses Safety and tolerability of ceftazidime/avibactam on local population in Pakistan.

ii. Studies should be conducted to know the antimicrobial susceptibility pattern of ceftazidime/avibactam against pseudomonas aeruginosa which is the also the leading cause of infection in our country.

iii. The detailed studies are needed that address the ceftazidime-avibactam as therapeutic option in both primary and secondary bacterial infections.

iv. The cost-effectiveness of ceftazidime-avibactam combination should be studied in detail on E. coli and K. pneumoniae infected patients.

v. Detailed analysis can be conducted that compare the susceptibility of the combination of ceftazidime-avibactam with ceftazidime-tazobactam. Most of the previous studies show that ceftazidime-tazobactam is also very effective drug against gram-negative rods.

vi. The future studies should be conducted on E. coli and K. pneumoniae infected patients having other health complications.

vii. Awareness programmers should be conducted about antimicrobial-resistant and proper use of antibiotics for patients on government level.

6.3 Strengths of the study

i. Ceftazidime – avibactam combination is promising therapy for treatment of E. coli and K. pneumoniae infection.

ii. Addition of Ceftazidime–avibactam facilitates the physicians to treat carbapenems resistant microorganisms. It is a valuable treatment option against ESBL producing gram-negative rods.

iii. Minimal inhibitory concentration of microorganism calculated by e-test, that is easy and effective method.

6.4 Limitations of the study

i. The study is being conducted at only one setting, it should be multicentered; keeping with the rampant level of infections by Escherichia coli and Klebsiella pneumonia in the general population and all age groups.

ii. Sample size is small.

iii. Susceptibility pattern and frequency of only two members of Enterobacteriaceae is being evaluated.

iv. Identification of organisms is done by conventional phenotypical methods.

CHAPTER 7

REFERENCES

Abduzaimovic, A., Aljicevic, M., Rebic, V., Vranic, S. M., Abduzaimovic, K., & Sestic, S. (2016). Antibiotic resistance in urinary isolates of Escherichia coli. *Materia socio-medica, 28*(6), 416.

Abid, M., Mirza, I. A., Bhatti, A., Malik, S., Hussain, A., & Khan, I. U. (2017). NTIMICROBIAL SUSCEPTIBILITY PATTERN OF POLYMYXIN B, TIGECYCLINE AND FOSFOMYCIN AGAINST CARBAPENAMASE PRODUCING ENTEROBACTERIACEAE (CPE). *Pakistan Armed Forces Medical Journal, 67*(6), 1026-1029.

Afzal, S. (2008). Spectrum of the microorganisms in children with urinary tract infection. *Journal of Rawalpindi Medical College, 12*(1), 44-46.

Ahmed, M., Moremi, N., Mirambo, M. M., Hokororo, A., Mushi, M. F., Seni, J., . . . Mshana, S. E. (2015). Multi-resistant gram negative enteric bacteria causing urinary tract infection among malnourished underfives admitted at a tertiary hospital, northwestern, Tanzania. *Italian Journal of Pediatrics, 41*(1), 44.

Akhavan, B. J., & Vijhani, P. (2019). Amoxicillin *StatPearls [Internet]*: StatPearls Publishing.

Aktaş, Z., Kayacan, C., & Oncul, O. (2012). In vitro activity of avibactam (NXL104) in combination with β-lactams against Gram-negative bacteria, including OXA-48 β-lactamase-producing Klebsiella pneumoniae. *International journal of antimicrobial agents, 39*(1), 86-89.

Alatoom, A., Elsayed, H., Lawlor, K., AbdelWareth, L., El-Lababidi, R., Cardona, L., . . . Mirza, I. (2017). Comparison of antimicrobial activity between ceftolozane–tazobactam and ceftazidime–avibactam against multidrug-resistant isolates of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. *International Journal of Infectious Diseases, 62*, 39-43.

Amani, J., Ahmadpour, A., Fooladi, A. A. I., & Nazarian, S. (2015). Detection of E. coli O157: H7 and Shigella dysenteriae toxins in clinical samples by PCR-ELISA. *The Brazilian Journal of Infectious Diseases, 19*(3), 278-284.

Ammar, E. M., Wang, X., & Rao, C. V. (2018). Regulation of metabolism in escherichia coli during growth on mixtures of the non-glucose sugars: arabinose, lactose, and xylose. *Scientific reports, 8*(1), 1-11.

Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. *The Journal of infectious diseases, 216*(suppl_3), S445-S451.

Armin, S., Fallah, F., Navidinia, M., & Vosoghian, S. (2017). Prevalence of blaOXA-1 and blaDHA-1 AmpC β-lactamase-producing and methicillin-resistant Staphylococcus aureus in Iran. *Archives of Pediatric Infectious Diseases, 5*(4).

Arsalan, A., Naqvi, S. B. S., Ali, S. A., Ahmed, S., & Shakeel, O. (2015). In vitro bactericidal activity of cefepime and cefpirome against clinical isolates at Karachi. *Pak. J. Pharm. Sci, 28*(3), 841-847.

Bartsch, S., McKinnell, J., Mueller, L., Miller, L., Gohil, S., Huang, S., & Lee, B. (2017). Potential economic burden of carbapenem-resistant Enterobacteriaceae (CRE) in the United States. *Clinical Microbiology and Infection, 23*(1), 48. e49-48. e16.

Bassetti, M., Taramasso, L., Giacobbe, D. R., & Pelosi, P. (2012). Management of ventilator-associated pneumonia: epidemiology, diagnosis and antimicrobial therapy. *Expert review of anti-infective therapy*, *10*(5), 585-596.

Berkhout, J., Melchers, M. J., van Mil, A. C., Nichols, W. W., & Mouton, J. W. (2015). In vitro activity of ceftazidime-avibactam combination in in vitro checkerboard assays. *Antimicrobial agents and chemotherapy, 59*(2), 1138-1144.

Bhardwaj, N., Khurana, S., Kumari, M., Malhotra, R., & Mathur, P. (2018). Pattern of antimicrobial resistance of gram-negative bacilli in surgical site infections in in-patients and out-patients at an apex trauma center: 2013–2016. *Journal of laboratory physicians, 10*(4), 432.

Bhowmik, D., Bhanot, R., Gautam, D., Rai, P., & Kumar, K. (2018). Osteomyelitis-Symptoms, Causes and Treatment. *Research Journal of Science and Technology, 10*(2), 165-177.

Bhunia, A. K. (2018). *Foodborne microbial pathogens: mechanisms and pathogenesis*: Springer.

Bradford, P. A. (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical microbiology reviews, 14*(4), 933-951.

Broom, A., Broom, J., Kirby, E., & Scambler, G. (2015). The path of least resistance? Jurisdictions, responsibility and professional asymmetries in pharmacists' accounts of antibiotic decisions in hospitals. *Social Science & Medicine, 146*, 95-103.

Brouwer, M., & Van De Beek, D. (2017). Management of bacterial central nervous system infections *Handbook of clinical neurology* (Vol. 140, pp. 349-364): Elsevier.

Bush, K., & Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbor perspectives in medicine, 6*(8), a025247.

Carmeli, Y., Armstrong, J., Laud, P. J., Newell, P., Stone, G., Wardman, A., & Gasink, L. B. (2016). Ceftazidime-avibactam or best available therapy in patients with ceftazidime-resistant Enterobacteriaceae and Pseudomonas aeruginosa complicated urinary tract infections or complicated intra-abdominal infections (REPRISE): a randomised, pathogen-directed, phase 3 study. *The Lancet Infectious Diseases, 16*(6), 661-673.

Carris, N. W., Pardo, J., Montero, J., & Shaeer, K. M. (2015). *Minocycline as a substitute for doxycycline in targeted scenarios: a systematic review.* Paper presented at the Open forum infectious diseases.

Castanheira, M., Arends, S. R., Davis, A. P., Woosley, L. N., Bhalodi, A. A., & MacVane, S. H. (2018). Analyses of a ceftazidime-avibactam-resistant Citrobacter freundii isolate carrying blaKPC-2 reveals a heterogenous population and reversible genotype. *Msphere, 3*(5).

Castro-Ibáñez, I., Gil, M. I., & Allende, A. (2017). Ready-to-eat vegetables: Current problems and potential solutions to reduce microbial risk in the production chain. *LWT-food science and technology, 85*, 284-292.

Chalopin, T., Dorta, D. A., Sivignon, A., Caudan, M., Dumych, T., Bilyy, R., . . . Gouin, S. (2016). Second generation of thiazolylmannosides, FimH antagonists for E. coli-induced Crohn's disease. *Organic & biomolecular chemistry, 14*(16), 3913-3925.

Chen, J., Wang, D., Ding, Y., Zhang, L., & Li, X. (2019). Molecular epidemiology of plasmid-mediated fosfomycin resistance gene determinants in Klebsiella pneumoniae Carbapenemase-Producing Klebsiella pneumoniae Isolates in China. *Microbial Drug Resistance, 25*(2), 251-257.

Chiotos, K., Han, J. H., & Tamma, P. D. (2016). Carbapenem-resistant Enterobacteriaceae infections in children. *Current infectious disease reports, 18*(1), 2.

Chmielarczyk, A., Wójkowska-Mach, J., Romaniszyn, D., Adamski, P., Helwich, E., Lauterbach, R., . . . Heczko, P. B. (2014). Mode of delivery and other risk factors for

Escherichia coli infections in very low birth weight infants. *BMC Pediatrics*, *14*(1), 274. doi: 10.1186/1471-2431-14-274

Cho, Y. H., Jung, S. I., Chung, H. S., Yu, H. S., Hwang, E. C., Kim, S.-O., . . . Park, K. (2015). Antimicrobial susceptibilities of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in health care-associated urinary tract infection: focus on susceptibility to fosfomycin. *International urology and nephrology*, *47*(7), 1059-1066.

Clegg, S., & Murphy, C. N. (2017). Epidemiology and virulence of Klebsiella pneumoniae. *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management*, 435-457.

Clements, A., Young, J. C., Constantinou, N., & Frankel, G. (2012). Infection strategies of enteric pathogenic Escherichia coli. *Gut microbes*, *3*(2), 71-87.

CLSI, C. (2016). Performance standards for antimicrobial susceptibility testing. *Clinical Lab Standards Institute*.

Connett, G. J., Pike, K. C., Legg, J. P., Cathie, K., Dewar, A., Foote, K., . . . Faust, S. N. (2015). Ciprofloxacin during upper respiratory tract infections to reduce Pseudomonas aeruginosa infection in paediatric cystic fibrosis: A pilot study. *Therapeutic advances in respiratory disease*, *9*(6), 272-280.

Crandon, J. L., & Nicolau, D. P. (2015). In vitro activity of cefepime/AAI101 and comparators against cefepime non-susceptible Enterobacteriaceae. *Pathogens*, *4*(3), 620-625.

Crandon, J. L., Schuck, V. J., Banevicius, M. A., Beaudoin, M.-E., Nichols, W. W., Tanudra, M. A., & Nicolau, D. P. (2012). Comparative in vitro and in vivo efficacies of human simulated doses of ceftazidime and ceftazidime-avibactam against Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy*, *56*(12), 6137-6146.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic Escherichia coli. *Clinical microbiology reviews, 26*(4), 822-880.

Cunha, B. A., & Brusch, J. L. (2018). Hospital-acquired pneumonia (nosocomial pneumonia) and ventilator-associated pneumonia. *Drugs and diseases*.

Deshpande, D., Srivastava, S., Chapagain, M., Magombedze, G., Martin, K. R., Cirrincione, K. N., . . . Gumbo, T. (2017). Ceftazidime-avibactam has potent sterilizing activity against highly drug-resistant tuberculosis. *Science advances, 3*(8), e1701102.

Devaney, B., Frawley, G., Frawley, L., & Pilcher, D. (2015). Necrotising soft tissue infections: the effect of hyperbaric oxygen on mortality. *Anaesthesia and intensive care, 43*(6), 685-692.

Dhillon, S. (2018). Meropenem/vaborbactam: a review in complicated urinary tract infections. *Drugs, 78*(12), 1259-1270.

Donnelly, P. C., Sutich, R. M., Easton, R., Adejumo, O. A., Lee, T. A., & Logan, L. K. (2017). Ceftriaxone-Associated biliary and Cardiopulmonary adverse events in neonates: a systematic review of the Literature. *Pediatric Drugs, 19*(1), 21-34.

Dos Santos, G., Solidonio, E., Costa, M., Melo, R., De Souza, I., Silva, G., & Sena, K. (2015). Study of the Enterobacteriaceae group CESP (Citrobacter, Enterobacter, Serratia, Providencia, Morganella and Hafnia): a review. *The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs, ed Méndez-Vilas A., editor.(Badajoz: Formatex,* 794-805.

Eichenberger, E. M., & Thaden, J. T. (2019). Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. *Antibiotics, 8*(2), 37.

Erdem, I., Ali, R. K., Ardic, E., Omar, S. E., Mutlu, R., & Topkaya, A. E. (2018). Community-acquired lower urinary tract infections: Etiology, antimicrobial

resistance, and treatment results in female patients. *Journal of Global Infectious Diseases, 10*(3), 129.

Falagas, M. E., Tansarli, G. S., Karageorgopoulos, D. E., & Vardakas, K. Z. (2014). Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerging infectious diseases, 20*(7), 1170.

Falagas, M. E., Vouloumanou, E. K., Samonis, G., & Vardakas, K. Z. (2016). Fosfomycin. *Clinical microbiology reviews, 29*(2), 321-347.

Falcone, M., Russo, A., Iacovelli, A., Restuccia, G., Ceccarelli, G., Giordano, A., . . . Venditti, M. (2016). Predictors of outcome in ICU patients with septic shock caused by Klebsiella pneumoniae carbapenemase–producing K. pneumoniae. *Clinical Microbiology and Infection, 22*(5), 444-450.

Fasugba, O., Gardner, A., Mitchell, B. G., & Mnatzaganian, G. (2015). Ciprofloxacin resistance in community-and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. *BMC infectious diseases, 15*(1), 545.

Flamm, R., Nichols, W., Sader, H., Farrell, D., & Jones, R. (2016). In vitro activity of ceftazidime/avibactam against Gram-negative pathogens isolated from pneumonia in hospitalised patients, including ventilated patients. *International journal of antimicrobial agents, 47*. doi: 10.1016/j.ijantimicag.2016.01.004

Flamm, R. K., Farrell, D. J., Sader, H. S., & Jones, R. N. (2014). Ceftazidime/avibactam activity tested against Gram-negative bacteria isolated from bloodstream, pneumonia, intra-abdominal and urinary tract infections in US medical centres (2012). *Journal of Antimicrobial Chemotherapy, 69*(6), 1589-1598.

Gandra, S., Tseng, K. K., Arora, A., Bhowmik, B., Robinson, M. L., Panigrahi, B., . . . Klein, E. Y. (2019). The mortality burden of multidrug-resistant pathogens in India: a retrospective, observational study. *Clinical Infectious Diseases, 69*(4), 563-570.

Garber, K. (2015). A β-lactamase inhibitor revival provides new hope for old antibiotics: Nature Publishing Group.

García-Castillo, M., García-Fernández, S., Gómez-Gil, R., Pitart, C., Oviaño, M., Gracia-Ahufinger, I., . . . Bou, G. (2018). Activity of ceftazidime-avibactam against carbapenemase-producing Enterobacteriaceae from urine specimens obtained during the infection-carbapenem resistance evaluation surveillance trial (iCREST) in Spain. *International journal of antimicrobial agents, 51*(3), 511-515.

García-Fernández, A., Gallina, S., Owczarek, S., Dionisi, A. M., Benedetti, I., Decastelli, L., & Luzzi, I. (2015). Emergence of ciprofloxacin-resistant Salmonella enterica serovar Typhi in Italy. *PloS one, 10*(6), e0132065.

Golan, Y. (2015). Empiric therapy for hospital-acquired, Gram-negative complicated intra-abdominal infection and complicated urinary tract infections: a systematic literature review of current and emerging treatment options. *BMC infectious diseases, 15*(1), 313.

Gonzales Zamora, J., & Murali, A. (2016). Rhinoscleroma with pharyngolaryngeal involvement caused by Klebsiella ozaenae. *Case reports in infectious diseases, 2016*.

Goodlet, K. J., Nicolau, D. P., & Nailor, M. D. (2016). Ceftolozane/tazobactam and ceftazidime/avibactam for the treatment of complicated intra-abdominal infections. *Therapeutics and clinical risk management, 12*, 1811.

Gu, D., Dong, N., Zheng, Z., Lin, D., Huang, M., Wang, L., . . . Zhang, R. (2018). A fatal outbreak of ST11 carbapenem-resistant hypervirulent Klebsiella pneumoniae in a Chinese hospital: a molecular epidemiological study. *The Lancet Infectious Diseases, 18*(1), 37-46.

Guedes, M., Duro, R., Fonseca, T., Abreu, I., & Rocha-Pereira, N. (2020). Carbapenemase-producing Klebsiella pneumoniae intra-abdominal infection successfully treated with ceftazidime/avibactam plus tigecycline. *IDCases*, e00745.

Hafeez, S., Izhar, M., Ahmed, A., Zafar, A., & Naeem, M. (2000). In-Vitro Antimicrobial Activity of Cefpirome: a new fourth-generation Cephalosporin against clinically significant Bacteria. *JPMA, 50*(250).

Hariharan, P., Bharani, T., Franklyne, J. S., Biswas, P., Solanki, S. S., & Paul-Satyaseela, M. (2015). Antibiotic susceptibility pattern of Enterobacteriaceae and non-fermenter Gram-negative clinical isolates of microbial resource orchid. *Journal of natural science, biology, and medicine, 6*(1), 198.

Harris, P. N., Tambyah, P. A., Lye, D. C., Mo, Y., Lee, T. H., Yilmaz, M., . . . Bassetti, M. (2018). Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with E coli or Klebsiella pneumoniae bloodstream infection and ceftriaxone resistance: a randomized clinical trial. *Jama, 320*(10), 984-994.

Hayden, M. K., & Won, S. Y. (2018). Carbapenem-Sparing Therapy for Extended-Spectrum β-Lactamase–Producing E coli and Klebsiella pneumoniae Bloodstream Infection: The Search Continues. *Jama, 320*(10), 979-981.

Heng, S. T., Chen, S. L., Wong, J. G., Lye, D. C., & Ng, T. M. (2018). No association between resistance mutations, empiric antibiotic, and mortality in ceftriaxone-resistant Escherichia coli and Klebsiella pneumoniae bacteremia. *Scientific reports, 8*(1), 1-10.

Higashino, M., Murata, M., Morinaga, Y., Akamatsu, N., Matsuda, J., Takeda, K., . . . Hasegawa, H. (2017). Fluoroquinolone resistance in extended-spectrum β-lactamase-producing Klebsiella pneumoniae in a Japanese tertiary hospital: silent shifting to CTX-M-15-producing K. pneumoniae. *Journal of medical microbiology, 66*(10), 1476-1482.

Jadhav, S., Sahasrabudhe, T., Kalley, V., & Gandham, N. (2013). The microbial colonization profile of respiratory devices and the significance of the role of disinfection: a blinded study. *Journal of clinical and diagnostic research: JCDR, 7*(6), 1021.

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M., Yan, T., & Ishii, S. (2017). Environmental Escherichia coli: ecology and public health implications—a review. *Journal of applied microbiology, 123*(3), 570-581.

Javed, H., Ejaz, H., Zafar, A., Rathore, A. W., & ul Haq, I. (2016). Metallo-beta-lactamase producing Escherichia coli and Klebsiella pneumoniae: A rising threat for hospitalized children. *JPMA, 66*(1068).

Judd, W. R., Ratliff, P. D., Hickson, R. P., Stephens, D. M., & Kennedy, C. A. (2016). Clinical and economic impact of meropenem resistance in Pseudomonas aeruginosa–infected patients. *American journal of infection control, 44*(11), 1275-1279.

Karlowsky, J. A., Biedenbach, D. J., Kazmierczak, K. M., Stone, G. G., & Sahm, D. F. (2016). Activity of ceftazidime-avibactam against extended-spectrum-and AmpC β-lactamase-producing Enterobacteriaceae collected in the INFORM global surveillance study from 2012 to 2014. *Antimicrobial agents and chemotherapy, 60*(5), 2849-2857.

Kaushik, M., Kumar, S., Kapoor, R. K., Virdi, J. S., & Gulati, P. (2018). Integrons in Enterobacteriaceae: diversity, distribution and epidemiology. *International journal of antimicrobial agents, 51*(2), 167-176.

Kayama, S., Koba, Y., Shigemoto, N., Kuwahara, R., Kakuhama, T., Kimura, K., . . . Ohge, H. (2015). Imipenem-susceptible, meropenem-resistant Klebsiella pneumoniae producing OXA-181 in Japan. *Antimicrobial agents and chemotherapy, 59*(2), 1379-1380.

Kaye, K. S., & Pogue, J. M. (2015). Infections caused by resistant gram-negative bacteria: epidemiology and management. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 35*(10), 949-962.

Kemnic, T. R., & Coleman, M. (2019). Trimethoprim Sulfamethoxazole *StatPearls [Internet]*: StatPearls Publishing.

Khalil, I. A., Troeger, C., Blacker, B. F., Rao, P. C., Brown, A., Atherly, D. E., . . . Kang, G. (2018). Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990–2016. *The Lancet Infectious Diseases, 18*(11), 1229-1240.

Khan, G. J., Khan, R. A., Majeed, I., Siddiqui, F. A., & Khan, S. (2015). CIPROFLOXACIN. *The Professional Medical Journal, 22*(01), 001-005.

Khan, I., Shah, M. A., Mehmood, F. S., Saeed, A., & Sualeh, M. (2015). Determination and identification of Enterobacteriaceae in street vended foods in Karachi, Pakistan. *Pakistan Journal of Nutrition, 14*(4), 225.

Khorshed, S. A. (2018). Molecular Epidemiology and Characterisation of Extended Spectrum Beta Lactamase Producing E. coli in Female Patients with Urinary Tract Infection. *International Journal of Medical Sciences, 1*(2), 62-63.

Kim, H.-S., Chon, J.-W., Kim, Y.-J., Kim, D.-H., Kim, M.-s., & Seo, K.-H. (2015). Prevalence and characterization of extended-spectrum-β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in ready-to-eat vegetables. *International journal of food microbiology, 207*, 83-86.

King, M., Heil, E., Kuriakose, S., Bias, T., Huang, V., El-Beyrouty, C., . . . Gardner, J. (2017). Multicenter study of outcomes with ceftazidime-avibactam in patients with carbapenem-resistant Enterobacteriaceae infections. *Antimicrobial agents and chemotherapy, 61*(7).

Kintz, E., Brainard, J., Hooper, L., & Hunter, P. (2017). Transmission pathways for sporadic Shiga-toxin producing E. coli infections: A systematic review and meta-analysis. *International journal of hygiene and environmental health, 220*(1), 57-67.

Kirac, S., Keskin, D., & Karahasanoğlu, F. B. (2018). Antimicrobial Resistance and Sensitivity among Isolates of Esherichia coli from Urine Samples in Denizli, Turkey. *Malaysian Journal of Medical and Biological Research, 5*(1), 13-18.

Kirmusaoglu, S., Yurdugül, S., Metin, A., & Vehid, S. (2017). The effect of urinary catheters on microbial biofilms and catheter associated urinary tract infections. *Urology Journal*, *14*(2), 3028-3034.

Krapp, F., Grant, J. L., Sutton, S. H., Ozer, E. A., & Barr, V. O. (2017). Treating complicated carbapenem-resistant enterobacteriaceae infections with ceftazidime/avibactam: a retrospective study with molecular strain characterisation. *International journal of antimicrobial agents*, *49*(6), 770-773.

Lachiewicz, M. P., Moulton, L. J., & Jaiyeoba, O. (2015). Pelvic surgical site infections in gynecologic surgery. *Infectious diseases in obstetrics and gynecology*, *2015*.

Lagacé-Wiens, P., Walkty, A., & Karlowsky, J. A. (2014a). Ceftazidime-avibactam: an evidence-based review of its pharmacology and potential use in the treatment of Gram-negative bacterial infections. *Core evidence, 9*, 13-25. doi: 10.2147/CE.S40698

Lagacé-Wiens, P., Walkty, A., & Karlowsky, J. A. (2014b). Ceftazidime–avibactam: an evidence-based review of its pharmacology and potential use in the treatment of Gram-negative bacterial infections. *Core evidence, 9*, 13.

Landersdorfer, C., Kinzig, M., Höhl, R., Kempf, P., Nation, R. L., & Sörgel, F. (2020). Physiologically-based population pharmacokinetic modeling approach for ciprofloxacin in bone of patients undergoing orthopedic surgery. *ACS Pharmacology & Translational Science*.

Levasseur, P., Girard, A.-M., Claudon, M., Goossens, H., Black, M. T., Coleman, K., & Miossec, C. (2012). In vitro antibacterial activity of the ceftazidime-avibactam (NXL104) combination against Pseudomonas aeruginosa clinical isolates. *Antimicrobial agents and chemotherapy, 56*(3), 1606-1608.

Levison, W. (2014). *Revew of Medical Microbiology and Immunology*: McGraw-Hill Education.

Li, B., Zhao, Y., Liu, C., Chen, Z., & Zhou, D. (2014). Molecular pathogenesis of Klebsiella pneumoniae. *Future microbiology, 9*(9), 1071-1081.

Liao, C.-H., Lee, N.-Y., Tang, H.-J., Lee, S. S.-J., Lin, C.-F., Lu, P.-L., . . . Hsueh, P.-R. (2019). Antimicrobial activities of ceftazidime-avibactam, ceftolozane-tazobactam, and other agents against Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa isolated from intensive care units in Taiwan: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan in 2016. *Infection and drug resistance, 12*, 545-552. doi: 10.2147/IDR.S193638

Lim, C., Takahashi, E., Hongsuwan, M., Wuthiekanun, V., Thamlikitkul, V., Hinjoy, S., . . . Limmathurotsakul, D. (2016). Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. *Elife, 5*, e18082.

Liscio, J. L., Mahoney, M. V., & Hirsch, E. B. (2015). Ceftolozane/tazobactam and ceftazidime/avibactam: two novel β-lactam/β-lactamase inhibitor combination agents for the treatment of resistant Gram-negative bacterial infections. *International journal of antimicrobial agents, 46*(3), 266-271.

Liu, S., Tang, Y., Wang, D., Lin, N., & Zhou, J. (2016). Identification and characterization of a new Enterobacter onion bulb decay caused by Lelliottia amnigena in China. *App Micro Open Access, 2*, 114.

Livermore, D. M., Mushtaq, S., Barker, K., Hope, R., Warner, M., & Woodford, N. (2012). Characterization of β-lactamase and porin mutants of Enterobacteriaceae selected with ceftaroline+ avibactam (NXL104). *Journal of Antimicrobial Chemotherapy, 67*(6), 1354-1358.

MacVane, S. H. (2017). Antimicrobial resistance in the intensive care unit: a focus on gram-negative bacterial infections. *Journal of intensive care medicine, 32*(1), 25-37.

Madappa, T., & Go, C. (2017). Escherichia coli (E. coli) Infections: Medscape.

Madhavan, T. V., & Sakellaris, H. (2015). Colonization factors of enterotoxigenic Escherichia coli *Advances in applied microbiology* (Vol. 90, pp. 155-197): Elsevier.

Magana, M., Sereti, C., Ioannidis, A., Mitchell, C. A., Ball, A. R., Magiorkinis, E., . . . Tegos, G. P. (2018). Options and limitations in clinical investigation of bacterial biofilms. *Clinical microbiology reviews, 31*(3).

Maina, D., Makau, P., Nyerere, A., & Revathi, G. (2013). Antimicrobial resistance patterns in extended-spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae isolates in a private tertiary hospital, Kenya. *Microbiology Discovery, 1*(5), 1.

Manges, A. R., Geum, H. M., Guo, A., Edens, T. J., Fibke, C. D., & Pitout, J. D. (2019). Global extraintestinal pathogenic Escherichia coli (ExPEC) lineages. *Clinical microbiology reviews, 32*(3).

Marais, B. J. (2016). The global tuberculosis situation and the inexorable rise of drug-resistant disease. *Advanced drug delivery reviews, 102*, 3-9.

Martin, A., Fahrbach, K., Zhao, Q., & Lodise, T. (2018). *Association between carbapenem resistance and mortality among adult, hospitalized patients with serious infections due to Enterobacteriaceae: results of a systematic literature review and meta-analysis.* Paper presented at the Open forum infectious diseases.

Mashaly, G. E.-S. (2016). Activity of fosfomycin in extended-spectrum beta-lactamases producing klebsiella pneumonae from hospital acquired urinary tract infections. *Open Journal of Medical Microbiology, 6*(03), 104.

Mawal, Y., Critchley, I. A., Riccobene, T. A., & Talley, A. K. (2015). Ceftazidime–avibactam for the treatment of complicated urinary tract infections and complicated intra-abdominal infections. *Expert review of clinical pharmacology, 8*(6), 691-707.

Menon, R., Gopinath, D., Li, K., Leung, Y., & Botelho, M. (2019). Does the use of amoxicillin/amoxicillin–clavulanic acid in third molar surgery reduce the risk of postoperative infection? A systematic review with meta-analysis. *International Journal of Oral and Maxillofacial Surgery, 48*(2), 263-273.

Meyer, E., Schwab, F., Schroeren-Boersch, B., & Gastmeier, P. (2010). Dramatic increase of third-generation cephalosporin-resistant E. coli in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. *Critical care, 14*(3), R113.

Mhmoud, N. A., Fahal, A. H., & van de Sande, W. W. (2014). The combination of amoxicillin-clavulanic acid and ketoconazole in the treatment of Madurella mycetomatis eumycetoma and Staphylococcus aureus co-infection. *PLoS Negl Trop Dis, 8*(6), e2959.

Moini, A. S., Soltani, B., Ardakani, A. T., Moravveji, A., Erami, M., Rezaei, M. H., & Namazi, M. (2015). Multidrug-resistant Escherichia coli and Klebsiella pneumoniae isolated from patients in Kashan, Iran. *Jundishapur journal of microbiology, 8*(10).

Morita, Y., Tomida, J., & Kawamura, Y. (2014). Responses of Pseudomonas aeruginosa to antimicrobials. *Frontiers in microbiology, 4*, 422.

Morrill, H. J., & LaPlante, K. L. (2015). Overconsumption of antibiotics. *The Lancet Infectious Diseases, 15*(4), 377-378.

Morrill, H. J., Pogue, J. M., Kaye, K. S., & LaPlante, K. L. (2015). *Treatment options for carbapenem-resistant Enterobacteriaceae infections.* Paper presented at the Open forum infectious diseases.

Nabipour, H., Hosaini Sadr, M., & Thomas, N. (2015). Synthesis, characterisation and sustained release properties of layered zinc hydroxide intercalated with amoxicillin trihydrate. *Journal of Experimental Nanoscience, 10*(16), 1269-1284.

Najjuka, C. F., Kateete, D. P., Kajumbula, H. M., Joloba, M. L., & Essack, S. Y. (2016). Antimicrobial susceptibility profiles of Escherichia coli and Klebsiella pneumoniae isolated from outpatients in urban and rural districts of Uganda. *BMC research notes, 9*(1), 235.

Naqvi, S. A. R., Roohi, S., Iqbal, A., Sherazi, T. A., Zahoor, A. F., & Imran, M. (2018). Ciprofloxacin: from infection therapy to molecular imaging. *Molecular biology reports, 45*(5), 1457-1468.

Nepal, K., Pant, N. D., Neupane, B., Belbase, A., Baidhya, R., Shrestha, R. K., . . . Jha, B. (2017). Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiella pneumoniae isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. *Annals of clinical microbiology and antimicrobials, 16*(1), 62.

O'Connor, A., & Eranki, A. P. (2019). Cefepime *StatPearls [Internet]*: StatPearls Publishing.

O'Ryan, M., Vidal, R., Del Canto, F., Carlos Salazar, J., & Montero, D. (2015). Vaccines for viral and bacterial pathogens causing acute gastroenteritis: Part II: Vaccines for Shigella, Salmonella, enterotoxigenic E. coli (ETEC) enterohemorragic E. coli (EHEC) and Campylobacter jejuni. *Human vaccines & immunotherapeutics, 11*(3), 601-619.

Oren, A., & Garrity, G. M. (2017). Notification that new names of prokaryotes, new combinations and new taxonomic opinions have appeared in volume 66, part 12, of the IJSEM. *International journal of systematic and evolutionary microbiology, 67*(3), 525-528.

Organization, W. H. (2017). Gonococcal antimicrobial resistance in the Western Pacific Region [fact sheet].

Paczosa, M. K., & Mecsas, J. (2016). Klebsiella pneumoniae: going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews, 80*(3), 629-661.

Peerayeh, S. N., Rostami, E., Eslami, M., & Rezaee, M. A. (2016). High frequency of extended-spectrum β-lactamase-producing Klebsiella pneumoniae and Escherichia coli isolates from male patients' Urine. *Archives of Clinical Infectious Diseases, 11*(2).

Peleg, A. Y., & Hooper, D. C. (2010). Hospital-acquired infections due to gram-negative bacteria. *New England Journal of Medicine, 362*(19), 1804-1813.

Persad, A. K. (2016). *Epidemiological studies on Non-O157 Shiga toxin-producing Escherichia coli.* The Ohio State University.

Philippon, A., Slama, P., Dény, P., & Labia, R. (2016). A structure-based classification of class A β-lactamases, a broadly diverse family of enzymes. *Clinical microbiology reviews, 29*(1), 29-57.

Pop, M., Paulson, J. N., Chakraborty, S., Astrovskaya, I., Lindsay, B. R., Li, S., . . . Walker, A. W. (2016). Individual-specific changes in the human gut microbiota after challenge with enterotoxigenic Escherichia coli and subsequent ciprofloxacin treatment. *BMC genomics, 17*(1), 440.

Raju, K. S., Reddy, K. N. K., & Vasu, K. (2017). Prescribing pattern for infectious diseases in tertiary care pediatric hospital. *Indian Journal of Research in Pharmacy and Biotechnology, 5*(1), 68.

Ramalheira, E., & Stone, G. G. (2019). Longitudinal analysis of the in vitro activity of ceftazidime/avibactam versus Enterobacteriaceae, 2012–2016. *Journal of global antimicrobial resistance, 19*, 106-115.

Rashid, T., & Ebringer, A. (2007). Ankylosing spondylitis is linked to Klebsiella—the evidence. *Clinical rheumatology, 26*(6), 858-864.

Reis, A. C. C., Santos, S. R. d. S., Souza, S. C. d., Saldanha, M. G., Pitanga, T. N., & Oliveira, R. R. (2016). Ciprofloxacin resistance pattern among bacteria isolated from patients with community-acquired urinary tract infection. *Revista do Instituto de Medicina Tropical de São Paulo, 58*.

Rizwan, M., Akhtar, M., Najmi, A. K., & Singh, K. (2018). Escherichia coli and Klebsiella pneumoniae sensitivity/resistance pattern towards antimicrobial agents in

primary and simple urinary tract infection patients visiting University Hospital of Jamia Hamdard New Delhi. *Drug research*, *68*(07), 415-420.

Roldan-Masedo, E., Sainz, T., Gutierrez-Arroyo, A., Gomez-Gil, R. M., Ballesteros, E., Escosa, L., . . . Méndez-Echevarría, A. (2019). Risk factors for gentamicin-resistant E. coli in children with community-acquired urinary tract infection. *European Journal of Clinical Microbiology & Infectious Diseases*, *38*(11), 2097-2102.

Ronald, A. (2015). 62. Genital ulcer adenopathy syndrome. *Clinical Infectious Disease*, 406.

Ross, J. J. (2017). Septic arthritis of native joints. *Infectious Disease Clinics*, *31*(2), 203-218.

Roxas, J. L., Monasky, R. C., Roxas, B. A. P., Agellon, A. B., Mansoor, A., Kaper, J. B., . . . Viswanathan, V. (2018). Enteropathogenic Escherichia coli EspH-mediated Rho GTPase inhibition results in desmosomal perturbations. *Cellular and Molecular Gastroenterology and Hepatology*, *6*(2), 163-180.

Ruppé, É., Woerther, P.-L., & Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Annals of intensive care*, *5*(1), 21.

Sader, H. S., Castanheira, M., & Flamm, R. K. (2017). Antimicrobial activity of ceftazidime-avibactam against Gram-negative bacteria isolated from patients hospitalized with pneumonia in US medical centers, 2011 to 2015. *Antimicrobial agents and chemotherapy*, *61*(4).

Sader, H. S., Castanheira, M., Flamm, R. K., Mendes, R. E., Farrell, D. J., & Jones, R. N. (2015). Ceftazidime/avibactam tested against Gram-negative bacteria from intensive care unit (ICU) and non-ICU patients, including those with ventilator-associated pneumonia. *International journal of antimicrobial agents*, *46*(1), 53-59.

Sader, H. S., Farrell, D. J., Flamm, R. K., & Jones, R. N. (2014). Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalised with

pneumonia in US and European hospitals: results from the SENTRY Antimicrobial Surveillance Program, 2009–2012. *International journal of antimicrobial agents, 43*(4), 328-334.

Saleem, A. F., Qamar, F. N., Shahzad, H., Qadir, M., & Zaidi, A. K. (2013). Trends in antibiotic susceptibility and incidence of late-onset Klebsiella pneumoniae neonatal sepsis over a six-year period in a neonatal intensive care unit in Karachi, Pakistan. *International Journal of Infectious Diseases, 17*(11), e961-e965.

Sastry, S., & Doi, Y. (2016). Fosfomycin: resurgence of an old companion. *Journal of Infection and Chemotherapy, 22*(5), 273-280.

Schwaber, M. J., & Carmeli, Y. (2007). Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy, 60*(5), 913-920.

Scott, L. J. (2019). Eravacycline: a review in complicated intra-abdominal infections. *Drugs, 79*(3), 315-324.

Shah, S. (2002). Susceptibility patterns of Escherichia coli: prevalence of multidrug-resistant isolates and extended spectrum beta-lactamase phenotype. *JPMA, 52*(407).

Shaikh, B. T. (2017). Anti-microbial resistance in Pakistan: a public health issue. *Journal of Ayub Medical College Abbottabad, 29*(2), 184-185.

Sharma, R., Park, T. E., & Moy, S. (2016). Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination for the treatment of resistant gram-negative organisms. *Clinical therapeutics, 38*(3), 431-444.

Shatalov, A. (2015). Prevalence and antibiotic resistance pattern of Escherichia coli and Klebsiella pneumoniae in urine tract infections at the La Paz Medical center, Malabo, Equatorial Guinea. *Open Journal of Medical Microbiology, 5*(04), 177.

Shiferaw, B., Gelaw, B., Assefa, A., Assefa, Y., & Addis, Z. (2015). Bacterial isolates and their antimicrobial susceptibility pattern among patients with external ocular infections at Borumeda hospital, Northeast Ethiopia. *BMC ophthalmology, 15*(1), 103.

Shirley, M. (2018). Ceftazidime-avibactam: a review in the treatment of serious gram-negative bacterial infections. *Drugs, 78*(6), 675-692.

Suhaeri, M., Noh, M. H., Moon, J.-H., Kim, I. G., Oh, S. J., Ha, S. S., . . . Park, K. (2018). Novel skin patch combining human fibroblast-derived matrix and ciprofloxacin for infected wound healing. *Theranostics, 8*(18), 5025.

Sumathy, J. (2018). A Study on The Enterobacteriaceae Pathogen Klebsiella Pneumoniae Isolated From Sewage And Drinking Water Environment. *4*, 1-5. doi: 10.9790/264X-0405020105

Sutherland, C. A., & Nicolau, D. P. (2015). Susceptibility profile of ceftolozane/tazobactam and other parenteral antimicrobials against Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa from US hospitals. *Clinical therapeutics, 37*(7), 1564-1571.

Sutherland, C. A., Verastegui, J. E., & Nicolau, D. P. (2016). In vitro potency of amikacin and comparators against E. coli, K. pneumoniae and P. aeruginosa respiratory and blood isolates. *Annals of clinical microbiology and antimicrobials, 15*(1), 39.

Tacconelli, E., Cataldo, M., Dancer, S., De Angelis, G., Falcone, M., Frank, U., . . . Rodríguez-Baño, J. (2014). ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clinical Microbiology and Infection, 20*, 1-55.

Tauzin, M., Ouldali, N., Lévy, C., Béchet, S., Cohen, R., & Caeymaex, L. (2019). Combination therapy with ciprofloxacin and third-generation cephalosporin versus third-generation cephalosporin monotherapy in Escherichia coli meningitis in infants:

a multicentre propensity score–matched observational study. *Clinical Microbiology and Infection, 25*(8), 1006-1012.

Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal Escherichia coli. *Nature Reviews Microbiology, 8*(3), 207-217.

Thompson, M. G., Truong-Le, V., Alamneh, Y. A., Black, C. C., Anderl, J., Honnold, C. L., . . . Hall, E. R. (2015). Evaluation of gallium citrate formulations against a multidrug-resistant strain of Klebsiella pneumoniae in a murine wound model of infection. *Antimicrobial agents and chemotherapy, 59*(10), 6484-6493.

Todd, P. A., & Benfield, P. (1990). Amoxicillin/clavulanic acid. An update of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs, 39*(2), 264-307. doi: 10.2165/00003495-199039020-00008

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-lactamase inhibitors in the 21st century. *Journal of molecular biology, 431*(18), 3472-3500.

Torres, A., Zhong, N., Pachl, J., Timsit, J.-F., Kollef, M., Chen, Z., . . . Stone, G. G. (2018). Ceftazidime-avibactam versus meropenem in nosocomial pneumonia, including ventilator-associated pneumonia (REPROVE): a randomised, double-blind, phase 3 non-inferiority trial. *The Lancet Infectious Diseases, 18*(3), 285-295.

Trevino, N. P., Ganta, D., Montes, W., & Guzman, C. (2019). *Disposable and Inexpensive Electrochemical Biosensor to Detect Ciprofloxacin.* Paper presented at the 235th ECS Meeting (May 26-30, 2019).

Tulara, N. K. (2018). Nitrofurantoin and fosfomycin for extended spectrum beta-lactamases producing Escherichia coli and Klebsiella pneumoniae. *Journal of Global Infectious Diseases, 10*(1), 19.

Tuon, F. F., Rocha, J. L., & Formigoni-Pinto, M. R. (2018). Pharmacological aspects and spectrum of action of ceftazidime–avibactam: a systematic review. *Infection, 46*(2), 165-181.

van Duin, D., & Bonomo, R. A. (2016). Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation β-lactam/β-lactamase inhibitor combinations. *Clinical Infectious Diseases, 63*(2), 234-241.

Vardakas, K. Z., Legakis, N. J., Triarides, N., & Falagas, M. E. (2016). Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. *International journal of antimicrobial agents, 47*(4), 269-285.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics, 40*(4), 277.

Wagenlehner, F. M., Sobel, J. D., Newell, P., Armstrong, J., Huang, X., Stone, G. G., . . . Gasink, L. B. (2016). Ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections, including acute pyelonephritis: RECAPTURE, a phase 3 randomized trial program. *Clinical Infectious Diseases, 63*(6), 754-762.

Wang, Q., Zhang, F., Wang, Z., Chen, H., Wang, X., Zhang, Y., . . . Wang, H. (2020). Evaluation of the Etest and disk diffusion method for detection of the activity of ceftazidime-avibactam against Enterobacterales and Pseudomonas aeruginosa in China. *BMC microbiology, 20*(1), 1-7.

Ward, D. V., Scholz, M., Zolfo, M., Taft, D. H., Schibler, K. R., Tett, A., . . . Morrow, A. L. (2016). Metagenomic sequencing with strain-level resolution implicates uropathogenic E. coli in necrotizing enterocolitis and mortality in preterm infants. *Cell reports, 14*(12), 2912-2924.

Wasey, A., & Salen, P. (2019). Escherichia coli (E coli 0157 H7) *StatPearls [Internet]*: StatPearls Publishing.

Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J., . . . Sievert, D. M. (2016). Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *infection control & hospital epidemiology, 37*(11), 1288-1301.

Wijetunge, D., Gongati, S., DebRoy, C., Kim, K. S., Couraud, P., Romero, I., . . . Kariyawasam, S. (2015). Characterizing the pathotype of neonatal meningitis causing Escherichia coli (NMEC). *BMC microbiology, 15*(1), 211.

Winkler, M. L., Papp-Wallace, K. M., Hujer, A. M., Domitrovic, T. N., Hujer, K. M., Hurless, K. N., . . . Bonomo, R. A. (2015). Unexpected challenges in treating multidrug-resistant Gram-negative bacteria: resistance to ceftazidime-avibactam in archived isolates of Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy, 59*(2), 1020-1029.

Woodford, N., Xu-McCrae, L., Mushtaq, S., Wu, H. H. T., Ellington, M. J., Lancaster, O., . . . Verma, A. (2018). Prevalence of carbapenem resistance and carbapenemase production among Enterobacteriaceae isolated from urine in the UK: results of the UK infection-Carbapenem Resistance Evaluation Surveillance Trial (iCREST-UK). *Journal of Antimicrobial Chemotherapy, 73*(3), 698-702.

Xu, Q., Jiang, J., Zhu, Z., Xu, T., Sheng, Z.-K., Ye, M., . . . Wang, M. (2019). Efflux pumps AcrAB and OqxAB contribute to nitrofurantoin resistance in an uropathogenic Klebsiella pneumoniae isolate. *International journal of antimicrobial agents, 54*(2), 223-227.

Yang, S.-C., Lin, C.-H., Aljuffali, I. A., & Fang, J.-Y. (2017). Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. *Archives of microbiology, 199*(6), 811-825.

Yaseen, M., Rashid, S., & Naqvi, S. (2020). Urinary tract infection in pregnant females attending antenatal clinics among middle socioeconomic settings. *The Professional Medical Journal, 27*(08), 1636-1641.

Younas, S., Ejaz, H., Zafar, A., Ejaz, A., Saleem, R., & Javed, H. (2018). AmpC beta-lactamases in Klebsiella pneumoniae: An emerging threat to the paediatric patients. *JPMA, 68*(893).

Zandi, H., Tabatabaei, S. M., Ehsani, F., Zarch, M. B., & Doosthosseini, S. (2017). Frequency of Extended-Spectrum Beta-lactamases (ESBLs) in strains of Klebsiella and E. coli isolated from patients hospitalized in Yazd. *Electronic physician*, *9*(2), 3810.

Zasowski, E. J., Rybak, J. M., & Rybak, M. J. (2015). The β-Lactams Strike Back: Ceftazidime-Avibactam. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *35*(8), 755-770.

Zhang, W., Guo, Y., Li, J., Zhang, Y., Yang, Y., Dong, D., . . . Hu, F. (2018). In vitro and in vivo bactericidal activity of ceftazidime-avibactam against Carbapenemase–producing Klebsiella pneumoniae. *Antimicrobial Resistance & Infection Control*, *7*(1), 142.

Zowawi, H. M., Sartor, A. L., Sidjabat, H. E., Balkhy, H. H., Walsh, T. R., Al Johani, S. M., . . . Al-Jardani, A. (2015). Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii isolates in the Gulf Cooperation Council States: dominance of OXA-23-type producers. *Journal of clinical microbiology*, *53*(3), 896-903.

CHAPTER 8

APPENDICES

(A) FRC Approval letter

Ref no: FRC/BUMDC-13/2019/Path-005 MS-11

Approval of Research Proposal

Mr/Miss/Ms/Mrs/ **Dr. Aafaq Khan**

Registration No. **59801**

Dear MS/MPhil Student,

I am pleased to inform you that your research proposal on **"Antimicrobial Susceptibility pattern of Ceftazidime-Avibactam among Different Clinical Specimen against Escherichia Coli and Klebsiella Pneumonia"** has been approved. You may, therefore, continue you research on this theme and produce a quality thesis, as per the HEC requirements.

I take this opportunity to remind you that you must complete your thesis, and defend it successfully, by **SPRING 2021**; this is the date which marks the end of the Extended Duration of your programme. However, to remain eligible for honours and awards, you must complete the thesis, and successfully defend it, by the end of 10 week into the next semester after the final semester.

I wish you every success.

Dated: 09/10/19

(CHAIRPERSON FRC)

Distribution:

- DG
- Principal
- Student's File (with the HOD/PGP Coordinator)
- Student

(B) ERC Approval letter

BAHRIA UNIVERSITY MEDICAL AND DENTAL COLLEGE

Defence phase II, Sailor Street, adjacent to PNS Shifa, Karachi. Tel: 021-35319491-9

ETHICAL REVIEW COMMITTEE

LETTER OF APPROVAL

Date: 09-Jan-2020

PATRON
Prof. Asad Ullah Khan
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Prof. Ambreen Usmani

SECRETARY
Dr. Quratul Ain
Omaeer

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
Ms Nighat Huda
Surg Cdre Amir Ejaz
Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Surg Lt Cdr Farah
Surg Lt Cdr Sadia
Dr. Ambreen Surti

Dr. Afaq Khan
M. Phil Student
Department of Pathology
BUMDC-Karachi

Subject: Institutional Approval of research study

Title of Study: " Antimicrobial Susceptibility Pattern of Ceftazidime- Avibactam Among Different Clinical Specimens Against Escherichia Coli & Klebsiella Pneumoniae "

Principal Investigator: Dr. Afaq Khan, M. Phil Student Department of Pathology, Bahria University Medical and Dental College.

Reference No: ERC 08/2020

Dear Dr. Afaq Khan

Thank you for submitting the above mentioned study proposal. ERC Bahria University Medical and Dental College has reviewed this project in the meeting held on 2nd-Jan-2020 and gives approval. Kindly notify us when the research is complete.

Regards,

10/1/2020

DR. QURATUL AIN OMAEER
Secretary ERC
BUMDC

PROF DR AMBREEN USMANI
Chairperson
BUMDC

Cc:
DG-BUMDC
Principal BUMDC
Chairperson ERC

Appendix C

(C) Informed Consent forms

INFORMED CONSENT FORM FOR PATIENT

I am giving my consent to participate voluntarily and at my own will in this research project which aims to determine effectiveness of new drug ceftazidime-avibactam against Enterobacteriaceae for the purpose of research.

I have been told that findings of my disease and my data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

I fully agree to give my samples at the beginning and end of study and when required in between.

I also agree to give all relevant information when needed, in full and to the best of my knowledge to the researcher. It is clarified to me that no incentive will be provided to me for participating in the study, whereas I do have the right to withdraw from the study at any time.

I am advised to contact Dr. Aafaq Khan on mobile number: 03132082308 or visit PNS Shifa Hospital in case of any query/ emergency related to my disease.

Name of Patient:_____ Sex _____

S/O, D/O, W/O_____ Age_____

Signature / Thumb impression of Patient: _____

Name of Researcher: _____

Signature of Researcher: _____ Date: _____

(آگاہ شدہ رضاکارانہ فارم برائےمریض)

میں رضاکارانہ طور پر اجازت دیتی /دیتا ہوں کہ میں اپنی مرضی سے اس تحقیقی منصوبے میں حصہ لے رہی /رہا ہوں جس کا مقصد پیٹ کی بیماریوں کی ایک نئ دوا کی تاثیر کا تعین کرناہے۔

مجھے بتایا گیا ہے کہ اس سلسلے میں میرے جسم سے ایک نمونہ (خون ، پیشاب اور پاخانہ) لیا جائے گا۔ مجھے یہ بھی بتادیا گیا ہے کہ میری بیماری کے نتائج اور اعدادوشمار کو خفیہ رکھا جائے گا اور صرف برادری،اشاعت اور مطالعہ کی پیشکش کے فا ئدے کے لیے استعمال کیا جائے گا ۔

میں مکمل طور پر اپنے نمونے دینے کے لیے اتفاق کرتی / کرتا ہوں چاہے مطالعہ کے شروع ، آخر یا بیچ میں کبھی ضرو رت پڑے ، ضرو رت کے تحت میں اپنی تمام تر معلومات فراہم کرنے پر بھی اتفاق کرتی / کرتا ہوں۔

مجھے بتایا گیا ہے کہ اس تحقیق کے لیے مجھے کوئی حوصلہ افزائی نہیں دی جائے گی اور مجھے اپنی خواہش کے مطابق اس مطالعہ سے نکلنے کا حق حاصل ہے ۔

مجھے مشورہ دیا گیا ہے کہ میں ڈاکٹر آفاق خاں سے انکے موبائل نمبر 03132082308 یا پھر PNS SHIFA ہسپتال میں کسی بھی سوال یا ہنگامی صورتحال میں رابطے میں رہو ں۔

مریض کا نام:_____

جنس :_____ (مرد / عورت)

والد/ شوہر کا نام:_____

عمر:_____

دستخط :_____

نشان انگوٹھا :_____

(D) Subject Evaluation Performa

Preliminary Data

Data: _____ ID No _____

Ward _____

Patients name _____

W/O, S/O, D/O, F/O _____

Sex_____

Age _____ Occupation _____

Address _____

Presenting complains _____

Past History _____

Laboratory test:

Sample _____

Gram staining _____

Bacterial growth on blood agar_____

Bacteria Growth on MacConkey _____

Biochemical test _____

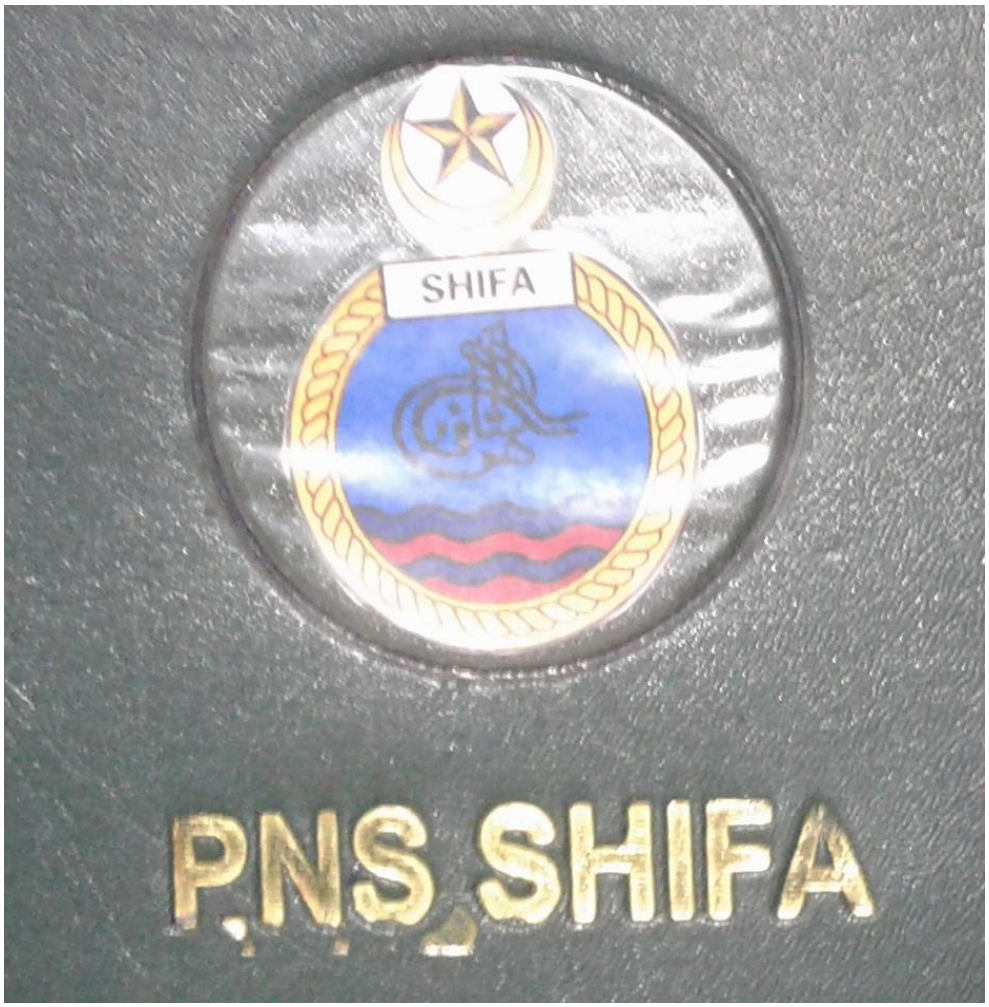
Disk diffusion test _____

E-test _____

ADVERSE EFFECTS

NA

(D) Hospital card



(E) Turnitin Plagiarism check report

ORIGINALITY REPORT

9%	5%	7%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	pesquisa.bvsalud.org Internet Source	1%
2	bmcmicrobiol.biomedcentral.com Internet Source	1%
3	pubmed.ncbi.nlm.nih.gov Internet Source	<1%
4	Yogesh Mawal, Ian A Critchley, Todd A Riccobene, Angela K Talley. "Ceftazidime–avibactam for the treatment of complicated urinary tract infections and complicated intra-abdominal infections", Expert Review of Clinical Pharmacology, 2015 Publication	<1%
5	"Posters", Clinical Microbiology and Infection, 2011 Publication	<1%
6	Evan J. Zasowski, Jeffrey M. Rybak, Michael J. Rybak. "The β-Lactams Strike Back: Ceftazidime-Avibactam", Pharmacotherapy: The Journal of Human Pharmacology and Drug	<1%