TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

DETECTION OF CARBAPENEMASES IN CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED FROM VARIOUS CLINICAL SAMPLES

FADUMO MOHAMED JAMA ALI

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

NICOSIA – 2020

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Prof. Dr. NEDIM ÇAKIR

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STATEMENT (DECLARATION)

Hereby, I declare that this thesis study is my study, I had no unethical behaviors in all stages from the planning of the thesis until writing there, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and, had no behavior of breaching patent rights and copyright infringement during the study and writing of this thesis

FADUMO MOHAMED JAMA ALI

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Special thanks to my Mom for her love and support throughout my life, thank you for giving me strength to reach for the stars and chase my dream, deep down everything I do is for you mom.

FADUMO MOHAMED JAMA ALI

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List of Abbreviations

%	Percentage
µg	Micro gram
µl	Micro letter
ATCC	American type culture collection
Bp	Base pair
CLSI	Clinical and laboratory standards Institute
DNA	Deoxyribonucleic acid
ESBL	Extended spectrum beta lactamase
HAP	Hospital acquired *Pneumonia*
HCl	Hydro chloric acid
I	Intermediate
Kb	Kilo base pair
kDa	Kilo Dalton
MDR	Multi Drug Resistant
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NCBI	National Center for biotechnological informations
PCR	Polymerase Chain Reaction
pH	Power of hydrogen
PIMS	Pakistan Institute of Medical Sciences
R	Resistant
Rpm	Revolution per minute
rRNA	Ribosomal Ribonucleic acid
S	Sensitive

SDS	Sodium dodecyl sulphate
Spp.	Species
ST	Sequence type
TBE	Tris Boric acid EDTA
TE	Tris EDTA
UTI	Urinary Tract Infection
WHO	World Health Organization
B	Beta

Thesis Title: Detection of carbapenemases in clinical isolates of *Klebsiella pneumonia* strains isolated from various clinical sample

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Department: Medical Microbiology and Clinical Microbiology

Abstract

Beta lactamases pose a significant global challenge rendering most bacterial infections difficult to treat. Among them carbapenemases are the most concerning, they hydrolyze several antibiotics including carbapenems and cephalosporins. The emergence of carbapenem resistance is an increasing issue for the both community and healthcare centers. The KPC and NDM enzymes are widespread mediated factors that contribute towards the emergence of carbapenem-resistant Enterobacteriaceae. The objective of this study was to screen carbapenems resistance strains and the frequency of NDM and KPC resistant enzymes from bacterial isolates of *K pneumonia* isolated from Pakistan Institute of Medical Sciences. For phenotypic detection of resistant KPC strains, 194 clinical specimens were collected from different wards and biochemical tests were done for detection of *K pneumonia*. Antibiotic susceptibility for these isolates was performed by Kirby Bauer method following CLSI guidelines against nine different antibiotics. The drug resistance profile of these isolates revealed that 94% were resistant to ampicillin followed by ceftriaxone 78%, gentamicin 65%, aztreonam 61%, levofloxacin 56%, imipenem, and meropenem 32%, Fosfomycin 31%, and polymyxin B 29%. Moreover, a double-disc synergy test was performed for the detection of extended-spectrum beta-lactamases that revealed the presence of ESBLs in 35% of total isolates. Phenotypic characterization of carbapenem-resistant isolates showed that 62% and 53% of isolates contain NDM and KPC enzymes respectively. Conclusively this study on clinical isolates of *K pneumonia* in the North region of Pakistan confirmed the presence of KPC and NDM resistant strains.

Tez Başlığı: Çeşitli klinik örneklerden izole edilen *Klebsiella pneumonia* suşlarının klinik izolatlarında karbapenemlerin tespiti

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ÖZET

Beta laktamazlar, çoğu bakteriyel enfeksiyonun tedavi edilmesini zorlaştıran önemli bir küresel zorluk oluşturmaktadır. Bunların arasında karbapenemazlar en önemlisidir, karbapenemler ve sefalosporinler dahil olmak üzere birçok antibiyotiği hidrolize ederler. Karbapenem direncinin ortaya çıkışı, hem toplum hem de sağlık merkezleri için artan bir sorundur. KPC ve NDM enzimleri, karbapeneme dirençli Enterobacteriaceae'nin ortaya çıkmasına katkıda bulunan yaygın aracı faktörlerdir. Bu çalışmanın amacı, Pakistan Tıp Bilimleri Enstitüsü ve Silahlı Kuvvetler Patoloji Enstitüsü'nden izole edilen *K pneumonia* bakteriyel izolatlarından karbapenem direnç suşlarını ve NDM ve KPC dirençli enzimlerin sıklığını taramaktı. Dirençli KPC suşlarının fenotipik tespiti için, farklı servislerden 194 klinik örnek toplandı ve *K pneumonia'* nin tespiti için biyokimyasal testler yapıldı. Bu izolatların antibiyotik duyarlılığı, dokuz farklı antibiyotiğe karşı CLSI yönergeleri izlenerek Kirby Bauer yöntemi ile gerçekleştirildi. Bu izolatların ilaç direnç profili,% 94 oranında ampisiline dirençli olduğunu, ardından seftriakson% 78, gentamisin% 65, aztreonam% 61, levofloksasin% 56, imipenem ve meropenem% 32, Fosfomisin% 31 ve polimiksin B% 29 olduğunu ortaya koymuştur. Ayrıca, toplam izolatların% 35'inde GSBL'lerin varlığını ortaya çıkaran genişletilmiş spektrumlu beta-laktamazların tespiti için çift disk sinerji testi yapıldı. Karbapeneme dirençli izolatların fenotipik karakterizasyonu, izolatların sırasıyla% 62 ve% 53'ünün NDM ve KPC enzimleri içerdiğini göstermiştir. Sonuç olarak, Pakistan'ın kuzey bölgesindeki *K pneumonia'* nin klinik izolatları üzerinde yapılan bu çalışma, KPC ve NDM dirençli suşların varlığını doğruladı.

CHAPTER ONE

1.0 INTRODUCTION

Klebsiella pneumonia (*K pneumonia)* from Enterobacteriaceae are Gram negative capsular bacilli, characteristically facultative anaerobes and lactose fermenters. Upon culture, it is identified as forming mucoid colonies. Residing on human skin, intestine and respiratory tract as normal flora, *K pneumonia* is an opportunistic pathogen and causes respiratory tract infections. In case of severe respiratory tract infections, complications such as bloody sputum results. *Klebsiella* is the second most important pathogen after *E coli* causing urinary tract infections, pulmonary disorders, rhinocerical, enteric pathogenicity and atrophy of nasal mucosa (1). Edwin Kleb and F. Loffler's work resulted in identification of Cory diphtheria, also known as Klebs-Loffler bacillus. Later Trevisan (1885) gave the genus its name *Klebsiella* in honor of the German bacteriologist's work (2).

Klebsiella species contain polysaccharide capsule as a virulence factor that involves repeating units of sugars and ironic acid. *K pneumonia'*s components of capsule are capsular polysaccharide and Lipopolysaccharide. They express two surface antigens; these are O and K O antigen have 9 different varieties while K have more than 80 varieties. Polysaccharide capsule provide protection to bacterium from bactericidal serum agents and polymorphonuclear granulocyte born phagocytosis (36). Some of the *Klebsiella* species have most virulent activity than other in group (14). As rhamnose and mannose are not found on K antigen so they are not recognized by lectin macrophages (36) while K2 antigen don't possess rhamnose structure and 63 mannose. That's may be the reason why K2 is more dominant serotype throughout the world (36). Cell membrane of Gram-negative bacteria have Lipopolysaccharide (LPS). LPS is composed of three components that are Lipid A, O antigen and the core oligosaccharide. Lipopolysaccharide is endotoxin and Lipid A has pathogenic properties which causes fever in the host. Complement system is activated by two pathways that are Lipid A pathway and the other one is alternative pathway through O Ag (antigen). As adhesion occurs due to Fimbriae, it is the cause of mostly hospital acquired infections as they form biofilm on medical devices and other surfaces. There are two types of fimbria that are type 1 and type 3. Type 1 commonly occurs in most of enterobacteriaceae while type 3 is found in less common strains that are only pathogenic to plants. Two kinds of siderophores

are produced by *K pneumonia* these are aerobactin and enterobactin, both of these have role in virulence (36).

Mainly, *K pneumonia* and *K oxytoca* are involved in causing human infections. It causes infections in immunocompromised individuals, involved both in community and hospital acquired infections (3) (4). It can survive on the skin of hospital staff for many hours which supports the HAI (5). It is estimated that approximately, 15% of the Intensive care unit (ICU) related infections are caused by *Klebsiella* spp. (3, 4). It is responsible for hospital acquired infections, specifically in NICUs where death rate is up to 70% (37). Jone *et al* in 2004 reported about the incidences in ICUs of five countries these are France, USA, Germany, Canada and Italy. It was reported that *K pneumonia* differs from country to country, it ranges from 3.5 to 5.8%. *K pneumonia* was found the third responsible organism regarding infection after *P aeruginosa* and *E coli* (38). One Year study in USA reported that 5.8% cause of HAIs is *K pneumonia* and also it is 7.7% cause of catheter related UTIs. In that study 21.2% resistance to cephalosporin and 10.1% resistance to carbapenems was reported (20).

Various different antibiotics are used for the treatment of infections caused by *Klebsiella* spp. Generally, these infections are treated by narrow and broad spectrum antibiotics such as carbapenems, cephalosporins, fluoroquinolones and aminoglycosides (6). Carbapenem is the drug of choice for the treatment of respiratory tract infections caused by *Klebsiella*. Currently, high levels of resistance have been observed against carbapenems which lead to multi-drug resistant (MDR) strains. Resistant strains of *K pneumonia* were isolated in 2013 (7). Recently these have shown resistance to the carbapenems drugs as these are in the last line therapy drugs so it shows an alarming situation in future (8). Diseases caused due to CRKP have only little cure options (9),(10) and these show ,rate of mortality up to 50% as a lot of resistance mechanisms are developed by bacteria (13). Antimicrobial resistance to carbapenems is the most serious and alarming situation for our community and infection control measures are needed to be taken on time to avoid the worse conditions (29). Since 1970, an increase in bacterial resistance is reported and in the last 2 decades, a prominent rise in the different bacterial multidrug resistant strains has been observed (30). MDR *K pneumonia* is one of the most causative agents of hospital acquired infections. It causes UTI, intra-abdominal infection and pneumonia in patients that have low immunity in hospitals (3). The spread of beta-lactam resistance in the 20th century, due to emergence of *K*

pneumonia strains that are now showing resistance to colistin and carbapenems, treatment options have been reduced to minimum (31). In recent CRKP is the most concerned issue for the community and it is mediated by *bla* NDM (32). Outbreaks are reported in several healthcare setups as well as in long term acute care hospital. Risk factors for infection and colonization are similar to others that are linked with MDR organisms. Mortality due to Carbapenem resistant *K pneumonia* ranges from 26% - 44%. But if bacteremia is involved then it reaches up to 70%. However, in case of *K pneumonia*, several cases were observed in which the patient have other associated complications. Thus, it is difficult to determine whether CRKP is the cause of mortality or not. Carbapenems resistance in America initially occurred due to plasmid mediated *K pneumonia* carbapenemases gene. An even worse situation is related to resistant *K pneumonia* species with high risk to hospitals out breaks Such potentially resistant strains are like silent carriers that enhance the possible chances of spread of transmission and render prophylactic measures ineffective Genome sequence techniques were used to identify outbreak of *K pneumonia* resistant to carbapenem in clinical setups of National Institute of Health in United States. 18 patients were infected by *K pneumonia* and resulted in 6 expiries. Emergence of Carbapenem resistance is a threat to the community as these are the last line therapy for life threatening disease earlier carbapenem-resistant *K pneumonia* were very rare. Before a decade outbreaks of *K pneumonia* have reported 10% in New York to National health care safety Network but in some regions, it was 30% these were related plasmid mediated carbapenemases in KPC. In recent research KPC resistance have been noted in areas other than United States.

In 2006, outbreaks of carbapenem-resistant *K pneumonia* were noted at multiple Israeli hospitals. The resistance was mediated by KPC and isolates were mostly susceptible only to colistin and gentamicin. A dominant clone producing KPC-3 was responsible for the outbreaks in numerous facilities and belonged to the genotype of a strain that had been implicated in multiple outbreaks in the United States. In the last decade, there have been reports of carbapenems resistant *K pneumonia* in different Israeli healthcare setups. These resistances were developed by KPC Drugs such as gentamycin and colistin were found sensitive to these isolates. KPC-3 gene was the responsible gene for causing outbreaks in many facilities and that was matched to the bacterial genotype of the strain that caused several outbreaks in US (28). In last 30 years, a wide dissemination of plasmid encoded beta lactamases is reported in Brazil. KPC-2 is the most variant gene reported till date in Brazil

and Polymyxin B resistance of around 27.1% was reported in Sau Paulo as it is the largest city of Brazil. In 2013 New Delhi Metallo-beta-lactamases was detected in different states of Brazil but it is not frequently distributed.

Beta lactam group have one subgroup of carbapenemases that are of most concerned in gram negative microbes. There members are divided into four classes. These include Class A, are the KPC, first found in 2001 in *K pneumonia* and now are found in many members of Enterobacteriaceae. Class B are the Metallo-carbapenemases. These are Verona integrons-encoded MBL (VIM). These were first reported in *P aeruginosa* in 1999 in Italy. Now they are found worldwide in different bacterial species Class C group are CMY-10, first reported in 2003 in Enterobacter aerogenes, this carbapenem is bounded and belongs to the few members of class C. Class D are OXA-type, Oxacillin hydrolyzing β-lactamases usually give limited activity but its wide activity is observed against carbapenems and oxyimino cephalosporins

Carbapenem resistant gene *bla*NDM-1 was first reported in isolates of *Klebsiella* in 2008 at Sweden. The patient had gone through treatment in hospital previously in Delhi (47). Presence of *bla*NDM1 has been reported in 15 patients out of 39, CRE that was 38.5% by Castenheira and these are the same as CRE reported in India in 2006 to 2007. Several publications have been reported on the occurrence of *bla*NDM1 gene in non-fermenting bacterial species from India and around (49-51). Carbapenem resistant gene KPC was initially discovered in United States in 2001. Virtually they spread worldwide and show resistant to entire available antibiotics (43). Lascol's reported about CRE isolated in India, in that study 34.8 % prevalence of *bla* NDM is reported (52).

Understanding mode of transmission can facilitate the control of *K pneumonia* epidemics. Several techniques like multi locus sequence (MLST) typing and gel electrophoresis (pulsed-field) are used for the classification and worldwide dissemination of *K pneumonia*. Clonality of *K pneumonia* shows difficulty in identification of nosocomial infection in healthcare organizations. In United States the *K pneumonia* carbapenemases are highly clonal and belong to 70 % to type ST. Sequencing of whole-genome is an important gold standard test in the typing of bacteria

The aim of the present study is to detect the carbapenem resistant *K pneumonia* in clinical isolates and to analyze gene of resistance in clinical carbapenem resistant *K pneumonia*

AIMS AND OBJECTIVES

The purpose of this study was to isolate and screen the CRKP from different clinical isolates for KPC and NDM in different clinical setting of Islamabad and Rawalpindi. Following are the details objectives;

1) Precise identification of *K pneumonia* from the clinical specimens of different hospitals by using biochemical and phenotypic methods.
2) Identification of Carbapenems and extended spectrum beta lactamases to find out frequency in clinical isolates of *K pneumonia*.
3) Frequency of NDM and NDM enzymes in CR *K pneumonia*.

CHAPTER TWO

2.0 LITERATURE REVIEW

K pneumonia was first discovered by Friedlander C Uber. It is a gram negative rod belonging to a group of facultative anaerobic bacteria and it causes pneumonia. Mostly hospital diseases are caused by *K pneumonia*, that's why it is very important. *K pneumonia* was first isolated in 2004 from a patient. It mostly occurs in the gut region and exposed skin of hospital staff like doctors and paramedics. The cause of *K pneumonia* virulence is its capsule that originates from cell membrane. This is not only found in humans but also in soil and sewage. In human they are not reserved to human skin but also found in nasopharyngeal region. Since 1970s, it has been observed that the resistance bacterial species are increasing in number due to the selective pressure exerted by antibiotics and there exists a dramatic rise in the number of multi-resistant observed over the period of last 20 years . Multidrug-resistant *K pneumonia* also known as MDR *K pneumonia* is one of the foremost causes of nosocomial infection globally. It is reported to cause pneumonia, urinary tract infections (UTIs) and intra-abdominal infections in immunocompromised patients that are hospitalized with severe underlying ailments and is responsible for roughly 15% of Gram-negative infections in hospital intensive care units (ICUs). The isolates of *K pneumonia* that are now showing resistance to colistin and carbapenems are decreasing treatment options plus infection containment owing to the spread of beta-lactam resistant strains by the end of the 20th century. In Turkey, for the last ten years, & for twenty years globally "Carbapenem-resistant *K pneumonia*" (CRKP) has been recognized as hospital acquired pathogen. CRKP still possess a big threat for public health due to high mortality rates and narrow treatment choices, notwithstanding novel improvements in the field of medicine in the current era. In the course of the past 10 years, carbapenem-resistant *K pneumonia* (CRKP) has transmitted globally, creating alarming concern for individuals. The first report of CRKP was made in 2001 in North Carolina, United States. Apart from the United States the first case of CRKP was reported in France, where a hospitalized patient from New York carried the strain with him when he travelled to France. Since then, there have been reported cases of CRKP in South America, Middle East, Far East and Europe. Multi-drug resistance is trending in the isolates of CRKP, meaning almost all of antimicrobial drugs available are of no use against CRKP strains. The studies showed that only polymyxins and tigecycline are effective against the CRKP isolates; some of the isolates show susceptibility to a few of left over

aminoglycosides, however resistance to these agents is of growing concern as it is increasing day by day. *K pneumonia*, an inhabitant of the gastrointestinal tract, skin and nasopharynx, can cause infection in many parts of the body, including urinary tract infections, hospital acquired pneumonia, intra-abdominal infections, wound infections, and primary bacteremia. Initially, CRKP seemed to be limited to causing hospital acquired infections, though further on, CRKP has spread in different health care systems, including long-term care facilities. The mortality rate of CRKP infections (mainly bacteremia) seems strikingly high, 30% - 50%

2.1 Risk factors for Carbapenem-Resistant *K pneumonia* infection

There have been various studies denoting risk factors for the attainment of CRKP infection by patients primarily colonized with carbapenem resistant *K pneumonia*. Their findings are; (i) Patients having diabetes mellitus, urinary catheter insertions, solid tumors, anti-pseudomonal penicillin therapy, previous invasive procedures and tracheostomy were at risk. Carbapenem was used for the detection of CRKP infection and it provided solid results. (ii) A study conducted by Wu et al showed that prior exposure to glycopeptides or carbapenems or ICU admission (within 2 weeks) were independent risk factors for catching hospital acquired CRKP infection. (iii) The use of antibiotics particularly colistin, ICU admissions, use of urinary catheters and invasive procedures like surgeries were risk factors for gaining a CRKP infection in a study reported by Shilo *et al*. In a study by Hussein *et al*, results of multivariate analysis showed that previous use of macrolides & any exposure of antibiotic (14 days) were the cause of a CRKP bacteremia, being the only linked independent factors. whereas in a univariate analysis in the same research, the CRKP bacteremia was linked with ICU stays, hematological malignancy, previous bone marrow transplantation, chronic renal failure, chronic liver disease, longer length of stay before the onset of bacteremia, central venous catheterization, mechanical ventilation experience, stay in the hematology department and dialysis. In a separate study by Schechner *et al* getting central venous catheters, receiving antibiotics, ICU admissions, and diabetes mellitus were independent predictors of succeeding infection of carbapenem-resistant Enterobacteriaceae (CRE). It is a matter of serious threat to public health owing to the dissemination of Carbapenem Resistant *K pneumonia* (CRKP). The control of CRKP infection by the health care society is becoming overbearing due to the fact that CRKP strains have now spread worldwide plus the deficiency of clinically proven efficacious antibiotics, and the phenomenon of having gene-encoded KPC enzymes riding on transmissible plasmids makes

the scenario worse for effective control strategy of CRKP dissemination. Carbapenem-Resistant Enterobacteriaceae (CRE) infections, specifically, carbapenem-resistant *K pneumonia* (CRKP) infections, are a noteworthy public health challenge internationally. The dissemination of these multi drug resistant pathogenic bacteria around the globe & the Mediterranean nations has been connected to several epidemiological factors one of them is travelling of people from endemic areas to non-endemic areas internationally.

2.2 Characteristics of *K. pneumonia*

K pneumonia, belongs to Enterobacteriaceae group and is normally present in the intestine of humans (part of microbial flora). It is frequently linked with nosocomial (hospital-acquired) infection. There exist certain risk factors or even diseases that can impair an individual's defenses against *K pneumonia* infection such as diabetes mellitus (DM), malignancy, alcoholism, cirrhosis and biliary tract disorders. After Escherichia coli, the 2nd most common cause of Gram negative bacteremia is *K pneumonia*. Bacteremia caused by *K pneumonia* produces significant general population morbidity and mortality rates. Metastatic infections for example Endophthalmitis, meningitis & pyogenic brain abscess are the most vital characteristics of *K pneumonia* infections. Earlier findings in East Asia exposed that 34-36% cases of bacteremia caused by *K pneumonia* is due to an underlying disease of diabetes myelitis.

2.3 Clinical and molecular physiognomies of *K. pneumonia*

K pneumonia is well-thought-out as an opportunistic pathogen, largely infecting hospitalized patients suffering from medical illnesses. Nevertheless, it has ability to cause severe diseases in healthy individuals as well. Such bacterial strains are frequently stated as hyper virulent. Rendering to the molecular Koch's postulates several virulence determinants were identified in *K pneumonia*. The first in the list is the polysaccharide capsule which is formed by the many strains of *K pneumonia* (KP) strains and thus makes it one of the chief virulence determinants of KP. The capsule provides resistance towards bactericidal activity of serum and it also impedes phagocytosis by the polymorphonuclear (PMNL) cells. There are at least 78 capsular serotypes (K-serotype) defined. The diverse K-serotypes vary in the degree of virulence. The serotypes K1 & K2 carrying KP strains are shown to be more virulent than non-K1/K2 carrying strains. The hyper-virulent strains from East of Asia are known to frequently show hyper muco viscosity (HV). It is due to production of exopolysaccharide web made up of fine fibers originating from the capsular polysaccharide.

Various genes such as magA, cps clusters and rmpA contribute towards the hyper muco viscous phenotype.

A retrospective study conducted by Guo S et al. took 70 isolates of mechanically ventilated patients with *K pneumonia*. It was found that the virulence determinants that contributed towards *K pneumonia* infection included hyper muco viscosity (HV)-positive phenotype, the presence of aerobactin genes & rmpA plus certain other serotypes. About 20 percent of the isolates were found to be hyper muco viscosity (HV)-positive strains and 80 percent of the isolates were HV-negative strains, amongst the seventy isolates of *K pneumonia* analyzed. The study also exposed that the patients with HV-positive strain showed a significantly higher frequency of Ventilator-associated pneumonia (100 percent; 14/14) as compare to patients with HV-negative strains (51.8 percent; 29/56). Findings of the study also included that there exists a high correlation amid HV-phenotype and presence of the aerobactin genes & rmpA. Out of 14 HV-positive isolates, 14.3 percent (2/14) were aerobactin & rmpA negative and around 85.7 percent (12/14) were aerobactin & rmpA positive. No HV-negative isolate showed an aerobactin or rmpA positive result. Of the HV-positive isolates, K2 & K1 serotypes accounted for 28.6 percent (4/14) and 14.3 percent (2/14), respectively. Serotypes K2 and K1 were not found amongst HV-negative isolates. The results further showed that all twelve of rmpA-positive isolates carried the aerobactin gene and all K2/K1 isolates showed HV-positive phenotype and carried the aerobactin gene & rmpA (115).

2.4 Other virulence determinants of *K. pneumonia*

When it comes to the structure of a Gram-negative bacteria, the lipopolysaccharide (LPS) layer is an essential component of its cell wall. LPS increases production of many pro-inflammatory mediators such as cytokines, chemokines and receptors of major histocompatibility complex (MHC) after binding to Toll-like receptor 4 (TLR4). Till date, in *K pneumonia* 9 dissimilar O-serogroups have been identified, among which O1 is the most widespread of all clinical isolates. Type 1 and Type 3 are the major adhesion factors recognized in *K pneumonia*. Among the various Enterobacteriaceae species, Type 1 fimbria is frequently spread & is encoded by gene cluster “fim”. The main function of Type 1 fimbria is adhesion; it helps bacteria to attach to mannose comprising structures located on host cells & extracellular matrix components (EMC). Type 1 fimbria of *K pneumonia* and *E coli* are

structurally similar yet they are not identical. Phase variation occurs in type 1 fimbria: During a lung infection and within gastrointestinal tract (GIT), its expression is turned off whereas during a urinary tract infection (UTI) its expression is turned on. In a murine UTI model, type 1 fimbria was presented to be a key virulence factor mrk gene cluster codes for Type 3 fimbria and it plays an important role formation of biofilms plus it is involved in attachment epithelial cells and endothelial cells of respiratory tract, collagen type V and cells of urinary *bla*dder. In catheter-associated UTIs, Type 3 fimbria was identified as an important colonization factor. Formation of biofilms upon urinary catheters, endotracheal tubes and intravascular catheters can aid in infection. Moreover biofilms inside water supplying pipelines and on environmental surfaces might add to the persistence of bacteria in hospital settings. Biofilm formation protects the bacteria from the harmful effects of antimicrobials, disinfectants and host defense mechanisms. Yet there exists an exchange of the genetic material amongst different bacterial species inside a biofilm. In addition to type 3 fimbria, which contributes majorly, there are still several other factors that are well-thought-out to have a role in biofilm formation of *K pneumonia*.

There is a limited availability of iron inside the host tissue and blood stream so it becomes crucial for the bacteria to obtain ferric ion for its normal functioning. In the context of *K pneumonia* pathogenesis, there are many iron acquisition systems identified till date. Enterobactin is the most commonly found siderophore amongst the isolates of K *pneumonia*, but lipocalcin-2 can disrupt its activity. Salmochelin is a glycosylated derivative of enterobactin is another iron binding molecule, together with Yersiniabactin, can resist binding by lipocalcin-2. Yersiniabactin was identified as a significant virulence determinant in pneumonia. As compared to enterobactin, the siderophore “aerobactin” has lower iron binding affinity, but it has better solubility and is more stable. In a murine subcutes & intraperitoneal infection model, the siderophore aerobactin was shown to be an important virulence factor. The non-hyper virulent strains of *K pneumonia* isolates seem to produce quantitatively less siderophores, than hyper virulent strains that produce mostly aerobactin. Furthermore, there was a discovery of a novel iron acquisition system, named *Klebsiella* Ferric ion Uptake system (kfu) in the hyper virulent strains of *K pneumonia*. There still exists many other factors apart from the well-defined virulence factors that may contribute to the pathogenicity of *K pneumonia*. Various studies indicate that no single virulence associated trait or virulence determinant can make a strain hyper virulent, rather the

concurrent manifestation of diverse virulence factors contribute to the virulence potential of an isolate or strain.

2.5 Significance of *K pneumonia*

The first report of KPC-producing *K pneumonia* isolate was made in North Carolina in 2001 (8). Back in 1994, Japan had reported a carbapenem hydrolyzing Class B Metallo-beta-lactamases (MBL) making KPC-1 (Ambler Class A beta-lactamases), not the first carbapenemases to be identified in K pneumonia. Nevertheless, in the United States, presence of Metallo-beta-lactamases in the *K pneumonia* are rare thus the production of KPC enzymes to resist carbapenem has become the most prevalent mechanism in US till date. KPCs are encoded by the gene *bla* NDM that is located on a type 3 transposon Tn4401. This explains the very potential of dissemination of *bla* NDM around the globe and even between different species. Tn4401 transposon is a mobile genetic element which has the capability of inserting into different plasmids of Gram-negative bacteria. The *bla* NDM inserted plasmids are frequently linked with resistance determinants for other antibiotics as well. Even though *K pneumonia* stands the most prevalent bacterial species hosting KPCs, the enzyme has been acknowledged in numerous other Gram-negative Rods. In 2009, a new term "Carbapenem-Resistant Enterobacteriaceae" (CRE) was introduced by the Centers for Disease Control and Prevention (CDC) in a report on KPC producing bacteria. Owing to the fact that many species of Gram-negative bacteria can harbor the *bla* NDM, this terminology was more suitable (132).

2.6 Epidemiology

Carbapenem resistance in *K pneumonia* was uncommon in the US before the first hospital outbreaks in New York City (133). The early outbreaks triggered a public health concern as these bacteria were showing resistance to currently available all of the beta-lactam antibiotics such as cephalosporins, penicillin, monobactam, & carbapenem. In in-vitro studies ninety-five KPC isolates were taken from Brooklyn hospitals during the period of 2003-2004. Their results showed that about half of the isolates showed susceptibility to aminoglycosides & very few were susceptible to fluoroquinolones. Subsequent to the early random outbreaks of KPC in New York City, the enzyme producing bacteria became endemic in numerous hospitals of New Jersey area & New York. Following that, KPC-producing bacteria disseminated throughout the US and globally within 10 years' time. According to an estimated data reported to CDC of the nosocomial infections from year 2000-2007, the

prevalence of carbapenem resistance *K pneumonia* had risen from <1% to 8% by the end of 2007. Another data source collected from an academic medical center in New York City showed that the percentage of carbapenem-resistant *K pneumonia* rose to 38 percent by year 2008 that was only 18 percent in 2004 and that itself had risen from 9% initially in 2002. So far, in the US, at least 33 states have reported KPC-positive bacteria in their isolates. The first report of a clinical isolate producing a KPC enzyme outside of the United States was made in France, in 2005. The source patient had a recent hospital stay in New York City. Following France, came Israel and Greece, both are now endemic countries. There has reports of Enterobacteriaceae-producing KPCs in Sweden, Brazil, Norway, India, China, Colombia, and lately, Finland & Italy has also reported KPC producing Enterobacteriaceae.

2.7 Molecular epidemiology

Molecular epidemiological studies of KPC indicate that there exist few suitable (fit) lineages that are playing a possible role in the dissemination of *bla* NDM gene. A research data from year 1996-2008 of all KPC-producing *K pneumonia* isolates sourced from 18 states of US plus from India and Israel was sent to the CDC. It exposed that ST258 (multi-locus sequence type 258), a single dominant strain covered almost 70 percent of the isolates in the CDC database including an isolate from the outbreak of Israel. A total of 7 variants (KPC 2-8) of the enzyme have been documented. Findings showed that most of the non-ST258 strains produce KPC-2 enzyme which is genetically identical to KPC-1 whereas most of the ST258 strains produce KPC-3 enzyme. There was also a confirmation of another fit strain called ST14, from the study conducted. It was isolated in the Midwestern states from various facilities. Moreover, Endimiani *et al* did clonal analysis of forty two KPC-producing *K pneumonia* isolates from 5 diverse institutions in the eastern US. The results indicated that 32 (76%) of the isolates were clonally related, proposing regional spread of a single dominant clone of KPC-producing *K pneumonia*.

2.8 Clinical features of KPCs

There exist certain health related downfalls contributed by the infections caused by KPC-producing *K pneumonia*. Among them increased length of stay in hospitals, more expense and frequent treatment failures are most observed. Old age, previous antibiotic exposure, organ transplantation, long hospital stays, mechanical ventilation is some of the risk factors for a KPC infection. There exists a controversy that whether infections caused by

a KPC-producing bacterium is linked with previous use of carbapenem drugs. However one study indicated that prior exposure to fluoroquinolones & extended-spectrum cephalosporin were both individually related with a KPC infection (134). An infection with a KPC-producing bacillus can lead to poor outcomes as it was indicated by the first outbreak report of KPC in the hospitals of New York City. In 2005, a report of KPC-producing bacteria causing blood stream infection in a small group of patients from New York hospitals showed patient mortality rates of 47percent to 66percent. The reports of KPC-producing bacterial infection outcomes were almost similar outside of US; a retrospective cohort study of 32 patients of Israel who were suffering bacteremia caused by carbapenem-resistant *K pneumonia* showed that when the comparison was made amid susceptible and resistant *K pneumonia*, results showed an attributable mortality rate of 50% and a crude mortality rate of 72%, in the patients who were suffering from KPC-producing *K pneumonia*. Furthermore KPC-production was linked with more than two-fold increased risk of demise.

It is not easy (fast enough) to detect KPC with routine tests performed in hospital laboratories as it takes time and that leads to poor outcomes such as delayed treatment of a bacterial infection caused by a KPC-producing bacterium. In a study by Weisenberg *et al*, 28 cases of confirmed KPC-producing *K pneumonia* were re-observed. It was found that 46 percent of clinical isolates had been reported wrongly as imipenem-sensitive, as a result of which majority of these 28 cases were treated with imipenem or meropenem. The administration of inappropriate antibiotics is one of the key causes of development of resistance in bacteria. In any clinical settings, KPC-producing bacteria creates a noteworthy problem as it is crucial to provide an effective empiric antibiotic therapy to prevent mortality. Only serious infections like bacteremia comes under this, but in other cases such as patients undergoing an organ transplant or having treatment for cancer etc. in all these cases the patients are immunocompromised so they require an effective empiric therapy with antibiotics. Another study by Mathers *et al* confirmed cause of death of two orthotropic liver transplant recipients; they died on the table due to the infections caused by KPC-producing *K pneumonia* as both of the patients were being treated with meropenem advised by the results of routine susceptibility tests of laboratory.

2.9 Current therapeutic options

For the time being, tigecycline and polymyxins stand a chance for the treatment of infections caused by the KPC-producing bacteria. It is suggested by some clinician that a high dose of carbapenem via continuous infusions might provide some relief but there is a lack of evidence for its efficiency. To treat KPC induced infections, there exists a need presently to find information that can help in prescribing antibiotics optimally with the help of other antibiotic classes or even solely.

There are five groups (A-E) of cyclic polypeptide antibiotics called Polymyxins, of which B and E (colistin) are at present accessible. Clinical isolates of KPC-producing bacteria show susceptibility of 90-100% to polymyxins, in-vitro. Currently Polymyxins are the only active agents that are able to achieve adequate bactericidal levels in the serum to treat serious blood stream infections caused by KPC-producing bacteria. Yet, there are certain risks associated with the use of polymyxins as it is associated with neurotoxicity and nephrotoxicity, the reason for their drop of use in the past. Use of polymyxins as monotherapy for the treatment of serious infections of KPCs is described in a small amount of retrospective data. During a Manhattan outbreak, caused by carbapenem-resistant *K pneumonia*, bloodstream infections of three isolates were treated with polymyxin B (susceptible to polymyxins), and only 1 survived (12). Polymyxins are usually used in blend with other antimicrobials, though there are no eventual data to assess the effectiveness of this method. The use of combination therapy may yield better results and could be helpful to put a stop to development of resistance in bacteria. In a study done by Lee *et al*, a total of 16 patients infected with KPC-producing *K pneumonia* were observed. Result showed that due to monotherapy with polymyxin 3/12 patients developed resistance to polymyxin during their treatment. Whereas when combination therapy (polymyxin tigecycline) was employed, none of the 4 cases established resistance to either of the antibiotic. Though there exists very little data of combination therapy outcomes for humans yet it is to be noted that combination therapy is still a better choice of treatment as compare to monotherapy as it gives rise to resistance, leaving clinicians with no option of treatment at all. The combination therapy still alone doesn’t provide efficient outcomes. In Greece, during KPC-2 outbreak, combination therapy was used to treat 88percent of the patients out of which failed cases were 22 percent.

A fresh glycylcycline, “Tigecycline” is frequently employed to treat infections caused by multidrug-resistant (MDR) Gram-negative bacteria including KPC-producing bacteria. Tigecycline has an outstanding in vitro activity (tested by the susceptibility breakpoint of a MIC <2 mg/L, Federal Drug Administration (FDA) approved) against KPC-producing bacteria. From year 2000-2005, around 73 KPC isolates from 7 diverse states and all 95 KPC isolates from Brooklyn hospitals in 2005 showed susceptibility to tigecycline, in vitro. Nonetheless, in the United Kingdom from year 2001-2006 there has been an increase in tigecycline resistance observed in *Klebsiella* species. There are few reports of tigecycline use outcomes in studies conducted on Multi-drug resistant Enterobacteriaceae infections treatment and even fewer have been devoted to infections caused by KPC-producing bacteria. In a study conducted by Kelesidis *et al* who reviewed ten studies in which there were only 33 infected patients with MDR-Enterobacteriaceae, it was concluded that only 70% of the cases showed favorable outcomes with tigecycline treatment. But, 49 percent of these were intra-abdominal infection cases, for which tigecycline has been permitted. When compared with other studies there were reports indicating need for high dose with longer period of administration to clear out the pathogen, recurrence of bacteria and delayed clearance of the organism. Tigecycline doesn’t achieve desirable concentrations in blood or urine, that’s why it is not recommended for treatment of such infections. There exists many reports that show development of tigecycline resistance during therapy so makes a concerning issue. In one New York City hospital, in 2009 (January -July), total of fourteen *K pneumonia* isolates were analyzed for sensitivity to tigecycline, they showed intermediate or resistant results. Six of the isolates were intermediate or resistant to polymyxin; 2/6 showed resistance to polymyxin, tigecycline and other antibiotics (140).

There is a growing concern of resistance to aminoglycoside amongst KPC-producing bacteria. However, there exists in vitro data showing quick bactericidal activity of gentamicin against gentamicin-susceptible strains of *K pneumonia*. Members of the efficacious ST258 clone frequently stay susceptible to Gentamicin. Nevertheless, lineages other than ST258 of KP strains might carry gentamicin-modifying enzymes thus other aminoglycosides such as tobramycin & amikacin have been known to be not very effective in treating infections caused by KPC-producing *K pneumonia* as compare to treatment of other forms of Multi-drug resistant *K pneumonia*. Aminoglycosides stand an important therapeutic choice for the treatment of KPC-producing bacteria only when their susceptibility is known. Still, there

exists reports of pan resistant that are resistant to all classes of antibiotics including tigecycline, polymyxin, & even aminoglycosides. Use of older antimicrobials such as nitrofurantoin & Fosfomycin have been deliberated for the treatment of non-invasive infections for example UTIs, however their clinical efficacy supporting data is missing.

2.10 Therapeutic options under development

Existing beta-lactamase inhibitors such as clavulanic acid might be helpful in restoring activity of beta lactams in vitro against KPC-producing bacteria, but MIC values of beta-lactam antibiotics can't be reduced by such additions of inhibitors so it is not advised for use. There is an ongoing processing of a novel beta-lactamase inhibitor called NXL104. It has a promising activity against the KPC enzyme but it is still in trial and developmental stages. Quite a few new synthetic derivatives of polymyxins are being manufactured for example NAB740 & NAB739 have been developed. There is an indication that they will be less nephrotoxic but will provide equal antimicrobial activity. There are two new members of existing antibacterial classes that are going through development stages, one is a novel tetracycline PTK-0796 and 2nd one is a novel aminoglycoside, ACHN-490 (8).

2.11 Carbapenemases

Carbapenemases fit in to molecular class B of Metallo-beta lactamases (VIM and IMP) or belong to class D Oxacillinase (OXA 23-7) plus also to class A clavulanic acid inhibitory enzymes (SME, GESS, IMI, and KPC) (145). There has been a growing concern regarding carbapenem resistance linked with the production carbapenem hydrolyzing enzymes called carbapenemases in Gram negative bacilli that is compromising treatment options and also increasing rate of nosocomial infections. Carbapenemases are encoded by many genes, such as serine carbapenemases; *bla*OXA-48 & *bla* NDM and Metallo--lactamases for example, *bla* NDM, *bla* IMP, & *bla* VIM. *Klebsiella pneumonia* carbapenemases (KPC) is the most commonly found in the United States, followed by the New Delhi Metallo-lactamase (NDM). These Genes are inserted on versatile plasmids that are spread efficiently in many organisms. There are numerous studies indicating ever growing resistance of *K pneumonia* towards carbapenems. In a latest study conducted by Moustafa *et al*. antimicrobial susceptibility test of 202 *K pneumonia* isolates were performed. Results showed that 26.2% (53) were multi-drug resistant plus showed resistance to imipenem and/or meropenem. Out of 53, 49 of the multi-drug resistant (MDR) isolates were resistant to

imipenem & meropenem whereas 4/53 MDR isolates exhibited resistance to meropenem only (149).

2.12 Prevalence of *bla* NDM and *bla* NDM-1

Various study portrays prevalence of *bla* NDM and *bla* NDM prevalence around the globe. In Italy, a study conducted by Mosca *et al* showed that all (100%) of the 38 *K pneumonia* isolates investigated were found to be positive for *bla* NDM gene. Another study by Metwally *et al* in Egypt who examined 20 *K pneumonia* isolates revealed that 14 of isolates showed positive results for *bla* NDM gene detection. In a latest study conducted by Moustafa *et al*, amongst the thirty-five isolates of K *pneumonia* which were EDTA disc synergy test- positive and Modified Hodge Test (MHT) -positive, 5 isolates (14.28 percent) gave positive results for *bla* NDM detected by PCR. In an early study done in Saudi Arabia, by Shibl *et al* , sixty *K pneumonia* isolates from diverse clinical specimens (Urine, blood, sputum, & wounds) were investigated for *bla* NDM gene detection but only 12 (20percent) isolates came positive (152). A study conducted by Chaudhary & Payasi in India revealed that of 150 *K pneumonia* isolates examined for *bla* NDM only twenty four isolates (16%) came positive for *bla* NDM gene detection. In one more study made in India, Bhaskar *et al* explored fifty nine blood isolates of *Klebsiella*, results revealed that 67.8% (40 isolates) were positive *bla* NDM detection.

In the study by Moustafa *et al*, 35 carbapenems resistant isolates which were positive for EDTA disc synergy and Modified Hodge tests were further put-on test for the detection of *bla* NDM and *bla* NDM genes using the PCR technique. Results showed that 10/35 of the isolates (28.57percent) displayed the presence of *bla* NDM gene at 150 bp and 5/35 of the isolates (14.28percent) showed the presence of *bla* NDM gene at 621 bp (149).

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1 Design of Study

The research was performed in the MICROBIOLOGY LABORATORY of PAKISTAN INSTITUTE OF MEDICAL SCIENCES (PIMS) in ISLAMABAD. A total of 194 samples for the study was executed from different clinical specimens of hospitalized patients from various hospital departments these include patients from OPD, ICU, CCU, Emergency and different general wards (Pulmonology, oncology, neurology, gastroenterology, cardiology, general wards of male and female etc.). The study protocol was accepted by the PIMS Research Assessment Committee. *Klebsiella pneumonia* organisms obtained from separate clinical specimens were used for testing and repeated isolates were removed from the same clinical specimen of the same patient.

3.2. Specimens Collection

In the microbiology laboratory, 194 clinical specimens of *Klebsiella pneumonia were* collected between December 2020 and March 2021. The isolated strains were kept in storage tubes for bacteria until they were used at -80°C.

3.3 Sample Processing

194 Samples collected from PIMS were cultured on Blood Agar and MacConkey agar plates, incubated for 24-48 hours at 37°C. After proper incubation, the colony color and different characteristics were observed using standard microbiological methods. Different characteristics included colony color, size, Gram staining and biochemical testing.

3.4 Colony Morphology

Culture characteristics of *K pneumonia* isolates were considers as, Color (Lactose fermenting), Consistency (Mucoid), Size (Moderate, Large size), Form (Circular, Irregular), Elevation (Dome shaped, convex, spreader) and, Margins (Curled, lobated, entire).

3.5 Gram Staining

Clean grease free slides were taken and a thin smear was prepared for gram staining. A drop of distilled water was placed to on slide in order to make a thin smear. The bacterial colony were picked from the isolated colony and thoroughly emulsified in the drop for making smear. Then it was air dried. Heat fixing is done on the spirit lamp. It was then flooded with crystal violet for about one minute and then rinsed with distilled water. Lugols iodine were applied for a minute then washed with tap water. It worked as a mordent. After complete washing decolorizer was applied for few seconds. Then the slide was washed with distilled water and then safranin was then poured for one minute. Safranin was used as a counter stain. This was then washed off with distilled water and the smear was allowed to dry in air. The slides were examined with oil immersion for identification of bacteria.

3.6 Quality Control

Sometime known gram positive and gram-negative bacteria were stained to compare with test organisms.

3.7 Biochemical Tests

Different biochemical tests were done to identify the organisms. Known control were performed with each biochemical test.

3.8 Turbidity Standard Solution (0.5 McFarland Standards)

In order to prepare 1% v/v solution of H_2SO_4 (sulphuric acid), 1 ml of concentrated H_2SO_4 was added to 99 ml of water. Exactly 2.35 g of dehydrated barium chloride was dissolved in 200 ml of distilled water to make 1.175% w/v solution of barium chloride (BaCl2.2H2O).0.5 McFarland (0.5% turbid) standard was set by mixing 0.5ml of barium chloride solution to 99.5ml of sulphuric acid solution with continuous stirring. This standard solution was then stored at room temperature in the dark.

3.9 Antibiotic Sensitivity Test:

3.9.1 Preparation of inoculum

A sterile loop of inoculum was used to pick 3 to 4 isolated, identical colonies of test microorganism from plates. These colonies were transferred to a sterile tube containing 2-3ml

of normal saline. Inoculum density was prepared with comparison to 0.5 McFarland turbidity standard. Suspension was used within 15 minutes after preparation.

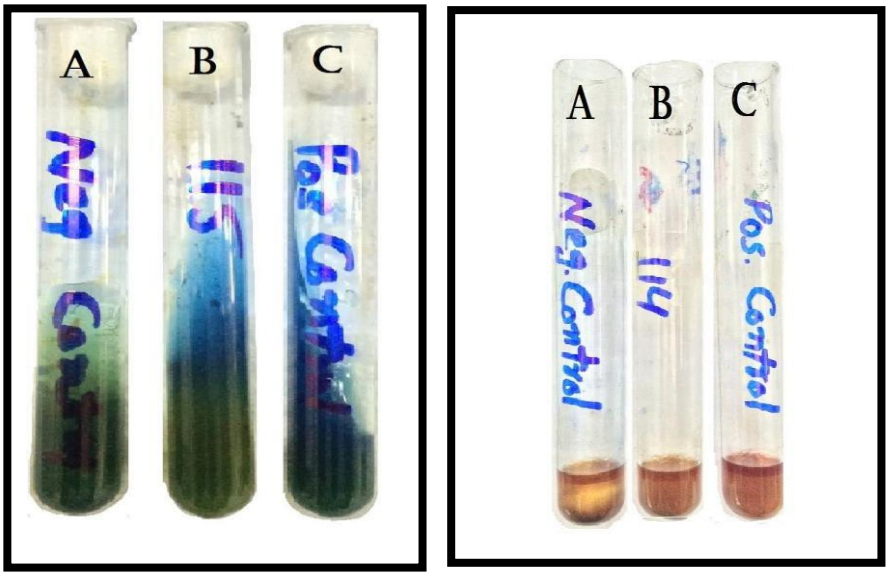


Figure 4.6: 1: Citrate test (Left) – Indole Test (Right) (A) Negative Control, (B) Test sample, (C) Positive Control

3.9.2 Inoculation of plates

Cotton swabs were sterilized for inoculation on media. These sterile swabs were dipped in the inoculum suspension and then swabbed over the media plate to yield a lawn culture. Swab was pressed and rotated with the wall of tube to avoid the extra liquid. Surface of MH agar was inoculated with swabs 4 to 5 times to make sure the proper spread of liquid. Plate was rotated by 60 degrees each time to ensure the proper distribution of inoculum on plate.

Figure No. 4.5 *K pneumonia* colonies on MacConkey agar

3.9.3 Application of discs

A pair of sterile forceps was used to carefully remove the discs from its vial. Lid of the petri plate was lifted from one side carefully and discs were placed gently on the agar plate one by one, pressing slightly to ensure thorough contact with agar surface. After application of the discs, plate lids were closed and plates were placed in an incubator in an inverted position at 36-37 degree Celsius for 24 hours.

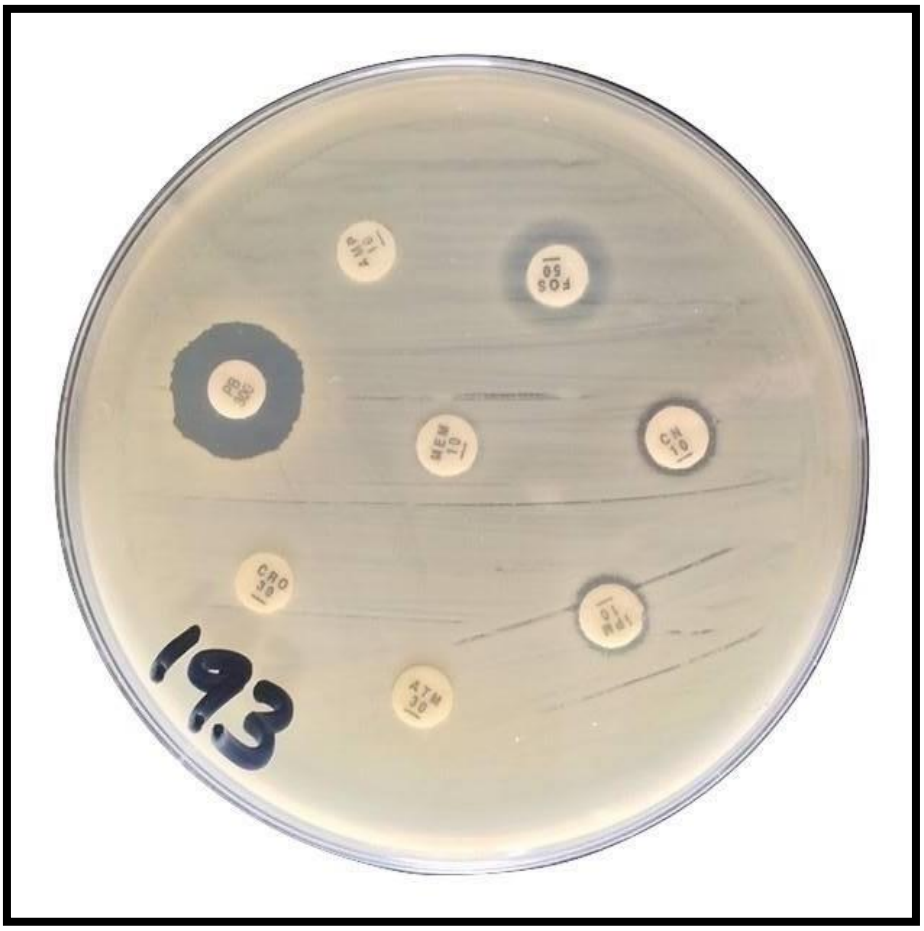


Figure No. 4.7: Antibiotic susceptibility profile of *K pneumonia* isolated from clinical samples.

3.9.4 Reading and interpretation of results

After incubation, diameter of zone of inhibition was measured to the nearest millimeter using a ruler, including the diameter of the disc. Measurements of the zones were recorded by viewing the back side of petri dish. Sensitive, resistant and intermediate sensitive results were recorded for all the sensitivity plates.

Table No.3.1 Panel of antibiotics used for *K pneumonia*

S. No	**Antimicrobial Agent**	**Class of antibiotics**	**Disc potency in (ug)**
1	Imipenem	Carbapenems	10
2	Meropenem	Carbapenems	10
3	Gentamicin	Aminoglycosides	10
4	Ciprofloxacin	Fluoroquinolone	5
5	Aztreonam	Monobactam	30
6	Ampicillin	Penicillin	10
7	Ceftriaxone	Cephalosporin	30
8	Polymyxin B	Glycopeptides	300
9	Fosfomycin	Phosphonic acid derivative	50

3.10 Quality Control Organism:

Following organisms were used as a control stains in the project:

Escherichia coli ATCC 25922

3.11 Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ±standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. All statistical analysis was conducted using SPSS version 25.00 statistical packages (SPSS Inc. Chicago, IL, USA). Significance level was accepted to be 0.05.

CHAPTER FOUR

4.0 RESULTS

Total number of 194 clinical samples were collected between the December 2020 and March 2021. Out of these 100 patients were *K pneumonia* positive.

Table No. 4.1 Total number of clinical samples collected on both centers

Total clinical samples collected from patients	194
K. pneumonia positive patients	100

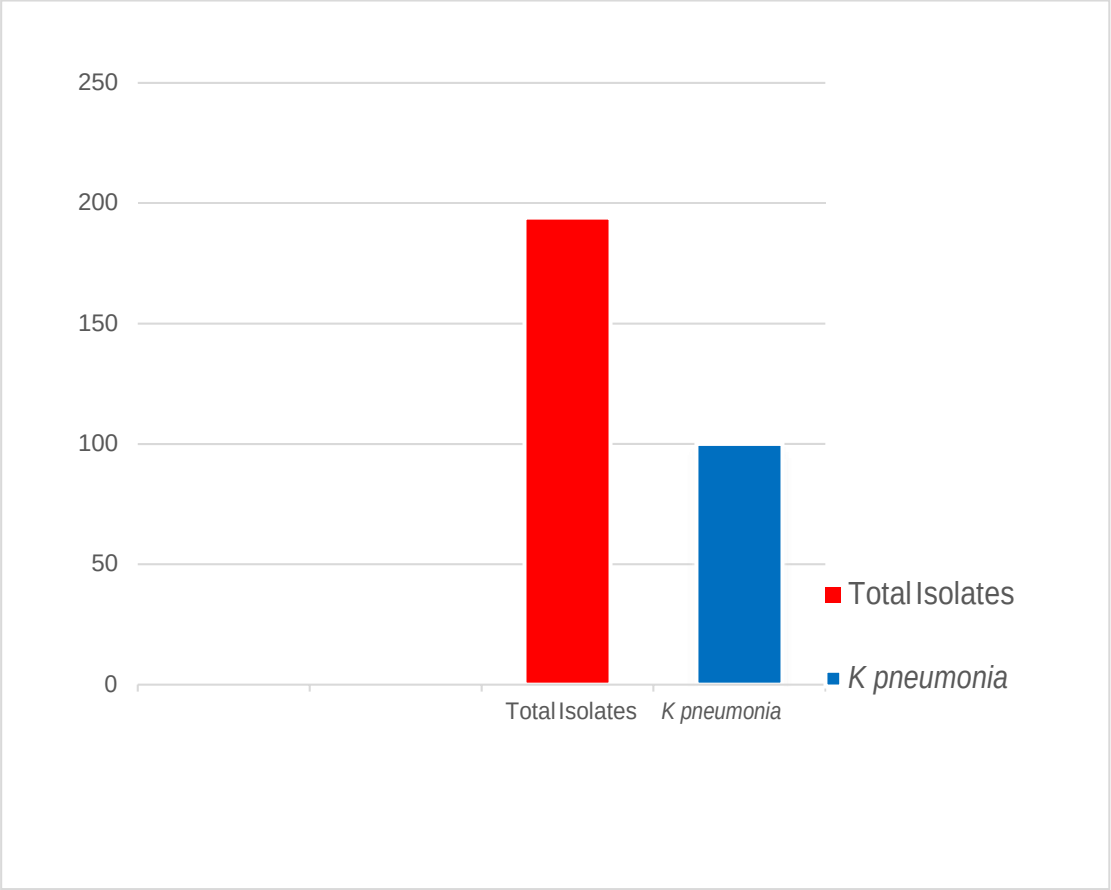


Figure 4.1: Number of *K pneumonia* isolated from different clinical samples

4.1 Gender distribution

Distribution of K pneumonia is dominant in male patients than female. These are 59% in Males while 41% covers the Females.

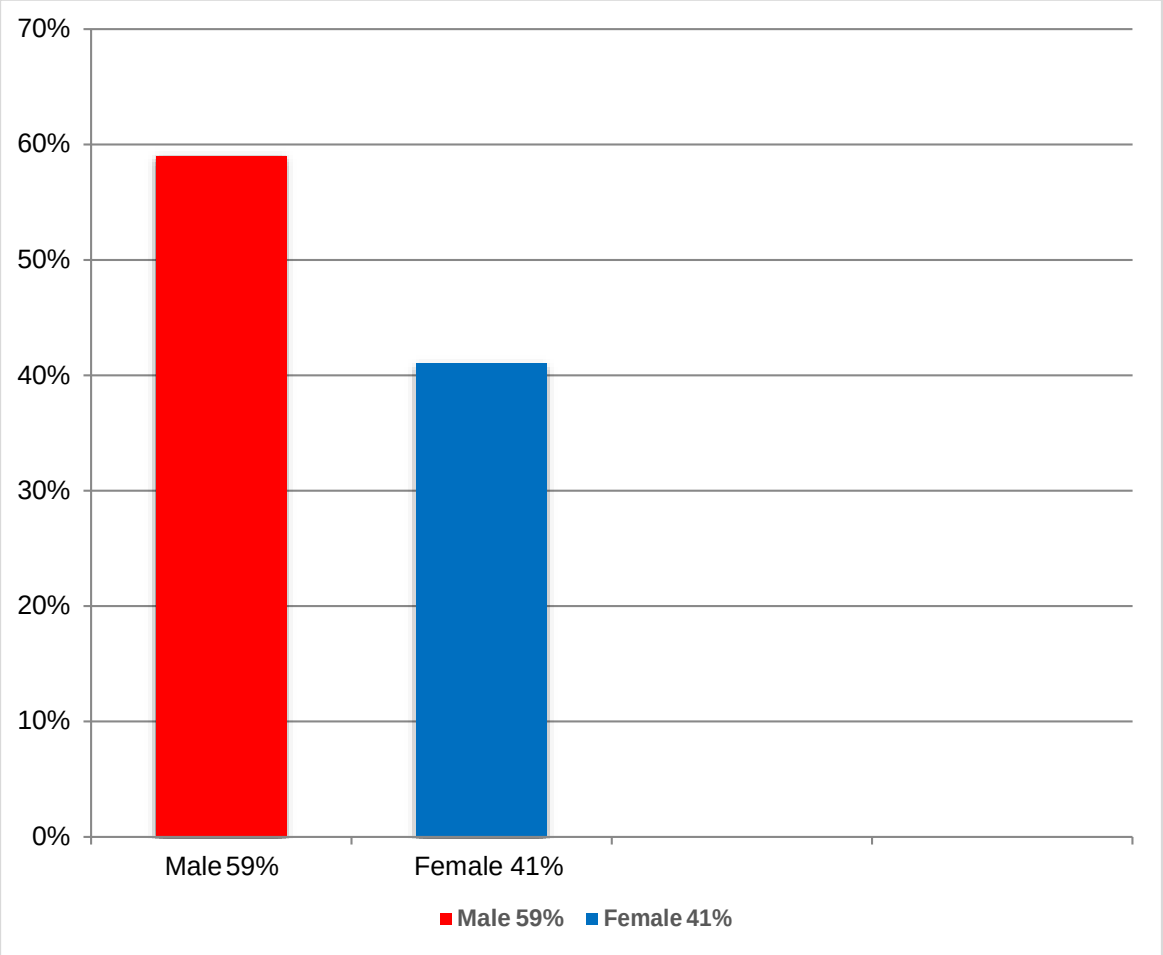


Figure No. 4.2: Gender based discrimination of *K pneumonia*e

4.2 Age Distribution

K pneumonia age wise distributions are found dominant in the ages between 40 years to 50. Total number of *K pneumonia* distribution in different age of patient are given below.

Table no. 4.2 Age wise distribution of *K pneumonia* patients

Ages (Years)	**No. of patients/100**	**Percentages %**
01 to 20	04	4
21 to 30	20	20
31 to 40	19	19
41 to 50	22	22
51 to 60	16	16
61 to 70	15	15
71 to 80	02	02
81 to 90	02	02

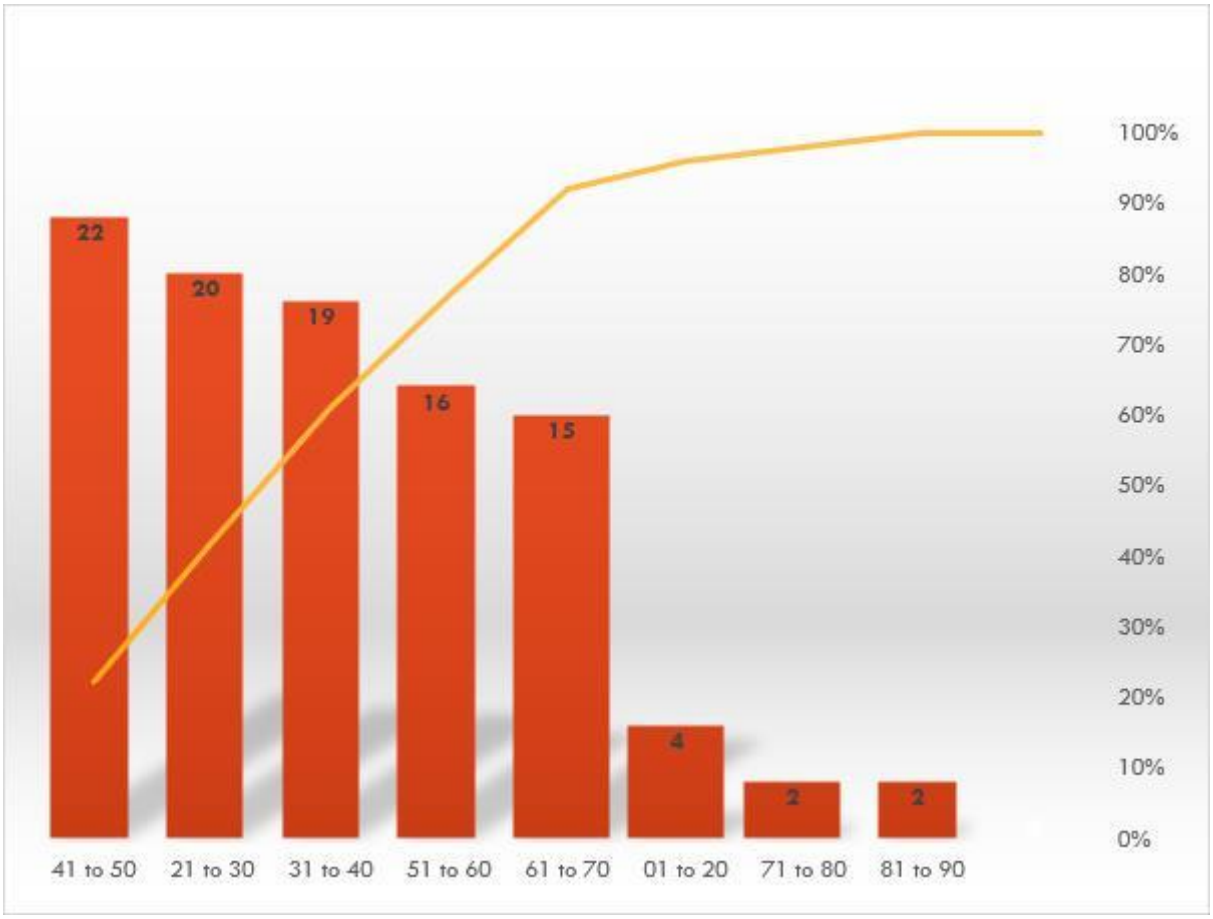


Figure No 4.3: Age distribution of *K pneumonia* in patients.

4.3 Types of specimens:

Clinical specimens of patients from different units of hospitals are collected aseptically. These include patients from OPD, ICU, CCU, Emergency and different general wards (Pulmonology, oncology, neurology, gastroenterology, cardiology, general wards of male and female etc.)

Table No. 4.3: Specimen wise distribution of different patients.

Specimen	Number
Urine	40
Pus	18
Blood	28
Tracheal	08
Miscellaneous (Nasal, Tissue, ETT tips)	06
Total	100

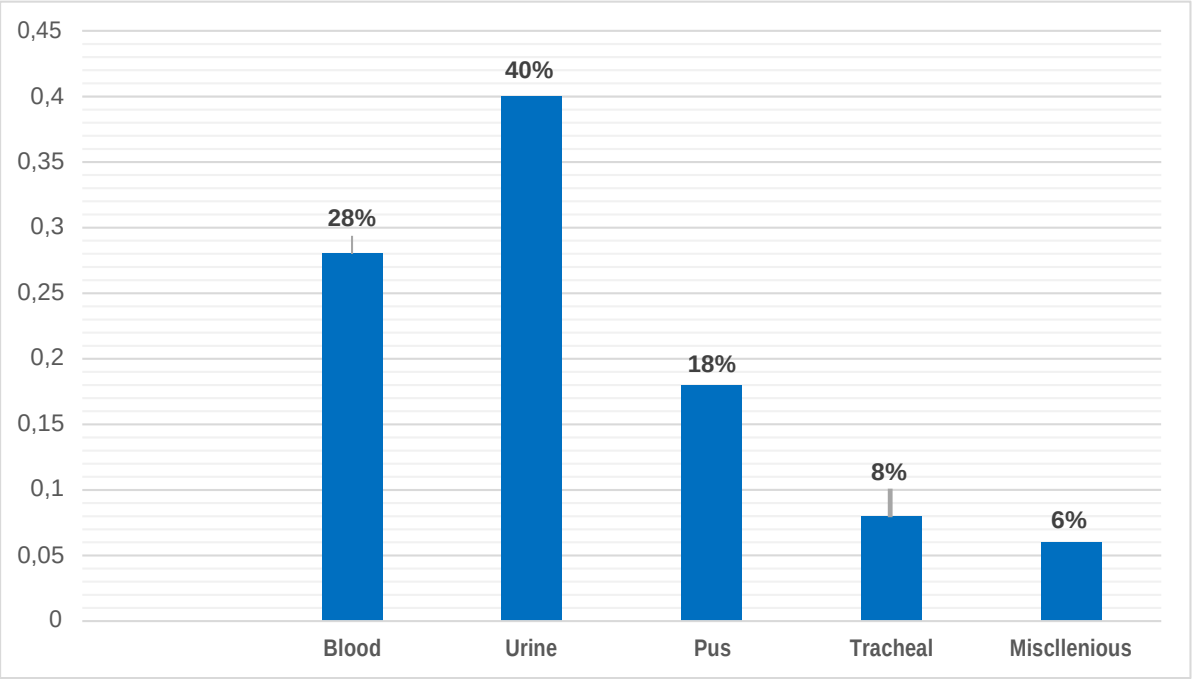


Figure 4.4: Specimen wise distribution among different patients.

4.4 Identification of *K pneumonia*

K pneumonia appear as a pink mucoid color large colony on MacConkey agar. In the result of Gram staining, it appears as rods shape. Different biochemical tests were performed for the *K pneumonia*, these include Citrate test and Indole test. Indole is performed to differentiate from *E coli.* As Indole is positive for *E coli* while negative for *K pneumonia*. On the basis of these two biochemical tests *K pneumonia* were identified. Indole is negative for *K pneumonia* while citrate is positive.

Table No. 4.4: Identification Index for *K pneumonia*.

S. No.	Testes	Results
1	Gram Staining	Single pink cooler rods, Gram Negative
2	Motile	Non-Motile
3	Colony morphology on MacConkey agar	Lactose Fermenting, Mucoid domed shape
4	Indole Test	Negative
5	Citrate Test	Positive

4.5 Antibiotic sensitivity testing (Double disc diffusion testing)

Kirby Bauer testing were used for the sensitivity of *K pneumonia* isolates. Different classes of antimicrobials were tested against KP. These include seven classes of different groups.

Antibiotics	***K pneumonia***					
	Sensitivity	**Percentage**	**Intermediate**	**Percentage**	**Resistant**	**Percentage**
Ampicillin	**6**	**6%**	**0**	**0**	**94**	**94%**
Levofloxacin	**42**	**42%**	**2**	**2%**	**56**	**56%**
Gentamycin	**32**	**32%**	**3**	**3%**	**65**	**65%**
Fosfomycin	**69**	**69%**	**0**	**0**	**31**	**31%**
Polymyxin B	**71**	**71%**	**0**	**0**	**29**	**29%**
Imipenem	**68**	**68%**	**0**	**0**	**32**	**32%**
Meropenem	**68**	**68%**	**0**	**0**	**32**	**32%**
Ceftriaxone	**20**	**20%**	**2**	**2%**	**78**	**78%**
Aztreonam	**36**	**36%**	**3**	**3%**	**61**	**61%**

Table No. 4.5: Antibiotic susceptibility results of *K. pneumonia*.

Antibiotics	Resistant %	CRB+	CRB-
Ampicillin	**94%**	**32%**	**62%**
Ceftriaxone	**78%**	**32%**	**46%**
Aztreonam	**61%**	**32%**	**29%**

Table No. 4.6: Antibiotic Resistance comparison between carbapenemase producing *K pneumonia* and carbapenemase Non-producing *K pneumonia.*

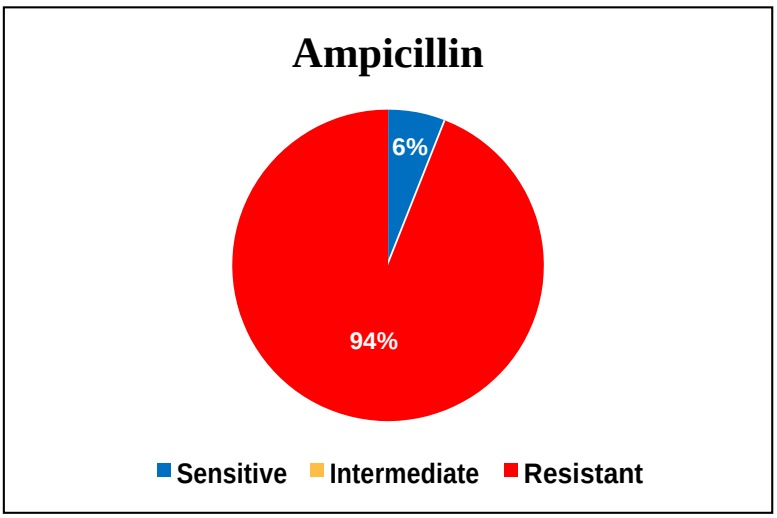
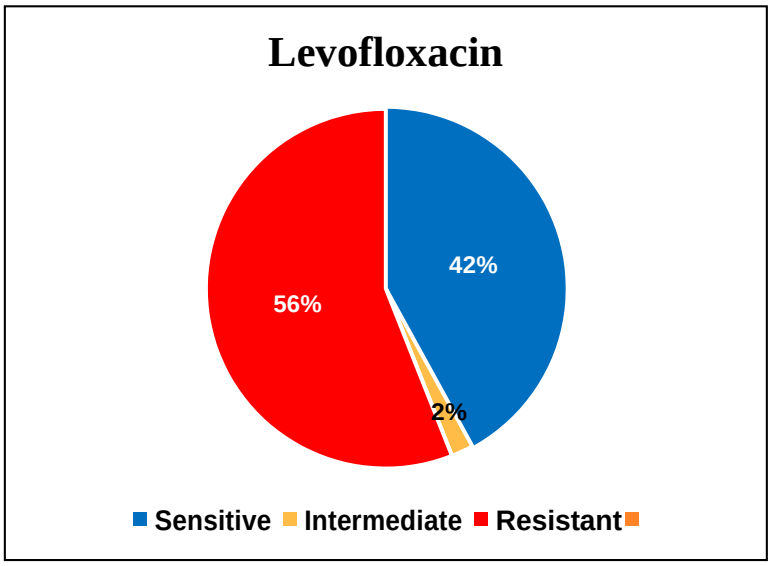


Figure No. 4.8.1



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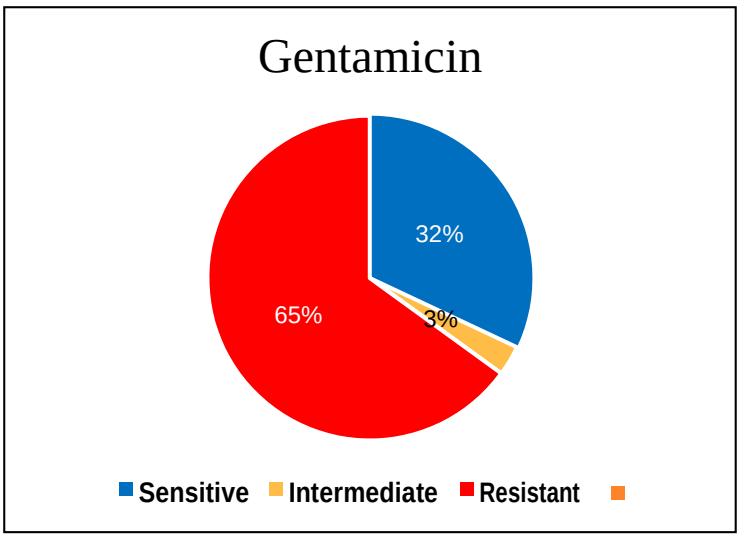

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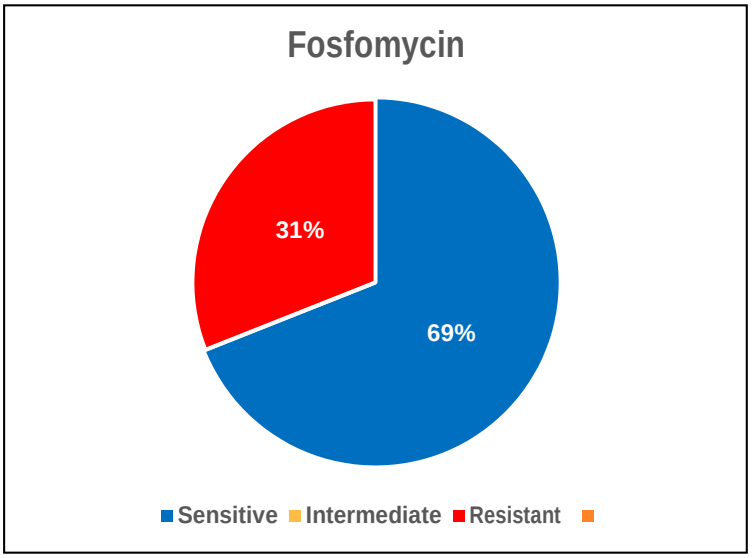

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Figure No. 4.8.5

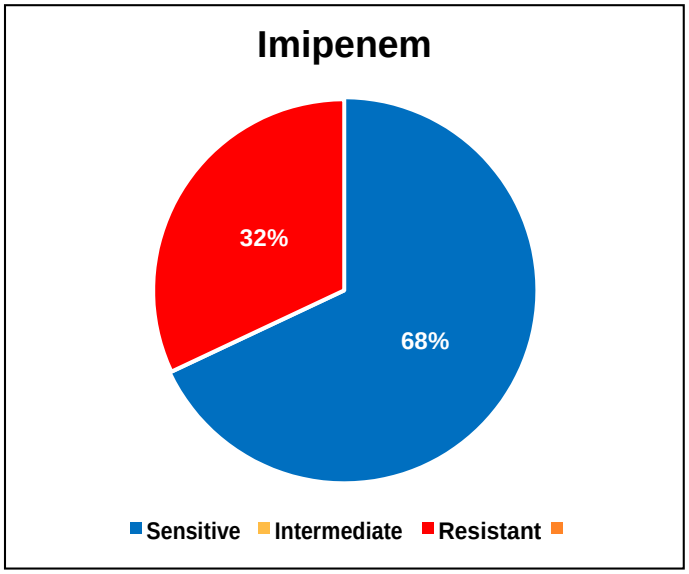

Figure No. 4.8.6

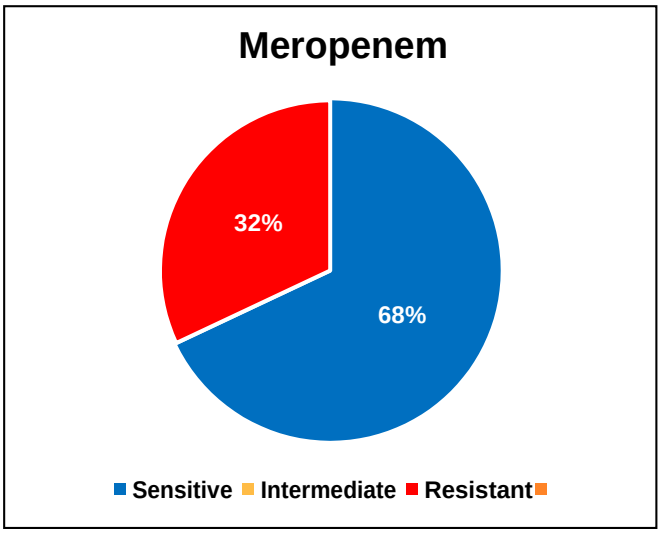

Figure No. 4.8.7

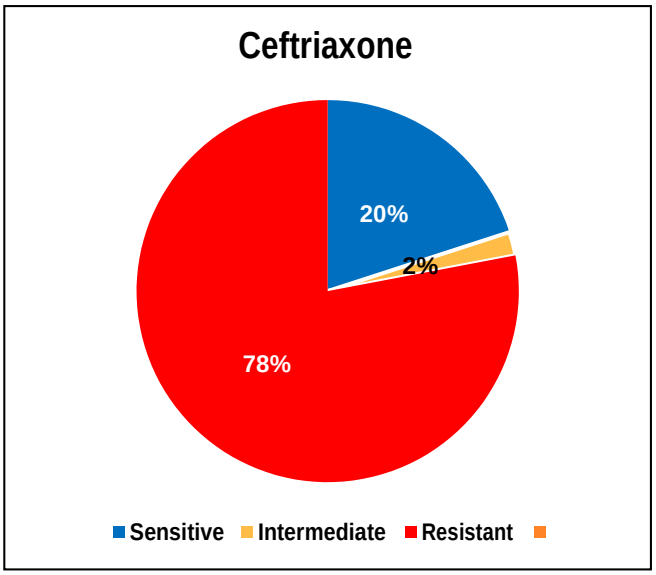

Figure No. 4.8.8

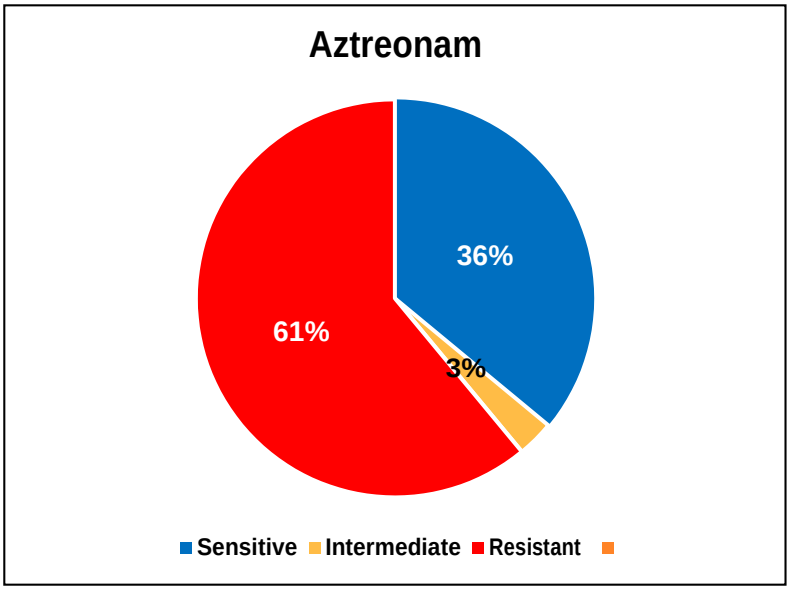

Figure No. 4.8.9

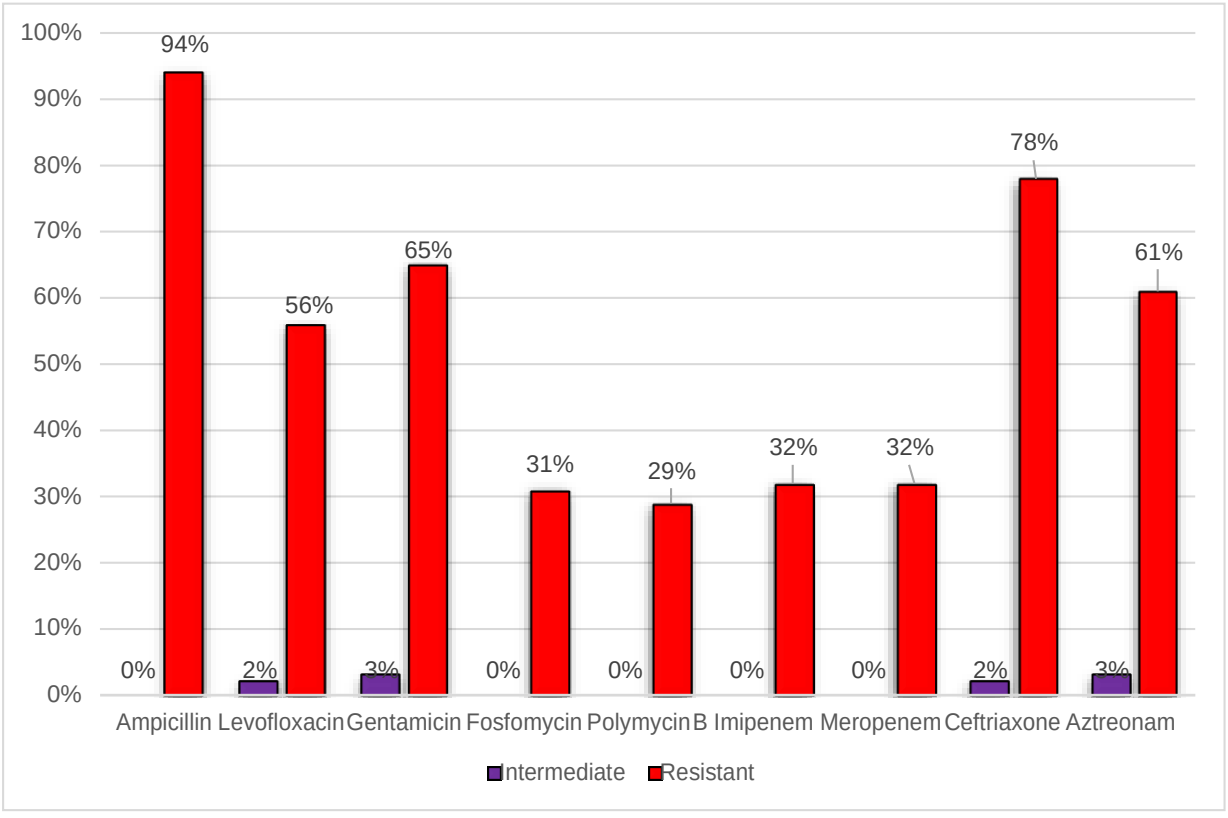

Figure no. 4.9: Over all antibiotic testing results.

CHAPTER FIVE

5.0 DISCUSSION

K pneumonia is one of the major etiological agents involved in hospital associated infections. The major infections caused by *K pneumonia* are pneumonia, UTI, septicemia and other tracheal infections. The most frequently recommended drugs against these infections are levofloxacin, gentamicin, aztreonam and ceftriaxone. However, in past decade there has been an alarming increase of resistance against these drugs particularly that of carbapenem class of drugs. Carbapenem resistant isolates have been found to carry mediated resistance enzymes that help in avid dissemination of resistance among isolates of either same species or different species. In the present study presence of two most prominent enzymes of KPC and NDM involved in carbapenem resistance was determined. This study is in line with a study conducted in England, where *K pneumonia* was detected in 89 out of 139 patients while our study reports presence of 100 *K pneumonia* isolates out of 200 samples. Similarly in our study the presence of NDM1 enzymes was found to be in positive 62% of isolates while in this study conducted by Jacqueline Findlay *et al* reports 61% of isolates carrying NDM1 enzymes. In one more study conducted in India, Bhaskar *et al* explored 59 blood isolates of *Klebsiella* confirms that 67.8% (40 isolates) were positive for NDM detection. Thus, so far now presence of NDM1 enzymes in our study is in accordance with previously reported data. However, in a study conducted in Saudi Arabia, only 20% of the isolates were found to carry NDM enzymes was detected, which is not in accordance with our findings (Sible *et al*). This difference in the prevalence of NDM enzyme can be due to limited use of carbapenems in Saudi Arabia. Study conducted by Manikandan *et al*. revealed resistance of 14% to carbapenems, 24.6% to fluoroquinolones while 48% resistance was observed against gentamicin. This study does not support our susceptibility pattern as in our study meropenem is 32%, levofloxacin is 56% and gentamicin is showing 65% resistance profile. The difference from current study is due to the fact that, the sample collection of the earlier study involves both hospital and community side whereas the present study achieves sample collection from hospital setup of two cities only. A strong indication of increased resistance in our study is may be due to the adverse use carbapenem antibiotics in the hospitals.

Discussing resistance pattern of *K pneumonia* in our study, most of the NDM1 positive isolates were imipenem, meropenem, ceftriaxone, ampicillin and aztreonam resistant, similar findings were reported by Jamal *et al*. (158) in their study reporting NDM1 positive isolates were found resistant to meropenem, imipenem, ampicillin, aztreonam and third generation cephalosporin. A study executed in US by Arnold *et al* (134), regarding carbapenem resistance inflation in healthcare centers, it was reported that 38% of the *K pneumonia* are resistant to carbapenem which is in agreement with our findings where we have 32% of isolates as carbapenem resistant. However, in a study by Bina *et al*, carbapenem resistant *K pneumonia* were only 15% of the total isolates, which is against the findings in our study. While it was reported in his study that highest resistance in the antibiotic profile was against gentamicin and cefepime which shows somewhat similarity to the results of the current study. If we talk about gender distribution in our isolates, it was found that 59% of the isolates were from females while rest of 41% were from males, which is in accordance with the findings of Bina *et al*, where the ratio was 57% and 43% among females and males respectively.

The present research scrutinized carbapenem resistant *K pneumonia* in the area to inspect the current trend of carbapenem resistance in *K pneumonia* within hospital acquired environments. Carbapenems resistance strains are widely disseminated across the globe. The rate of resistance is arising in Asia, Europe and in the Middle East as well. In this research many parameters are correlated to other studies. In Asia there is high prevalence of KPC and NDM. *K pneumonia* is responsible for many complications which includes, 7-14% for pneumonia, 6 - 17% for UTI, 2 - 4% for wound infections, 4 - 15% for septicemia, 4 -17% for nosocomial infections in intensive care units, and lastly 3-20 % of all neonatal septicemia. Spread of *K pneumonia*, carrying resistance to carbapenem drugs are increasing with time as route of transmission have a significant role in the production and spread of resistance. In a hospital environment, blood transmission is an important factor whereas when both community and hospital setups are apprehended, transmission through aerosols may also give rise to increase carbapenem- resistant *K pneumonia*.

In our study the number of carbapenemase producing *K pneumonia* was found to be 32% but the number of carbapenemase Non-producing *K. pneumonia* from Ampicillin 62% and for Aztreonam was 29% while the ceftriaxone was 46%.

CONCLUSION

In this study, it is concluded that clinical isolates of *K pneumonia* are increasingly getting resistant to carbapenems. One of the important causes of resistance is the production of carbapenemases enzyme.

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