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Study of Multi Drug Resistance of *Klebsiella Pneumoniae* Isolated from Hospitalized Patients in Hilla City

A Thesis

Submitted to the Council of College of Medicine-University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Master in Pharmacology / Pharmacology and Toxicology

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﴿ وإذ تأذن ربكم لئن شكرتم لأزيدنكم ولئن كفرتم إن عذابي لشديد﴾

صدق الله العلي العظيم

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Dedication

This thesis is dedicated to **my beloved parents** , who has been a constant source of support and encouragement during the challenges of graduate and life.

I am truly thankful for having them in my life. This thesis is also dedicated to **my lovely wife** , who had always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.

Who supply me: **my brothers**

Finally I dedicate it to **my son**, **Ali** whom God has blessed me with this year and who has made life more beautiful.

I dedicated this work.....

Mustafa Ewad

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I would like to express my thanks to " **Allah**" the Most Gracious and Most Merciful, and to His Prophet **" Mohammad**"

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SUPERVISION CERTIFICATION

We certify that this thesis was prepared under our supervision**(Study of Multi Drug Resistance of Klebsiella Pneumoniae Isolated from Hospitalized Patients in Hilla City)** at the College of Medicine, University of Babylon as partial fulfillment of the requirements for the Degree of Master in Pharmacology and Toxicology.

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We certify that we have read this thesis entitled **"Study of Multi Drug Resistance of Klebsiella Pneumoniae Isolated from Hospitalized Patients in Hilla City"** Mostafa Mohammed Ghany in its contents and that in our opinion it meets the standard of a thesis for degree of Master in pharmacology and Toxicology.

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Summary

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections . β-lactam antibiotics are antibiotics that contain a beta-lactam ring in their chemical structure. This includes penicillin derivatives , cephalosporins , monobactams and carbapenems.

Klebsiella species are ubiquitously found in nature including water, soil and animals, and they can colonise medical devices and the healthcare environment . *Klebsiella* species are considered opportunistic pathogens colonising mucosal surfaces without causing pathology; however, from mucosae *Klebsiella* may disseminate to other tissues causing life-threatening infections including pneumonia, UTIs, bloodstream infections and sepsis . *K. pneumoniae* infections are particularly a problem among neonates, elderly and immunocompromised individuals within the healthcare setting.

The study aimed was to determine the patterns of antibiotic resistance in *K. pneumoniae* and the most effective antibiotic for treating *K.pneumoniae* infections.

This is cross sectional study by collecting clinical samples (155) specimens from patients of both sexes when obtained at Hilla city's four largest hospitals between September 2021 and December 2022. Only 32 isolates, or 20.6%, were confirmed to be *Klebsiella pneumoniae* based on cultural features and biochemical assays.7(22%) sputum, 9(28.1%) urine, 5(12.6%) stool, 3(9.3%) blood, 5(12.6%) wound, and 3% CSF. The(155) specimens according to gender revealed 63 specimens (40.6 %) for males and 92 (59.3 %) for other gender . Participating patients' ages ranged from one to seventy. The age of patients was ranged from 4-

70 years (mean, 39.86 ± SD 15.7). Based on age, the patients were separated into four distinct cohorts. Female was more than male in general; in urine was 5, blood 3, sputum 5, only in stool, male was more than female.

The antibiotic susceptibility tests of the bacterial isolates against 13 type antibiotics , using disc diffusion method according to CLSI(2022). Results showed that the percentages of the resistant toward these antibiotics were as follow: augmentin (81%), aztreonam(88%), ceftazidim (75%),cefixime (75%), cephalotin (84%), ceftriaxone (72%),cefotaxime(72 %), doripenem (50%),ertapenem (56%), imipenem(47%), meropenem(56%),piper./tazo.(78%), penicillin (100%). In the current investigation, all of the isolates were found to be Pencillin-resistant, and 9(28.1%) were found to be resistance to all antibiotics. This study also showed that most of *K. pneumoniae* were 100 % multi-drug resistant .Carbapenemase gene was positive in 87% (28/32) of the *K. pneumoniae*, **including blaNDM** in 15(47%) was identified and absent in 17(53%) isolates. The highest resistance rate to most antibiotics was observed in the isolates carrying blaKPC then blaNDM gene, the isolates that have blaNDM, blaKPC, and blaOXA-48 genes was resistance to all antibiotiacs (100%) , the isolates that have blaNDM, and blaKPC genes were resistance to 6 antibiotiacs (100%) , the isolates that have blaNDM, and blaOXA-48 genes was resistance to 3 antibiotiacs (100%) , the isolates that have blaKPC, and blaOXA-48 genes was resistance to 10 antibiotiacs (100%) , the isolates that haven't any genes was resistance only to penicillin(100%). **Regarding blaKPC** gene it was identified in 12 isolates of *K.pneumoniae* (37.5%) and absent in 20 (62.5%) isolates. **While blaOXA-48** gene was identified in 19 isolates of *K. pneumoniae* (59%) and absent in 13(41%) isolates. The highest percentage of blaKPC was found in Sputum 4(12.5%) followed by urine 3(9.3%),CSF 2(6.2%), blood, wound 1(3.1%) ,stool (0%), The highest percentage of blaOXA-48 gene was found in urine 9(28.1%) followed by wound , sputum 3 (9.3%),CSF 2(6.2%), blood and stool 1(3.1%), the highest percentage of blaNDM

was found in urine 5(15.6%) followed by Sputum 3(9.3%), blood, stool , CSF 2 (6.2%), wound 1(3.1%).

In conclusions , the result show all carbapenems antibiotic are the most effective against *Klebsiella pneumonia* in Beta-lactam while the resistance to pencilline was 100% . Bacterial isolates that contain blaNDM, blaKPC, and blaOXA-48 were resistance to all antibiotics .

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List of abbreviations

Abbreviation	Meaning
AST	Antibiotic susceptibility test
BSIs	Bloodstream infections
CRE	Carbapenem-resistant Enterobacteriaceae
CSF	Cerebrospinal fluid
CIs	Chromosomal integrons
DNA	Deoxyribonucleic acid
DAP	Diaminopimelic acid
DDT	Disk diffusion test
EDTA	Ethylenediaminetetraacetic
ESBL-EC	Extended-spectrum beta-lactamase-
KPC	*Klebsiella pneumoniae* carbaenamase
MBL	Metallo-beta-lactamase
MIC	Minimal inhibitory concentration
MIs	Mobile integrons
MHA	Muller Hinton agar
MDR	Multiple drug resistance
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
blaNDM	New Delhi metallo-β-lactamase
OXA	Oxacillinase
PBP	Penicillin binding proteins
PDR	Pandemic Drug Resistance
PCR	Polymerase chain reaction

RNA	Ribonucleic acid
SHV	Sulf-hydryl variable active site
SIs	Super-integrons
UTI	Urinary tract infections
WHO	World Health Organization
XDR	Extensive drug Resistance

1.1 Introduction

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections .It may either kill or inhibit the growth of bacteria. β-lactam antibiotics are antibiotics that contain a beta-lactam ring in their chemical structure. This includes penicillin derivatives , cephalosporins , monobactams and carbapenems. (Džidic *et al.*, 2008)

Resistance of antibiotic is closely associated with the use of antimicrobial agents in clinical practice. Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt gradually and develop resistance to antibiotics. When an antibiotic attacks bacteria, bacterial cells susceptible to it will die, but those that have some insensitivity will survive (Džidic *etal.*, 2008).

The genus Klebsiella is Gram-negative, non-motile, non sporulating, lactose-fermenting, oxidase-negative, and having a conspicuous polysaccharide capsule, the genus *Klebsiella* is also characterized by its lack of spore formation. *Klebsiella* are ubiquitous in the natural world and can be found colonizing the skin, throat, gastrointestinal (GI) tract, sterile wound, urine, and can in certain cases be considered "typical flora" in the colon, intestines, and biliary system of humans (Martin and Bachman., 2018). Human mucosal surfaces, such as the GI tract and oropharynx, can be colonized by *K. pneumoniae* with seemingly no ill effects. Strains of *K. pneumoniae* can spread to different parts of the human body via these

entry points (Paczosa and Mecsas, 2016). Some examples of virulence factors include biofilm development, hypermucoviscosity, capsule synthesis, adhesions, iron uptake, and lipopolysaccharides production; real virulence genes; virulence-associated genes; and virulence lifestyle genes (Mirzaie and Ranjbar., 2021). Gram-negative bacteria are resistant to Beta-lactam antibiotics because of the hydrolytic action of Beta-lactamases. It was previously considered that *Klebsiella* spp. isolates resistant to Beta-lactam antibiotics had high levels of extended-spectrum beta-lactamase (ESBL) (AL-Muhanna., 2013). These enzymes originate from broader-spectrum Beta-lactamases like TEM-1, TEM-2, and SHV-1, and are mostly transmitted through plasmids (Jyoti *et al*.,2010). Among them, *Klebsiella pneumoniae* has gained notoriety for its propensity to accumulate and spread of extended-spectrum Beta-lactamase (ESBL). The results of a study (Shaikh *et al*., 2015). It is well-known that *K. pneumoniae* can acquire and spread drug-resistance determinants like extended-spectrum Beta-lactamase . Because of their rising resistance to numerous medicines, which may be produced by exceedingly complex mechanisms, ESBL-producing *K. pneumoniae* infections are notoriously difficult to treat (Shaikh *et al*.,2015).

Aim of the study

This research aimed to identify *K. pneumoniae* antibiotic resistance trends. This research needs to find the best antibiotic for treating *K. pneumoniae* infections.

Study Objectives :

1. Collection of different clinical specimens (urine, sputum, wound, stool, blood, and CSF) from hospitalized patients at Hilla hospitals.
2. Cultivation of clinical materials on selective media for isolation of bacteria in pure culture, then identification of *K.pneumoniae* using traditional biochemical tests
3. Screening test for antibiotic resistance by antibiotic susceptibility test(AST) using disk diffusion test (DDT) against beta-lactams and carbapenam agents.
4. Detection of ESBL using β-lactam antibiotic susceptibility test.
5. Detection of carbapenamase (KPC) by CHROM agar Technique.
6. Detection of KPC genes by molecular technique (using PCR) for Antibiotic resistance by β-lactamases(KPC).

1.2. Literatures review

1.2.1. Antibiotic

It is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections .They may either kill or inhibit the growth of bacteria. β-lactam antibiotics are antibiotics that contain a beta-lactam ring in their chemical structure. This includes penicillin derivatives , cephalosporins , monobactams and carbapenems. (Džidic *etal.*, 2008)

The emergence of a phenotype resistant to antimicrobial agents depends on various factors of a host: degree of resistance expression, capability of a microorganism to tolerate resistance mechanism, initial colonization site, and other factors. When resistance determinants are on plasmids, they will spread quickly within the genus and even unrelated bacterial genera. When resistance is associated with genes on chromosomes, resistant microorganisms will spread more slowly (Džidic *etal.*, 2008). An important cause of the spread of antimicrobial resistance is a failure to apply infection control measures in a hospital and outside it. It has been established that methicillin-resistant Staphylococcus aureus (MRSA) in a hospital and MRSA in the community are often genetically related. Resistant bacteria are transmitted by aerosol transmission, especially during periods of viral upper respiratory infections, frequent hand-nose contacts, and poor hand washing among health care workers (Rice *etal.*, 2003). Antibiotic

use in nonhuman niches is another important reason for the spread of resistant bacteria (Martínez and Baquero ., 2002).

1.2.2. Multi Drug Resistance

Multi drug resistance (MDR) is antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories. Antimicrobial categories are classifications of antimicrobial agents based on their mode of action and specific to target organisms. The MDR types most threatening to public health are MDR bacteria that resist multiple antibiotics. .(Magiorakos, *et al.*, 2014)

Recognizing different degrees of MDR in bacteria, the terms extensively drug-resistant (XDR) and pandrug-resistant (PDR) have been introduced. Extensively drug-resistant (XDR) is the non-susceptibility of one bacteria species to all antimicrobial agents except in two or less antimicrobial categories. Within XDR, pandrug-resistant (PDR) is the non-susceptibility of bacteria to all antimicrobial agents in all antimicrobial categories.(Magiorakos., *et al* 2014)

Infections with multidrug-resistant bacteria are hard to treat since few or even no treatment options remain. In some cases health care providers have to use antibiotics that are more toxic for the patient.

Multidrug-resistance facilitates spread of antibiotic resistance. When multidrug-resistance plasmids are transferred to other bacteria, these become resistant to

many antibiotics at once. In environments where bacteria are continuously exposed to antibiotics, like in hospitals or some large production animal farms, multidrug-resistance may be favorable and therefore selected and spread further.(Woerther, *etal* , 2013)

1.2.3. General Characteristics of *Klebsiella* :

Klebsiella species can colonize healthcare facilities and medical equipment, and they are found in a wide variety of natural habitats, including water, soil, and animals (Martin and Bachman, 2018). Despite the fact that *Klebsiella* species can colonize mucosal surfaces without causing pathology, they are classified as opportunistic pathogens because they can spread from mucosae to other tissues and cause serious, sometimes fatal infections such as pneumonia, urinary tract infections (UTIs), bloodstream infections (BSIs), and septic shock (Paczosa and Mecsas, 2016). Those who are newborns, old, or immunocompromised in a healthcare setting are more likely to get a *K. pneumoniae* infection (Magill *et al*. 2014). The propensity to metastasize and substantial morbidity and mortality are hallmarks of these illnesses (Paczosa and Mecsas, 2016).

1.2.4. *Klebsiella* Species :-

Human infections have been linked to three different *Klebsiella* species: *K. pneumoniae, K. oxytoca, and K. granulomatis.* There are two non-fermenting subspecies of *K. pneumoniae, K. ozaenae and K. rhinoscleromatis*, each with its own set of symptoms. All of the strains in this genus are non-motile and ferment lactose, with the following exceptions: the majority of the strains in this genus

generate highly mucoid colonies on plates because they produce a rich polysaccharide capsule; and all of the strains in this genus ferment lactose (Mandell *et al* .,2009).

1.2.4.1 *Klebsiella pneumoniae :*

It is Rod-shaped, non-motile, gram-negative, lactose-fermenting, *Klebsiella pneumoniae* bacteria.. Being a facultative anaerobe, it can thrive in environments with or without oxygen. In addition, this particular bacteria is protected by a capsule, which acts as a physical barrier that stops the host's immune system from identifying it and so contributes to the bacterium's pathogenicity (Guo *et al.*, 2012).This enclosing membrane also prevents the cell from drying out. It can live on the skin, in the mouth, and in the intestines with no ill effects at first. *K. pneumoniae* is a member of the microbiota, yet it can cause serious diseases such as pneumonia, blood poisoning, wound infections, urinary tract infections, and even meningitis. Individuals who rely on medical devices, such as catheters or ventilators, are especially vulnerable to infections. If a patient is given a course of broad-spectrum antibiotics, the body's usual flora of bacteria will be altered, making it more vulnerable to diseases (Nordmann *et al* .,2009).

Klebsiella pneumoniae is a common bacterium that spreads in hospitals and is responsible for pneumonia and other life-threatening respiratory tract infections.Diarrhea and inflammation are two other symptoms of infections induced by this microbe (Hackstein *et al* .,2013). Nosocomial infections in 2009 were dominated by *Klebsiella* (Nordmann *et al* .,2009). The serum's bactericidal

effect is mediated in great part by complement proteins, and phagocytosis by polymorphonuclear granulocytes is essential for host defense against bacterial invasion. The bacteria use a variety of strategies to circumvent the host's natural defenses. Their pathogenicity is mostly determined by the presence of a polysaccharide capsule. The capsule is made up of acidic polysaccharides that are quite complicated. The bacterium is shielded from polymorphonuclear granulocyte phagocytosis by this thick coating.

The capsule also protects the bacteria from the serum factors that would normally kill them (Won *et al* .,2011).Bacterial cells grown in 37C° produce dome-shaped, glittering colonies with different degrees of stickiness when cultured in meat extract media. Glucose is fermented to produce acids and gases, and 2,3-Butanediol is a prominent end product of glucose fermentation in most strains; the Vogas-proskauer test is invariably positive when testing these strains (Drancourt *et al* .,2001).

Surface water, sewage, soil, and plant surfaces are all possible environmental habitats for *Klebsiella* pneumoniae, but the mucosal surface of mammals like humans, horses, and swine is also a likely host. *Klebsiella* pneumoniae is a bacterium that is found in people but not in nature, like Enterobacter but dissimilar to Shigella spp. and Escherichia coli. *Klebsiella* species are uncommon on human skin because gram-negative bacteria do not thrive there. Thus, these bacteria are typically considered to be temporary residents of the skin's flora (Dong *et al*.,2006)

1.2.5. Medical Important of *Klebsiella pneumoniae*

Nosocomial infections are a common manifestation of opportunistic infections caused by organisms such as *Klebsiella* pneumoniae, which target mostly those whose immune systems have been suppressed. (Li, *et al*., 2014). *K. pneumoniae* serotypes that produce more capsule polysaccharide than usual can infect otherwise healthy people and lead to potentially fatal community-acquired diseases (Shon *et al*., 2013). Virulence factors utilized by *K. pneumoniae* for the purpose of infection-related survival and immune-system evasion include, but are not limited to, capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins, determinants for iron acquisition, and determinants for nitrogen source utilization. These are just some of the virulence factors (Bei *et al.*, 2014).

1.2.6. Epidemiology of *Klebsiella pneumoniae*

The Propagation of a bacterium that causes infection *Klebsiella* pneumoniae strains that produce *K. pneumoniae* carbapenemases (KPC-KP) are something that experts in the medical field have learnt to be careful of because of the potential for fast in-hospital transmission and increased mortality owing to carbapenem resistance.

1.2.7. Antibiotic Treatment and Resistance of *Klebsiella pneumoniae*

One of the most pressing issues nowadays is the emergence of resistance to many antimicrobials among Gram-negative bacteria, in particular among Enterobacteriaceae like *Escherichia coli* and *Klebsiella pneumoniae*. These bacterial infections are associated with increased mortality and prolonged hospital

stays (Munoz *et al*., 2013). The hydrolytic activity of -lactamases is the primary factor responsible for the development of resistance to -lactam antibiotics in Gram-negative bacteria. These enzymes are responsible for the ineffectiveness of the antibiotics because they hydrolyze the peptide link on the four-membered beta-lactam ring (Majiduddin *et al*., 2002).

Among the most challenging problems to manage is the dissemination of plasmids, which aid in the multiplication of essential resistance genes (Carattoli, 2013). Plasmids with genes for antibiotic resistance can be passed between bacteria and pose a threat because they can make the recipient strain resistant to many antibiotics. This includes -lactams, quinolones, aminoglycosides, tetracycline, sulfonamides, trimethoprim, macrolides, and chloramphenicol, to name a few (Carattoli.,2009). Most of the commonly used antibiotics, including -lactams, aminoglycosides, tetracyclines, quinolones, sulfonamides, and others, were ineffective against *Klebsiella* . It was hypothesized that *Klebsiella* spp. isolates resistant to -lactam antibiotics were also likely to be highly producing ESBLs; hence, all bacterial isolates are tested for their ability to manufacture ESBLs (AL-Muhanna.,2013).

The bacteria *Klebsiella pneumoniae* are resistant to amoxicillin and triacillin, but they are susceptible to the other -lactam compounds that were tested. Two grams of calvulante per milliliter is the dose that is effective against these germs (Granier *et al* .,2002;Walcker *et al* .,2004). Many antibiotics are ineffective against *Klebsiella* bacteria. It is hypothesized that this is a plasmid-mediated trait. Prolonged hospitalization and invasive procedures both increase the likelihood of

contracting these pathogens. What organs are affected by a disease determines how that disease is treated. In most cases, doctors will use their best judgment when coming up with a treatment plan for patients suspected of having bacteremia. A region's susceptibility patterns should be considered before selecting an antibiotic. Changes to treatment may be made after bacteremia has been verified. Patients in critical condition should be treated with agents that have strong intrinsic activity against *K pneumoniae*. These medications can be used alone or in combination to treat a variety of conditions. Treatment of non-ESBL-producing isolates has been suggested as a combination of an aminoglycoside and a third-generation cephalosporin by some specialists. A counterargument proposes a single form of treatment instead (Van Duin *et al*.,2016).ESBL producers are a novel form of *Klebsiella* spp. that has been linked to hospital-wide epidemics of multidrug-resistant *Klebsiella* spp., notably in newborn wards (Sadeta *et al*.,2012).

The development of *Klebsiella pneumoniae* carbapenemase (KPC) is a unique method among the most frequent carbapenemases in enterobacteria. These are the carbapenemases that are produced by genes that belong to the blaKPC, blaIMP, blaVIM, and blaNdm families (Lascols *et al*.,2012).Cephalosporins, penicillins, monobactams, and carbapenems are all rendered ineffective against bacteria harboring this enzyme. The latter group is broad-spectrum and is typically used to treat illnesses brought on by multi resistant bacteria. As a result, the treatment choices for infections caused by *Klebsiella* pneumoniae carbapenemase-producing bacteria are quite restricted. Hospitals and other healthcare facilities throughout the globe worry that KPC might spread rapidly because of its plasmid location, which allows transfer to the interspecies gene (Cai *et al*.,2012). Each and

every antibiotic drug that has a beta-lactam ring in its chemical structure belongs to the class of broad-spectrum antibiotics known as beta-lactams. Cephalosporins, monobactams, carbapenems, and derivatives of penicillin are all in this category. The most popular class of antibiotics is called -lactams, and they function by blocking the production of cell walls in bacteria (Holten *et al*.,2000).

Even if a bacteria produces beta-lactamase, it may still be treatable with other beta-lactam medicines. To prevent the bacteria from becoming resistant to the antibiotic, a -lactamase inhibitor may be given alongside the -lactam. To give just one example, the antibiotic combination Augmentin consists of the beta-lactam amoxicillin and the beta-lactamase inhibitor clavulanic acid. The clavulanic acid is intended to act as an antagonist to prevent the -lactamase enzymes from neutralizing the amoxicillin. Studies are being conducted on other -Lactamase inhibitors, such as boronic acids, which bind irreversibly to the active site of -lactamases. This is an advantage over clavulanic acid and other competing beta-lactams since they are not rendered ineffective by hydrolysis. Substantial studies are being conducted to generate specific boronic acids to inhibit various beta-lactamase isozymes (leonard *et al*.,2013).

When an illness that might be caused by a bacteria that produces -lactamase is suspected, it is imperative that a suitable β-lactam antibiotic be thoroughly investigated prior to beginning therapy. The correct choice of β-lactam-antibiotic treatment is especially important when dealing with organisms that express -lactamase at even a low level. The selection of bacteria with greater levels of -lactamase expression after an inadequate first line of -lactam antibiotic treatment

may reduce the efficacy of future attempts with different -lactam antibiotics (Macdougall ,2011).

1.2.8. Classification of B-Lactam Antibiotics

Many consider the introduction of penicillin to the clinic in the 1940s to be a pivotal milestone in medical history because of the dramatic improvement it brought to the treatment of bacterial infections. Despite the fact that there have been significant advances in the field of antimicrobial chemotherapy in the more than 70 years since the first use of penicillin, the beta-lactams continue to be the foundations of the antibacterial arsenal as we move closer to the centenary of Fleming's development. This is the case despite the fact that we are approaching the centenary of Fleming's development (Aminov ,2010).

There are four major groups of beta-lactam antimicrobials now used in medicine. They include penicillins (1), which have a thiazolidine ring fused to a -lactam ring, cephalosporins (2), which contain a six-membered dihydrothiazine as the fusion partner, and carbapenems (3), which contain a five-membered pyrroline to round out the bicyclic system. The monobactams (4) are a monocyclic system that forms a fourth class (Tooke *et al*., 2019).

Consequently, action occurs from the -lactam ring combining with the nucleophilic serine of target penicillin binding proteins (PBPs). This reaction opens the ring and produces irreversible PBP acylation, which stops the production of peptidoglycan trans peptide crosslinks (Sauvage and Kerff, 2008).Hence, the C6-penicillins, C7,C3-cephalosporins, C2-carbapenems, and C3-monobactams are

only few examples of sites on the -lactam scaffold that may be modified without losing antibacterial activity.Aminopenicillins, like ampicillin, were developed as a result of these adaptations and expanded penicillin's spectrum of activity to encompass Gram-negative bacteria (Tooke *et al*.,2019).

• Penicillins. Most antibiotics with the -cillin suffix have a 6-animopenicillanic acid (lactam plus thiazolidine) ring as their central molecular structure, along with additional chains. Natural penicillins, agents resistant to beta-lactamase, aminopenicillins, carboxypenicillins, and ureidopenicillins are all part of this class.

• Cephalosporins. A 7-aminocephalosporanic acid core is present, along with a 3,6-dihydro-2 H-1,3- thiazane ring side-chain. Traditional classification of cephalosporins into five generations or classes is not universally accepted.

• Carbapenems. Their resistance to these chemicals is a major problem, and it occurs most frequently in gram-negative organisms , which manufacture various kinds of beta-lactamases together known as carbapenemase.

• Monobactams. Independent of any other ring structure, the beta-lactam ring is present.

For example, beta-lactamase inhibitors. Their primary mechanism of action is to prevent the action of serine beta-lactamases, enzymes that hydrolyze the beta-lactam ring and render the antibiotic ineffective (especially in gram-negative bacteria).

• Effective against carbapenemases like *Klebsiella* pneumoniae carbapenemase (KPC) are both first-generation beta-lactamase inhibitors and second-generation beta-lactamase inhibitors (Carcione *et al*., 2021).

1.2.8.1.Mode of Action of β-Lactam

Antibiotics belonging to the β-lactam family work by preventing the formation of the bacterial cell wall (Bradford, 2001). They exert their effects on bacteria by binding to peptidoglycan-binding proteins (PBPs), which are enzymes essential for cell wall production (Sauvage *et al* ., 2008). PBPs are localized on the outside of the cytoplasmic membrane. In Gram-negative bacteria, this is found in the periplasmic space. These antibiotics block terminal transpeptidation in the bacterial cell wall by interfering with the structural cross linking of peptidoglycans. This helps prevent the growth of resistant bacteria (Andes and Craig , 2005). In bacteria, the cell wall is a network of peptidoglycan cross-links that helps the cell retain its form (Tailor,2011).

The peptidoglycan has a repeating structural unit made up of discontinuous chains of the disaccharides N-acetylglucosamine-(NAG) and N-acetylmuramic acid-(NAM), which are connected together via -(1,4)-glycosides. Muramic acid typically has a chain of four amino acids substituted for its carboxyl group. The most common examples are L-alanine, D-alanine, glutamic acid, glutamine, and lysine (5-diaminopimelic acid) (DAP). In the presence of -lactam antibiotics, transpeptidases and carboxypeptidases react with acyl-D-alanyl-D-alanine to create an alethal serine-ester-linked acyl (penicilloyl, cephalosporoyl) enzyme complex.

This complex is lethal to bacteria. Because of how stable the -lactam-enzyme complex is, it prevents the typical transpeptidation reaction from occurring. Because of this, the manufacturing of the cell wall is disrupted, leaving the rapidly expanding bacteria extremely vulnerable to cell lysis and death (Wilke, *et al*. 2005).

1.2.9. Mechanism of Action For β-Lactam Antibiotics Against *K. Pneumoniae*

Peptidoglycan, often known as murein, is a structurally important component of bacterial cell walls. This highly conserved part of the envelope is present in both gram-positive and gram-negative bacteria. Nevertheless, peptidoglycan in gram-negative bacteria is a thin structure (only 2 layers) but in gram-positive bacteria it is a dense structure (10 layers). To stop the last stage in peptidoglycan production, beta-lactam antibiotics acylate the transpeptidase responsible for cross-linking peptides. Penicillin and other beta-lactam antibiotics like it function by attaching to specialized proteins in the cell membrane (PBPs). This binding interferes with the final transpeptidation process, which in turn reduces bacterial cell viability and ultimately kills the cell via autolytic processes (Eckburg & Lister, 2019).

1.2.10. β- Lactam Antibiotic Resistsnce

Occasionally, bacteria will develop a natural resistance to an antibiotic. The usage of antibiotics is a direct outcome of bacterial structural or functional abnormalities. (Blair *et al*.,2015) for instance, some microbes' outer membranes may be structurally incapable of being penetrated by an antibiotic, and others may

be able to permeate antibiotics but be rendered ineffective by the usage of efflux pumps. The bacteria may either acquire resistance by chromosomal mutations or horizontal gene transfer (when one bacterium receives antibiotic resistant genes from another bacteria through moveable plasmids or transposons) (Eckburg & Lister, 2019).

Four distinct molecular pathways and four distinct modalities of expression contribute to the development of antibiotic resistance: cell wall production, protein synthesis, RNA polymerase and DNA gyrase, the folate mechanism, and membrane structure (prevent cell penetration, expel via efflux pumps, inactivate proteins, and modify the targ).The majority of MDR bacteria produce -lactamases, which renders them resistant to B -lactams. bacterium with a negative charge Penicillin, cephalosporins, monobactams, and carbapenems are all rendered useless against their therapeutic target, penicillin-binding proteins, by the hydrolysis catalyzed by these enzymes. There are four distinct classes of B-lactamases (classes A, B, C, and D) Classes A, C, and D utilise serine-based enzymes, whereas class B - lactamases are Zn2 hydrolases (Bush K. 2013). Extended-spectrum B -lactamase (ESBL)-producing bacteria are a public health concern due to their ability to render bacteria resistant to third-generation cephalosporins, which in turn increases the need for the use of carbapenems as a last resort (Pitout and Laupland ,2008) .

Yet, after the year 2000, there has been an uptick in the number of cases where these were discovered in the general population. Although the widespread presence of ESBL-EC and pAmpC-EC in livestock (particularly poultry), the

extent to which they are transmitted to people via chicken flesh and companion animals (Baede *et al*., 2015) remains debatable (Dierikx *et al*., 2013). In a 2011 study (Van L. ,*et al*.2011) Nevertheless, the proportional contributions of the several potential routes of exposure to ESBL-EC and pAmpC-EC for humans—animals, food, the environment, and interpersonal transmission—remain unknown (Egbule *et al*., 2020).

1.2.11. Mechanisms of Resistance to Beta - Lactam Antibiotics

Antibiotic resistance in bacteria can either be present at the time of infection (intrinsic resistance), or it can develop over time (acquired resistance) this is usually due to mutation . Rapid emergence of resistance is a common feature of microbial populations. Antimicrobial resistance is a result of chromosomal mutation that can be acquired by microorganisms via transferable genes like plasmids, transposons, and integrons. If a microbe has developed drug resistance, it can potentially spread this trait to similar microbes through the horizontal gene transfer system or the vertical gene transfer process (Elufisan *et al*., 2012 and Soares *et al*., 2012).

1- Changes in Target Site

Most antibiotics exert their effects on germs by binding to and inhibiting the activity of a specific location on the microbes known as the drug target site. Antibiotics have a strong preference for the microbial sites and can therefore outcompete any other substrate that may rely on this site. Yet, if this site is changed, the bacteria that carry the change become resistant to antibiotics since they no longer have an affinity for the drugs. One strain of Staphylococcus aureus

is resistant to -lactam antibiotics because its cell wall contains a modified penicillin binding protein. Mutation can have an impact on a wide variety of target locations, including penicillin-binding proteins. These locations, which are often protein molecules, are referred to as the microbe's receptor site (Elufisan *et al*., 2012).

2- Reduction in Cellular Permeability

In order to exert their effects, antibiotics must first breach the host cell membrane, a mechanical barrier. Antibiotics including aminoglycosides and -lactams, chloramphenicol, bacitracin, and isoniazid are ineffective against certain bacterial mutants because they have developed a protective layer that blocks or delays the cellular absorption of these drugs (Elufisan *et al*., 2012). While the loss of porin and the subsequent production of beta-lactamase work together to increase resistance to beta-lactams, the outer membrane alterations in K.pneumoniae are not the deciding factors in this process (Hernandez- Alles *et al*.,2000).

3- Increased Efflux Pumps

Antibiotic resistance can be achieved by the use of efflux mechanisms, which entail the export of waste products or toxins from a microorganism's cell (Van Bembeke *et al*.,2000). This method is seen in bacteria and involves the use of efflux pumps to selectively expel antibiotics. Most of them are multidrug transporters that can pump multiple classes of antibiotics, including fluoroquinolones, tetracyclines, and macrolides (Dzidic *et al*, 2008 and Elufisan *et al*., 2012). The efflux pump's ability to confer resistance is significantly influenced by the synergy between outer membrane permeability and the active efflux family (Elufisan *et al*., 2012).

4- Enzymatic Inactivation

B-lactamases inactivate penicillins and cephalosporins, acetyltransferases change chloramphenicol and aminoglycosides, and enzymes that metabolize tetracyclines and macrolides contribute to antibiotic resistance to a lesser extent (Pallasch, 2003). As soon as the - lactam ring is opened, the - lactam antibiotics are rendered ineffective, and betalactamases hydrolyze them into a linear molecule that cannot attach to their receptors, PBPs (Koch. , 2000).

1.2.12. β- Lactamases

Bacterial resistance to the medically relevant -lactam antibiotics is most often caused by -lactamases in Gram-negative bacteria (Babini and Livermore., 2000). The hydrolyzing activity of these enzymes may vary and they may have a wide host range. B - lactamase enzymes, which hydrolyze the - lactam ring before binding to PBPs on the bacterial cytoplasmic membrane, are typically responsible for mediating resistance (Thirapamnethee , 2012). - lactamase is an exoenzyme that is secreted from Gram-positive bacteria into the external membrane environment. They do not move into the cytoplasm but instead stay in the periplasmic region, where they assault the antibiotic before it reaches the receptor site in Gram-negative bacteria (Stratton, 2000).

1.2.12.1. Evolution and Classification of Beta-lactamase

Each of the many different types of -lactamases is named by the organism or geographic place where it was originally found or the substrate that it is most

efficient against (Thomas,2007). In 1993, TEM-1 from E. coli, a B-lactamase that is produced by Gram-negative bacteria and is mediated by plasmids, was discovered for the first time. SHV-1 is a plasmid-mediated B-lactamase that may be detected in *Klebsiella* pneumoniae and Escherichia coli. SHV-1 is an abbreviation for "Sulfhydryl variable" (Paterson and Bonomo, 2005). Classification schemes for these enzymes have been suggested in many different ways since the early 1970s, including according to phenotype, gene, or amino acid protein sequences and function (Hall and Barlow.,2005). Ambler's approach for categorizing proteins based on their amino acid sequences has found widespread use. Depending on their molecular makeup, the -lactamases were classified as either group A, B, C, or D. Classes A, C, and D enzymes have structural similarities, suggesting they descended from a common ancestor and all hydrolyze their substrates by creating an acyl (Penicilloic or cephalosporoic) group in the active site (Hall and Barlow , 2004).

1.2.12.2 β-Lactam Enzymes and *Klebsiella pneumoniae* carbapenemase

Increasing -lactamase identification and the availability of protein, and then nucleotide, sequence information, led to the realization that -lactamases are not a monolithic group but rather may be broken down into several distinct categories. Furthermore, as reports of enzyme activity against various -lactam substrates emerged, it became clear that -lactamases contain a range of biochemical features. The number of known beta-lactamases has increased almost exponentially alongside the availability of sequence data; as of this writing, more than 4,300 of these enzymes have been characterized in the beta-lactamase database (Naas *et al*.,

2017). At present, two systems exist for classifying these enzymes: the activity-based Bush-Jacoby-Medeiros system and the sequence-based Ambler system. The latter divides -lactamases into four subgroups—A, B, C, and D—based on common and distinct sequence motifs and fundamental differences in their hydrolytic function. The oxacillinase (OXA) family of Class D enzymes is responsible for carbapenem resistance in Acinetobacter baumannii and Enterobacteriaceae. (As has been shown, the field is made much more difficult by long-standing inconsistencies in nomenclature that have their roots in the past. Carbapenem resistance has been identified as a major clinical concern due to the continued dearth of novel medicines having anti-Gram-negative efficacy (Logan, and Weinstein , 2017).

1- Class A Carbapenemases-

Penicillins, cephalosporins, and aztreonam are only some of the beta-lactams that can be hydrolyzed by class A carbapenemases, which feature a serine in their active site (Queenan, 2007). principally made up of the enzymes carbapenemase (KPC) from *Klebsiella* pneumoniae, imipenemase/non-metallocarbamylase (IMI/NMC-A) from Serratia marcescens, and metallo-beta-lactamase (MBL) from Salmonella typhimurium (SME), KPC is the most commonly seen carbapenemase in the United States (Van-Duin.,2017). Antibiotic combinations including meropenem-vaborbactam, ceftazidime-avibactam, and imipenem-relebactam contain novel B-lactamase inhibitors that prevent the majority of KPC enzymes from doing their damage . The blaKPC gene, which codes for KPC, is situated on plasmids that are easily transferred across bacteria of various species because they are bordered by transposable elements(Logan and Weinstein,2017). In contrast to

KPC, which is acquired through horizontal gene transfer, the SME and IMI/NMC-A enzymes are chromosomally encoded (Naas *et al* .,2016)

2-Class B Carbapenemases-

Class B carbapenemases, also known as Ambler MBLs, require zinc as a cofactor for B-lactam ring cleavage (Meini *et al*., 2015). Except for the monobactams (like aztreonam), the class B MBLs hydrolyze every type of B-lactmase (Marshall *et al*.,2017) In contrast to B-lactmase inhibitors like clavulanate, tazobactam, and avibactam, metal chelating compounds like ethylenediaminetetraacetic acid (EDTA) are ineffective against MBLs because they are not approved for use in humans (Meini *et al*.,2015)

3-Class C β-lactamases

In Gram-negative bacteria, class C beta-lactamases are found all throughout the chromosomes. The E. coli enzyme has a special place in the field since it was the first beta-lactamase discovered. Several significant opportunistic Gram-negative pathogens have dormant copies of the genes for class C enzymes (ampC) on their chromosomes. Nevertheless, derepression of these, either by mutation or activation by certain -lactams, may lead to high expression and a rise in MICs for susceptible -lactams (Juan *et al*., 2017). As several members of the class C enzyme family are found on mobile genetic elements in Enterobacteriaceae and nonfermenting organisms like Pseudomonas aeruginosa, their clinical importance is enhanced (Jacoby.,2009).

4-Class D Carbapenemases-

Class D serine-carbapenemases include the OXA Beta-lactamase, which may hydrolyze carbapenems. In Enterobacteriaceae, the OXA-48-like enzymes are the most common type of class D B-lactamase, and they only weakly hydrolyze carbapenems while usually sparing the expanded range cephalosporins (Poirel *et al*.,2012) In 2001, researchers in Turkey discovered that certain Enterobacteriaceae produced an OXA-48-like compound (Poirel *et al*.,2007)

1.13. Treatment of Carbapenemase- producing *K.pneumoniae*

The widespread usage of carbapenems has led to the development of resistance mechanisms in Gram-negative bacteria, making them less and less effective as antimicrobials. Moreover, diminished susceptibility to other types of antimicrobials, such as quinolones and aminoglycosides, is often the result of the simultaneous carrying of other resistance determinants. As a result, there are no clear standards for the therapy of infections caused by bacteria resistant to the antibiotic carbapenem (Boucher *et al*.,2009) But, extremely resistant illnesses may benefit from a combination of antibiotics. Doripenem and ertapenem together were more effective than either treatment alone against KPC producer, as demonstrated by (Bulik and Nicolau .,2011). It appears that colistin, tigecycline, fosfomycin, and aminoglycosides are the best treatments for *K. pneumoniae* infections at the moment (Kosmidis *et al*., 2012). In addition to the advent of fosfomycin and aminoglycosides resistance, the neurotoxic and nephrotoxic effects of colistin and the low serum and urine concentrations of tigecyclines make them less desirable treatment alternatives (Nordmann *et al*.,2009; Neuner *et al*.,2011and Kosmidis *et*

al., 2012). Some new medicines are in research and have showed good in vitro action against carbapenemase generating microbes. Avibactam (NXL-104), is a carbapenemase inhibitor which has shown excellent action against β- lactamase generating Enterobacteriaceae, including KPC- types and OXA- 48 (Mushtaq *et al*., 2010). Plazomicin (ACHN-490), a new aminoglycoside; NAB739 and NAB7061, experimental polymyxin derivatives with the potential to be less nephrotoxic than colistin; and BAL30072, a novel monobactam; may all be viable possibilities in combination with one another (Patel and Bonomo, 2011). Another drug , BAL30376 is a monobactamclavulanic acid combination with efficacy against MBLs (Livermore *et al*., 2010).

1.14. Mechanisms of Resistance Transfer

Bacteria may transfer B-lactamase genes to other bacteria by conjugation, transduction, or transformation on plasmids, transposons, insertion sequences, or integrons. Neither the donor nor the recipient genome is present in the resulting cell (Courvalin , 2006).

1-Conjugation

Here, bacteria of the same or other species exchange genes with one another unilaterally. The R- plasmid is transferred from the donor cell to the recipient cell through a cytoplasmic bridge. A plasmid or transposon plays the role of mediator. Several types of Gram-negative bacteria have this behavior. While plasmid transfer also happens across Gram-positive bacteria, the processes by which this is accomplished are fundamentally different. Instead of pili to initiate conjugation, it

seems that bacterial cells aggregate and transmit genetic material by the help of structures on their cell membranes (Elufisan *et al*., 2012).

2-Transformation

By means of transformation, bacteria may acquire segments of bare DNA from their surroundings and incorporate them into their own chromosome. This may happen naturally, as when a bacterial chromosome leaks out of a donor cell and into a recipient cell, or artificially, as when the chromosome is extracted using chemical methods. Bacteriophage lysis may also cause cellular rupture, which might be the cause. Only between competent cells can the process be completed. Only bacteria are capable of transfer; Gram positive bacteria, which can absorb large molecular weight DNA from water, are particularly frequent (Elufisan *et al*., 2012).

3-Transduction

In transduction, bacterial DNA is substituted for phage DNA during packaging of the phage particles (van-Hoek *et al*., 2011). Bacteriophages are a kind of bacteriophage, which is an extrachromosomal genetic element (DNA, RNA) that can infect and spread across bacterial cells on its own. It's possible that only bacteria with a certain surface receptor can be infected by a bacteriophage since the bacteriophage has to bind to it before it can enter the host cell. Infected cells may either integrate into the host genome, allowing the host to survive, or reproduce excessively, leading to cell lysis (McDermott *et al*., 2003).

1.15. Horizontal Gene Transfer of Resistance Genes

The acquisition, maintenance, and dissemination of antimicrobial resistance genes have been linked to many different forms of mobile genetic elements, which have been identified to date. Plasmids, integrons, and transposons are the most crucial components in this respect. (Van-Hoek *et al*., 2011)

1-Plasmids

Antibiotic resistance in bacteria is often caused by plasmids, which are additional chromosomal components with their own origin of replication (van - Hoek *et al*., 2011, Elufisan *et al*., 2012). Plasmids are similar to chromosomes because of their ability to replicate independently, although they are smaller than bacterial chromosomes, accounting for only around 0.1 to 10% of the bacterial genome (Elufisan *et al*., 2012). Up to 10% of the chromosome in the host cell is encoded by the majority of these molecules, which are circular, double-stranded DNA (Giedraitiene *et al*., 2011). Some plasmids have a broad host range and may initiate their own transfer in a variety of species, whereas others are particularly specialized to a single genus or species and cannot do so on their own (Van- Hoek *et al*., 2011).Plasmids have little effect on how a bacterial cell normally functions, but they may bestow traits on bacteria that increase their chances of survival under particular circumstances, such as when they are exposed to antibiotics. Bacterial plasmids may also encode for the creation of toxins, the use or fermentation of uncommon sugars or foods such as camphor, and the formation of pili for the attachment of a cell to a substrate (Elufisan *et al*., 2012).

2. Integrons

An integron is a kind of gene capture system that may be located on plasmids, chromosomes, or transposons. They can identify and collect several cassettes of genes. Most of the genes in integrons have not been described, however a gene cassette could encode antibiotic resistance genes. Integrons were previously thought to function as a reservoir of antimicrobial resistance genes within microbial populations, and this hypothesis has now been confirmed (Elufisan *et al*., 2012). Recombinase encoded by the integron recognizes the cassette and catalyzes its integration into a particular spot within the integrons, conferring mobility in this way (Giedraitiene *et al.*,2011). Mobile integrons (MIs) and chromosomal integrons (CIs) are the two most common forms of integrons (MIs). CIs may be found on the chromosomes of hundreds of different types of bacteria (Cambray et al., 2010). Super-integrons (SIs) are another name for CIs due to the fact that they may include as many as 200 cassettes, the vast majority of which encode proteins with as-yet undiscovered functions (Cambray et al., 2010).While MIs are not themselves transposable, they are found on other types of mobile genetic elements that aid in their spread among bacterial populations (Stalder *et al*., 2012).

3-Transposons

Evidence for the existence of transposons (or transposable element), also known as jumping gene, has shed light on the widespread prevalence of bacterial resistance to antimicrobial drugs (Elufisan *et al*., 2012). Transposons are DNA segments that randomly insert themselves into new DNA sequences by

recombination, a process facilitated by an enzyme called transposase that is encoded inside the transposon itself (Thomas , 2007). Although they have some characteristics with plasmids, such as having originated from a circularized intermediate, they do not undergo independent replication and are instead often integrated into the bacterial chromosome (Mullany *et al*., 2002).

Via conjugation, transduction, or transformation, transferable B-lactamase genes may be passed from a bacterium to a recipient on plasmids, transposons, insertion sequences, and integrons. The genome of the product cell is distinct from that of either the donor or the receiver (Courvalin , 2006).

2.1. The Materials:

2.1. Equipment and Instrument:

The equipment which are utilized in this study are shown in Table (2-1).

Table (2-1) Equipment and instruments in present study

No.	Equipments/instrumint	Company	country
1	Incubator , Oven	Memmert	Germany
3	Laboratory Distilator	GFL	Germany
4	Centrifuge	Hettich	Germany
5	Micropipetts 5-50 µl ,100-1000 µl 0.5-10-µl Multi channel pipete	Capp	Germany
6	DNA extraction tubes 100 µ, P.C.R Tube 50µl.	Capp	Germany
7	Millipore filter (0.22mm)	Filter Gm, BH, W.	Japan
8	Millipore filter (0.45mm)	Satorins membrane	Germany
9	Multi-channel pipete	Slamed	Japan
10	Autoclave	Stermite	Japan
11	Digital Camera	Panasonic	Japan
12	Plain tube 10 ml	Afco-Dispo	Japan
13	Microwave	Panasonic	Japan
14	Petri dishes	Afco-Dispo	Japan
15	Vortex	Omni	USA

16	Gel electrophoresis system	Clever	USA
17	UV-trans illuminator	Clever	USA
18	Sensitive Balance	Denver	USA
19	PCR Thermal Cycler	Clever	USA
20	Refrigerator	Concord	Italy
21	Laminar Hood	Labogene	Denmark
22	Sterile swab	Lab.Service	Spain
23	Freezer	Kelon	Korea
24	Plastic Test Tube	Citotest	USA
25	Ependrofe tube	Citotest	USA
26	Transport collection swabs	Citotest lab ware	USA
27	Glassware	Slamid U.K Hysil	England

2.1.2. Chemical Materials:

The stains and chemical ingredients that used in present study were listed in Table (2-2)

Table. (2-2) Chemicals compound.

No	Chemicals	Company	Country
1	95%,Ethanol (C_2H_5OH)	BDH	England
2	70% alcohol	BDH	England
3	Glucose($C_6H_{12}O_6$)	BDH	England
4	Glycerol ($C_3H_8O_3$)	Bioneer	Korea
5	Nuclease Free Water	Bioneer	Korea
6	Simple safe (Red safe)	Promega	USA
7	100ubp- DNA laddr	Promega	USA
8	Loading dye (bromophenolemblue)	Promega	USA
9	Agarose	Promega	USA
10	Tris –EDTA buffer. (TE)	Promega	USA
11	Tris-Borate-EDTA (TBE) buffer	Promega	USA

2.1.3. Biological Materials:

The most important biological components employed in this investigation are listed in the Table. (2-3).

Table (2-3) : Culture media

Culture Media	Country	Company
Blood agar base	India	Himedia
Brian- heart infusion agar	India	Himedia
Brian- heart infusion broth	India	Himedia
Eosin methylene blue agar	India	Himedia
Kligler's iron agar	USA	Diffco -Michigan
MacConkey agar,	Belgium	Tulip Diagnostic
MR-VP broth	USA	Diffco -Michigan
Muller-Hinton Agar	India	Himedia
Nutrient agar	USA	Oxoid
Nutrient broth	USA	Oxoid
Peptone water	USa	Oxoid
Simmons citrate agar	USA	Diffco -Michigan
Chromo UTI agar	India	TM Media

2.1.4. Antibiotic discs

The antibiotics used in this study, were listed in table (2-4).

Table (2-4): Antibiotic Disc, Symbol and Potency

The antibiotics	Symbol	Potency	Country	Company
Meropenem	MRP	10 mcg	India	HIMEDIA
Ceftazidim	CAZ	25mcg	India	HIMEDIA
Doripenem	DOR	30mcg	India	HIMEDIA
Cefotaxime	CTX	10 mcg	India	HIMEDIA
Pipercilln./Tazobactam	PIT	30mcg	India	HIMEDIA
Ertapenem	ETP	30mcg	India	HIMEDIA
Agumentin	AMC	30mcg	India	HIMEDIA
Cefixime	CFM	10mcg	India	HIMEDIA
Cephalotin	KF	10mcg	India	HIMEDIA
Ceftriaxone	CIT	10 mcg	India	HIMEDIA
Pencillin	P	30 Iu	India	HIMEDIA
Imipinem	IMI	30mcg	India	HIMEDIA
Aztreonam	ATM	30mcg	India	HIMEDIA

2.1.5. Primers :

The primers used in this study showed in table (2-5).

Table (2-5) : The primers used in the present study

Markers	Oligonucleotides sequence 5`-3`	Size of amplicon	Thermal cycler conditions	Reference
blaNDM-1-F blaNDM-1-R	AAA ACG GCA AGA AAA AGC AG AAA ACG GCA AGA AAA AGC AG	439 pb	5 min at 95C 35 cycles, denaturation at 95 _C for 1 min, annealing at 52 _C for 1 min, extension at 72 _C for 1 min and final extension at 72 _C for 5 min	Salloum,, 2017
blaKPC-F blaKPC-R	AAA ACG GCA AGA AAA AGC AG AAA ACG GCA AGA AAA AGC AG	340 pb	5 min at 95C 35 cycles, denaturation at 95_C for 1 min, annealing at 56 _C for 1 min, extension at 72 _C for 1 min and final extension at 72 _C for 5 min	Salloum,, 2017

blaOXA-48-like- F	TTGG TGGC ATCG ATTA TCGG	597 pb	5 min at 95C 35 cycles, denaturation at 95 _C for 1 min, annealing at 56 _C for 1 min, extension at 72 _C for 1 min and final extension at 72 _C for 5 min.	Salloum,, 2017
blaOXA-48-like- R	GAGC ACTT CTTT TGTG ATGG C			

2.1.6. Commercial kits:

The commercial kits used in this study ,were listed in Table (2-6) and its appendices:

Table (2-6) Commercial kits used in the present study :

No .	Type of kits	Company	Country
1.	DNA extraction kit	Favorgen	USA
3.	DNA ladder	Favorgen	USA
2.	Green master mix 2x kit	Favorgen	USA
4.	Primers	Alpha	USA

2.1.6.1. DNA extraction kits :

DNA extraction for PCR used the present study were shown in table (2-7)

Table (2-7) : Content of DNA extraction kit (Favorgen -USA):

Composition	Company	Country
Ethanol alcohol (96-100%), Proteinase K solution for precipitation proteins, Wash buffer, W1 buffer solution, FABG buffer, ddH2O, Elution buffer, RNase solution.	Favorgen	USA

Table (2-8) : Genomic extraction

DNA extraction Kit	Company	Country
1.Lysis cells solution: Nuclei lyses solution - RNase solution -	Favorgen	USA
2. Protein precipitation solution		
3. DNA precipitation alcohol - Ethanol alcohol		
4. DNA rehydration solution		

2.1.6.2. Green master mix kit

Table (2-9) : Green master mix kit used in the study present.

Green master mix Kit	**Company**	**Country**
1.DNA polymerase enzyme (Taq)	Favorgen	USA
2.dNTPs (400 µm dATP, 400µm d GTP,400µm d CTP, 400µm d TTP)		
3.MgCl2 (3Mm)		
4.Reaction buffer (pH 8.3)		

2.1.6.3. DNA ladder

Table (2-10) : DNA ladder

DNA ladder	**Company**	**Country**
1.ladder consist of 11 double –stranded DNA with size 100-1500bp	Favorgen	USA
2.Loading dye has a composition (15% ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G , 10mM Tris-HCL(pH 7.5) and 50mM EDTA)		

2.2 Methods

2.2.1. Preparation of Reagents and Solutions :

2.2.1.1. Catalase Reagent :

This reagent is made by adding 3% H_2O_2 to 100ml distilled water and should be kept in a dark container (Forbes *et al* .,2007).

2.2.1.2. Oxidase Reagent :

This reagent was prepared directly by dissolving 0.1gm of tetramethyl–p-paraphenylene diamine dihydrochloride in 10ml of distilled water to be stored in a dark container . Every time used , the reagent has been freshly prepared (Forbes *et al* .,2007).

2.2.1.3. Methyl Red Reagent :

The reagent was prepared by dissolving 0.1 gm of methyl red in 300 ml of 99% ethanol , and then , the volume was completed to 500ml by distilled water (MacFaddin ,2000).

2-2-1-4. Kovac`s Reagent:

It was prepared by dissolving 5gm of (p-dimethylamine benzaldehyde) in 75 ml of amyl alcohol and then 25 ml of concentrated HCL was added . This reagent was used for the detection of indole production (MacFaddin,2000).

2.2.2. Solutions :

2-2-2-1. Phosphate Buffer Solution :

It was prepared by dissolving 8gm of NaCl, 0.34gm KHPO4 and 1.12 gm K2HPO4 in 1 liter D.W, the pH was adjusted to 7.3 , autoclaved and stored until use (Collee *et al* .,1996).

2.2.2.2. Normal Saline Solution (0.85%):

It was prepared by dissolving 0.85 gm of NaCl in a small volume of 100 ml distilled water , and then completed to 100 ml ,pH fixed at 7.2 and sterilized in autoclave at 121C for 15 minutes, then kept at 4 °C (Macfaddin,2000).

2.2.2.3. Gram Stain Solution :

The solutions were prepared according to the required microbiological methods. These solutions included : four solutions crystal violet , iodine, absolute alcohol, and safranine the prepared according to method that mentioned in Collee *et al* .,(1996).

2.2.2.4. MacFarland Tube Standared (0.5×10^8) solution:

A barium sulfate turbidity standard solution equivalent to a 0.5 MacFarland standard was prepared as described by (CSLI,2022) as follows :

- A volume of 0.5 ml aliquot of 0.048 M $BaCl_2.2H_2O$ (1.175 % w/v) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring to maintain a suspension .
- Correcting density of the turbidity standard was verified by using the absorbance wave at 625 nm. The absorbance should be 0.08 to 0.10 for the MacFarland standard.

2.2.3. Preparation of culture media:

2.2.3.1. MacConkey agar medium:-

MacConkey agar medium was prepared according to the method recommended by the manufacturing company. It was used for primary isolation of most Gram-negative bacteria and to differentiate lactose fermenter from non-lactose fermenter.

2.2.3.2. Urea Agar Medium :

This medium was prepared by adding 15 ml of urea solution sterilized by filtration , to 100 ml of urea agar base sterilized by autoclaving at 121C° for 15 minutes, and cooled at 50C° . the pH was adjusted to 7.1, and the medium was distributed into sterilized test tubes and allowed to solidify in a slant form. This medium was used to test the ability of bacteria to produce urease enzyme (MacFaddin,2000).

2.2.3.3: Blood Agar Medium :

Blood agar medium was prepared by dissolving 40 gm blood agar base in 1000ml D.W. the medium was autoclaved at 121°C for 15 min, cooled to 50°C and 5% of fresh human blood was added. This medium used as enrichment medium for the cultivation of the bacterial isolates and to determine their ability of blood hemolysis (MacFaddin,2000).

2.2.3.4. Brain Heart Infusion Glycerol Medium :

Brain heart infusion glycerol medium as basal medium as basal medium supplemented with 15% glycerol. After autoclaving it was distributed in 5ml amount in sterile tubes, them kept at 4C° until use. This medium was used to preserve the bacterial isolates at – 4C° for long term stronger several months (MacFaddin ,2000).

2.2.3.5. Kligler Iron Agar Medium :

The basal medium of KIA is composed of casein and meat peptones with the addition of lactose and dextrose and preparation by suspended 52 gm of the medium in 1000 ml of distilled water, then distributed into test tubes, and autoclaved. The production of acid by lactose and/or dextrose fermentation results in color changes of the phenol red pH indicator. Presence of the carbohydrates thus enables the differentiation of species of enteric bacilli.

Kligler Iron Agar was used for determining glucose and lactose fermentation and possible hydrogen sulfide (H_2S) production (MacFaddin.,2000).

2.2.3.6. Peptone Water Medium :

This medium was prepared by dissolving 8 gm peptone in 1000 ml of distilled water , then distributed into test tubes, and autoclaved. It was used for the demonstration of the bacterial ability to decompose the amino acid tryptophan into indole (MacFaddin ,2000).

2.2.3.7. MR-VP Medium :

Methyl Red and Voges- Proskauer test are among the two various tests used in the biochemical identification of bacterial species. This media was prepared by Suspending 17 grams in 1000 ml of distilled water. Heat if necessary to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. MR-VP medium was prepared and used to detect the partial and complete hydrolysis of glucose (MacFaddin,2000).

2.2.3.8. Semi-Solid Medium :

This medium was prepared by adding 0.5 gm of agar to 100 ml of nutrient broth, then it was sterilized by autoclave at 121C° for 15 minutes . It was distributed in tubes , this medium used to detect bacterial motility (MacFaddin,2000).

2-2-3-9. Nutrient Agar Medium :

Nutrient agar medium was prepared (28 gm/1 L). it was used for general tests, cultivation and activation of bacterial isolates when it is necessary (MacFaddin,2000).

2.2.3.10. Muller –Hinton Agar Medium :

Muller –Hinton agar was prepared according to the manufacturing company. The medium was used in anti-bacterial susceptibility testing.

2.2.4. Specimens

155 clinical specimens were collected from patients from the two genders and diverse age who admitted to the four main hospitals in Hilla city/Babylon province (General Teaching Hospital of Hilla, Merjan Medical Teaching Hospital/Babylon Hospital for Maternity and Pediatrics and Al-Sadiq Hospital during a period from September 2021 to December 2021.

2.2.4.1. Collection of specimens :

The different specimens(sputum, stool, wound, urine and CSF). were taken by a sterile containers, then stored in a cool place until transport to the laboratory.

2.2.4.2. Sputum sample :

The 5ml sputum samples were collected from patients in sterile wide mouth containers at morning from patients. The swab was inserted into sputum and inoculated on culture media and incubated aerobically at 37°C for 24h (Girma and Aemiro, 2022).

2.2.4.3. Urine sample:

Generally,10ml specimen was taken from individuals suffering from urinary tract infections (UTIs). Midstream urine samples were collected in sterilized screw-capped containers, spun at 5000 rpm for

15 minutes, inoculated on culture media, and incubated aerobically at 37°C (Ng and Martin, 2005).

2.2.4.4. Wound Sample

In burns and wound, swabs were taken from the depth of burn or wound.

2.2.4.5. Blood Sample

A 2-5 ml of blood withdrawn aseptically using a disposable syringe. Blood samples were promptly transferred to a sterile blood culture bottle with brain heart infusion broth. Following that, the blood culture bottle was incubated for 24-72 hours at 37°C. (Ng and Martin, 2005).

2.2.4.6. CSF Sample

The 5ml specimen was generally collected from patients suffering from meningitis CSF samples were collected in sterilized screw capped containers, then centrifuged at 5000 rpm for 15 min . Then the samples were inoculated on culture media and incubated aerobically at 37°C. (Ng and Martin, 2005).

2.2.4.7. Stool Sample

Suitable amount by spatula were collected in sterile containers and suspended in normal saline

2.3. Cultivation and Isolation :

For primary isolation of pathogenic *Klebsiella pneumoniae*, a loopful of material was streaked on MacConkey agar. The plates were incubated at 37°C overnight. The colonies that had grown to a certain size, color, and mucoid

phenotype were moved to a new plate for further testing. (Girma and Aemiro, 2022).

2.3.1. Ethical Issues:

All adult patients and family members parents of children patients signed a written informed consent before taking samples from them . The study was performed and made facilitated with a permission from Babylon university College of medicine and the general health director of Babylon province .

2.3.2. Identification of *Klebsiella pneumoniae* isolates:

2.3.2.1. Staining and microscopic examination:

Gram stain used in microscopic examination to differentiate gram negative from gram positive bacteria and to identify their shape and arrangement in steps declared in (Collee *et al.*,1996).

2.3.2.2.Characterization of colonies :

Mucoid colonies were demonstrating by string test when touched by wire loop

- Lactose ferment or mucoid appear as pink colour on MacConky agar

2.3.2.3. Biochemical Tests :

2.3.2.3.1. Catalase Test:

A small amount of bacterial growth was transferred by a sterile wooden stick into the surface of a clean dry glass slide , one drop of 3% H_2O_2 was added to it. The formation of gas bubbles indicated the positive result, (Collee *et al.*,1996).

2.3.2.3.2. Oxidase Test :

A piece of filter paper was saturated in a petri dish with oxidase reagent (freshly prepared), A small portion of the bacterial colonies was spread on the filter paper by a wooden stick . Turning the color of the smear from rose to purple within 10 seconds indicated a positive result, the name of reagent is tetramethyle-p-pheylene diamine (Forbes *et al* .,2007).

2.3.2.3.3. Urease test

Urea agar slant was inoculated with tested bacterial culture by sterile loop, and then incubated at 37 Cº for 24 hours; existence of pink color indicates a positive result. This test was used to detect bacterial capacity to produce urease enzyme which hydrolyzes urea to ammonia and carbon dioxide (Cappuccino and Welsh, 2018).

2.3.2.3.4: IMVIC Test

1:Indole Test :

The tubes of the peptone water broth were inoculated with selective bacterial colonies and were incubated at 37°C for 24-48 hours. Five drops of kovac`s reagent were then added to it . The appearance and observation of red ring means a positive result and a complete hydrolysis of tryptophan (MacFaddin,2000).

2: MR-VP Test:

The tubes of the MR-VP broth were inoculated with selective bacterial colonies and were incubated at 37°C for 24-48 hours. Five drops of methyl red reagent were then added to it . The appearance and observation of red color means a positive result and a complete hydrolysis of glucose (MacFaddin.,2000).

3:Vogues- Proskauer Test :

The tubes of the VP broth were inoculated with selected bacterial colonies and were incubated at 37°C for 24 hours. The result was then read by adding 0.6 ml of alpha nephthol (reagent A) and 0.2 ml of 40% KOH solutions (reagent B). the appearance of red color after 15 minutes means a positive result due to the partial hydrolysis of glucose , which produced acetoin (Acetyl methyl-carbinol) (MacFaddin ,2000).

4:Citrate (Simmon`s) utilization test :

Simmon`s Citrate slant was inoculated and incubated with a young culture at 37°C for 48-72 hrs. the color change of medium from green to blue indicates a positive result (MacFaddin ,2000).

2.3.2.3.٥. Sugar fermentation:

This test was done to detect the ability of bacteria to ferment different types of carbohydrate include (mannitol, lactose, sucrose and glucose). This test was performed as the following :

Sugar fermentation medium was inoculated by the suspected bacterial colonies and the tubes were incubated 35-37°C for 18-24hrs, looking for the result next day .

a- Change the color of broth from red to yellow indicates positive result (acid formation i.e. sugar fermenter).
b- No change in color indicating result (no sugar fermentation)

2.3.2.3.6. Motility test :

This test was done by inoculating the tube that contained semisolid media with tested bacteria by a stabbing method and was incubated at 30°C for 24-48 hours. The disseminating of growth out of the stab line was an indication of positive result (MacFaddin ,2000).

2.3.3. Preservation and maintenance of bacterial isolates :

The bacterial isolates were preserved on nutrient agar slants at 4°C. The isolates were maintained monthly during the study by sub culturing on new culture media. For long preservation, brain heart infusion broth supplemented with 15%glycerol was used, and the isolates were maintained frozen 3 months at -4°C (Collee *et al*., 1996).

2.4 :Antimicrobial Susceptibility Test(AST):

susceptibility tests of *K.pneumoniae* have been performed for detection of resistance to antibiotics by Using disc diffusion method. The Antimicrobial susceptibility testing (AST) was performed by disk diffusion method to 13 different antibiotics.

After adjustment of inoculum to 0.5 according to the Clinical Labrotary Standared Instituite (CLSI-2022) The antibiotic used in the styde were show in table (2-4).

1-Activation of isolates were performed using nutrient broth for 18 hrs at 37°C

2-The growth was adjusted to 0.5 McFarland's standard then spread on Muller Hinton agar (MHA) with a sterile cotton swab.

3-Antibiotic disks were placed onto MHA, gently pressed down to ensure complete contact with the agar inoculated with bacteria and then incubated for 24 hrs. at 37°C

4-inhibition zone diameter was measured by in millimeters (mm) recorded. Interpretation of results as a sensitive or resistant were achieved according to (CLSI, 2022).

2.5. Detection of β- Lactam enzyme :

Antibiotic susceptibility testing was done to three types of 3rd generation cephalosporins antibiotics on Muller Hinton Agar that inoculated with fresh culture: cefotaxime, ceftriaxone, ceftazidime. If inhibition zone for bacterial isolates were ≤ 27mm for cefotaxime, ≤ 22mm for ceftazidime and ≤ 25mm for Ceftriaxone, this result considered as positive result for production of extended spectrum beta lactamase(CLSI, 2022)

2.6. Detection of ESBL

This test was carried out as modified by Coudron *et al*. (1997). Antibiotic disks containing 30 μg (cefotaxime, ceftazidime, ceftriaxone) and azetreonam were placed 15mm (edge to edge) around a central disk of amoxi- clav (20 μg amoxicillin plus 10 μg clavulanate) on Mueller- Hinton agar plates inoculated with organism being tested for ESBL production.The plates were incubated aerobically at 37 °C for 24 hrs. Any augmentation (increase in diameter of inhibition zone) between the central amoxi-clav disk and any of the Beta - lactam antibiotic disks showing resistance or intermediate susceptibility was recorded, and the organism was thus considered as an ESBL producer (CLSI,2022).

2.6.1. Detection of KPC

K.pneumoniae carbapenemase (KPC) Chromagar plates were streaked in the same day of preparation by overnight growth of K.pneumoniae , and incubated at 37 °C for 24 hrs according to manufacturer procedure. Growth of blue colonies indicated to suspected KPC producer. The reference strain of E.coli ATCC 25922 was inhibited and used as negative control.(CLSI,2020).

2.7. Molecular detection of antibiotic resistance genes:

The Favorgens DNA purification kit was used to extract DNA, and the procedures laid forth by Geneaid were followed. Genomic DNA was isolated from bacterial cells using the following procedures :

1-Sample Preparation

1 ml of bacterial suspension that have been cultivated for 18 hours and transferred to microcentrifuge tube , centrifugation (10,000 rpm /1 min), and the floatable was then discarded.

2-Steps of Cell Lysis

A.The tube was filled with 180 ml of GT (guanidium thiocyanate) buffer, the cell pellet was suspended by a vortex, 20 ul (Proteinase K) was added, and incubated at 60 C /10 min. The mixture tubes were turned over every three minutes during the incubation periods.

B-Each tube received 200 ul of the GB buffer, which was then vortexed (10 sec) at 60 °C /10 min, inverted every three minutes.

3-Steps in DNA Binding

A- 200 l of pure ethanol were added, quickly mixed by vortex, and any precipitates that formed were broken up with pipetting.

B. All mixtures (including any precipitate) were transferred to a GD column, which was positioned in a 2 ml collection tube. then spun the mixture for one minute at 10,000 rpm. The flow-through-containing 2 ml collection tubes were discarded, and a fresh 2 ml collection tube was used to hold the GD column.

2.7.1. Polymerase chain reaction (PCR):

PCR reactions were created using the PCR master mix that created in accordance with the provided company instructions as shown in Table 2-7. The standard Maxime PCR PreMix kit tubes, which also contained every other

component required for the PCR reaction, such as those in the table above, were then mixed with the PCR master mix components.

Using a conventional PCR thermocycler, the following conditions methodology was used for each gene as in Table 2-5:

Table 2-11: PCR thermocycler conditions protocol

Gene	PCR products (bp)	Initial denaturation ^{0}c/min	Denaturation ^{0}c/min	Annealing ^{0}c/min	Extension ^{0}c/min	cycles	Final extension ^{0}c/min	Hold ^{0}c/time
blaNDM-1	439	95/5	95/1	52/1	72/1	35	72/5	4/∞
blaKPC	340	95/5	95/1	56/1	72/1	35	72/5	4/∞
blaOXA-48	597	95/5	95/1	56/1	72/1	35	72/5	4/∞

2.7.5. Detection of PCR product by Gel-electrophoresis:

PCR results were examined using the agarose gel electrophoresis method in the manner described below:

1- 1.5% Agarose gel was made by dissolving it in 0.5IX TBE (Tris/Borate/EDTA) for 5 minutes in the microwave, and then allowing it to cool at 50°C.

2- The agarose gel solution was then stained with 3 ul of ethidium bromide dye.

3- Removed the comb after the agarose gel solution had hardened in the tray (15 min). at room temperature following placement of the comb in the proper area and pouring the solution into the tray. PCR results were

examined using the agarose gel electrophoresis method in the manner described below:

4- A 0.5IX TBE buffer was added to tray. Each well received 5ml of a liter of PCR product, with the first well receiving 5ml of DNA marker ladder (100-1500 bp).

5- UV transilluminators were used to see the PCR results (Suchman, 2016).

2.6: Statistical analysis :

The statistical analysis was done using Chi-square ($P \leq 0.01$) and LSD in one way ANOVA method at ($P \leq 0.05$) as well as histograms analysis were performed by using SPSS,2010 VERSION Statistics software (Paulson, 2008).

3.1 Isolation and Identification of *K. pneumoniae* isolate :-

A total of (155) clinical specimens were collected from different patients and from both gender who attended or admitted to the main four hospitals in Hilla city (General Teaching Hospital of Hilla, Merjan Medical City , Al-Imam Alsadiq Hospital and Babylon Hospital for Maternity and Pediatrics) during a period from September 2021 to December 2021.

Only thirty two isolates (20.6%) of *Klebsiella pneumoniae* were identified according to the cultural characteristics and biochemical tests as presented in Figure (3-3) and Table (3-2).

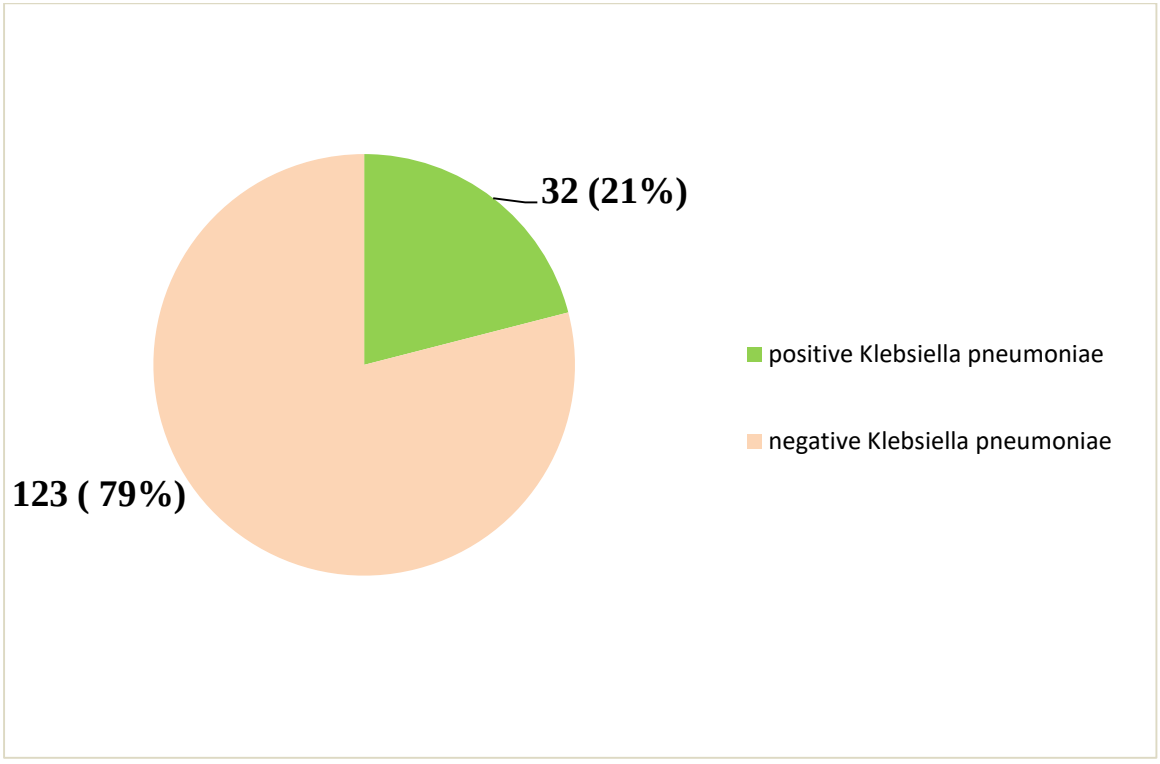


Figure 3-1: Distribution of *Klebsiella pneumoniae* isolates out of total clinical samples (N=155).

A 32 (20.6%) specimens were positive culture for *Klebsiella pneumoniae* . These clinical specimens included, blood 3 (9%),sputum 7 (22%), stool 5 (15%), urine 9 (28%), CSF 4 (13%),wound 4 (13%). As in figure (3-1)

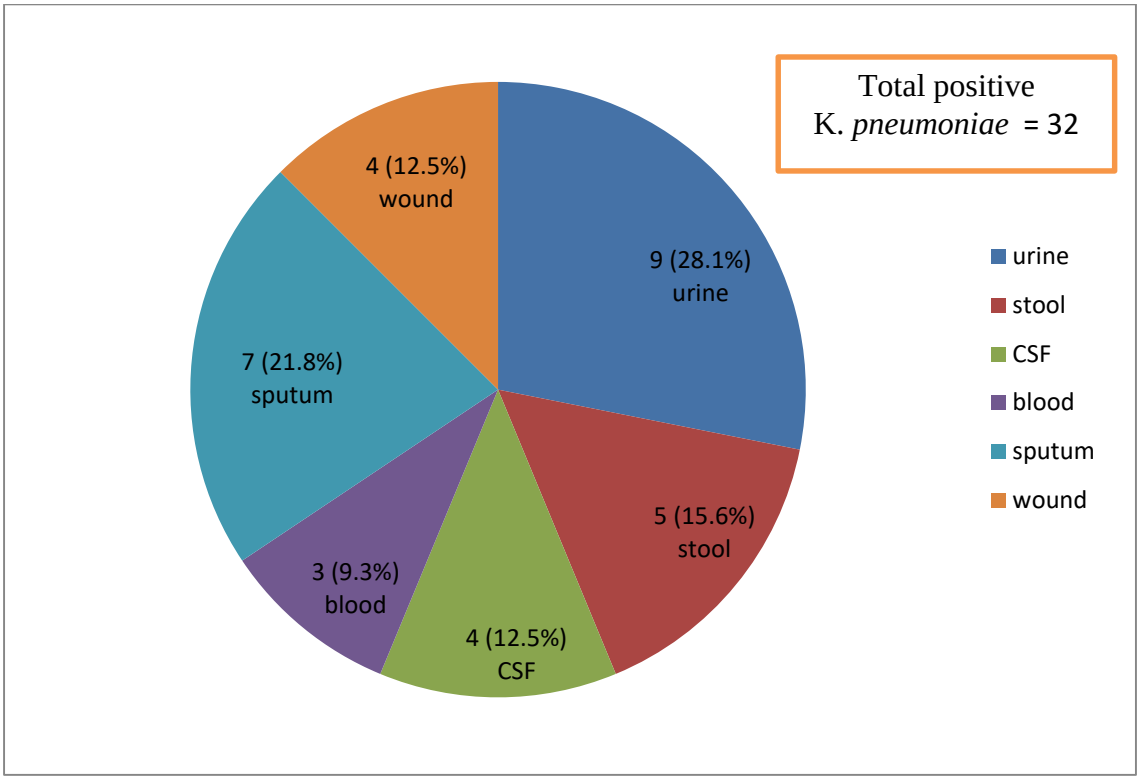


Figure (3-2). Distribution of positive culture of *Klebsiella pneumoniae* isolated from different clinical specimens

The clinical specimens were randomly selected and examined for the detection of *Klebsiella pneumoniae* from the hospitals of Hilla City included : Al-Hilla Teaching Hospital 10 (31%), Mirgan Medical City 5 (15%) , AL-Imam Alsadiq 10 (31%) and Babylon Hospital for Maternity and Pediatrics 7 (21%) as detailed in table(3-1).

Table (3-1): Distribution of isolated *Klebsiella pneumoniae* with their percentages in the collected specimens.

Specimen	Al-Hilla Hospital		Alsadiq Hospital		Babylon Hospital		Mirgan Hospital		total	
	No	%	No.	%	No.	%	No.	%	No.	%
Blood	1	3	0	0	0	0	2	6	3	9
Sputum	0	0	4	13	0	0	3	9	7	22
Stool	0	0	4	13	1	3	0	0	5	15
Urine	5	15	2	6	2	6	0	0	9	28
CSF	0	0	0	0	4	13	0	0	4	13
Wound	4	13	0	0	0	0	0	0	4	13
Total	10	31.25	10	31.25	7.5	21.8	5	15.6	32	100

3.1.1 Identification of *Klebsiella pneumoniae* on different media.

The morphological features of colonies on culture media (EMB agar,MacConkey agar, and Chromogenic agar) were checked. On MacConkey agar, *Klebsiella* colonies were lactose fermenting colonies and gave pink color, regular edge. and the colonies were metallic blue on Chromogenic agar orientation, on the EMB agar appear in metallic. Microscopic examination exhibited gram negative rods.

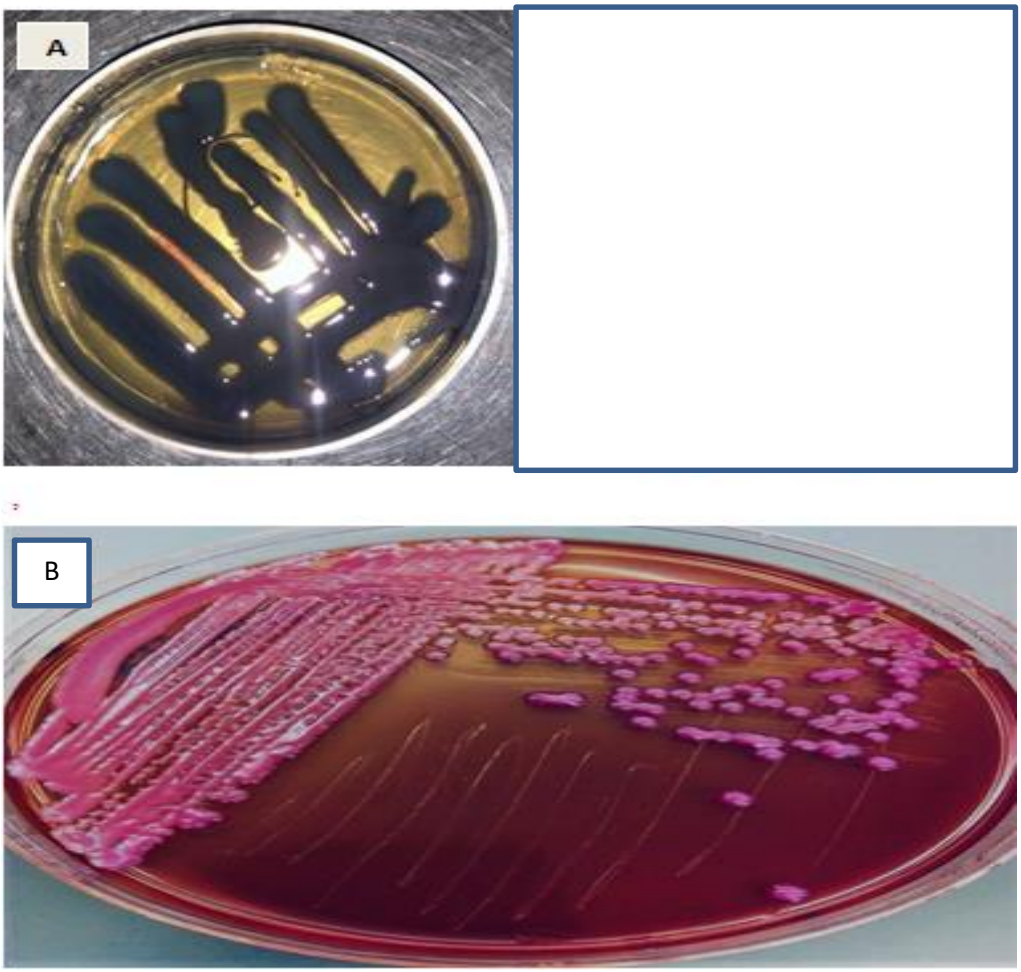


Figure (3-3): *Klebsiella pneumoniae* colonies on (A) Chromogenic agar and (C) MacConkey agar.

3.1.2. Identification of *Klebsiella pneumoniae* using biochemical tests

Some biochemical tests were performed for more validation (Table 3-2). *Klebsiella pneumoniae* isolates have given in biochemical tests; a positive result for urease and catalase test, while have given negative results to the Oxidase test. The isolates showed negative results for indole, hemolysin, methyl red (MR) and H2S production tests(without gas production), but have given positive result on Vogas – Proskaur ,Citrate utilization, Lactose, Sucrose, glucose fermentation .

Table (3-2): Cultural and Biochemical characteristics of *K.pneumoniae*

Tests	Result
Gram stain	Gram –ve Rods
Colonies	Mucoid (+)
Catalase	+
Oxidase	-
H_2S production	-
Indole	-
Methyl red	-
Vogas – Proskaur	+
Citrate utilization	+
Fermentation of Lactose	+
Sucrose	+
Glucose	+
Mannitole	+
Hemolysin	-
Urease	+
Motility	-

Abbreviations : (+), positive test; (-), Negative test.

3-2: Gender Factor.

The distribution of specimens according to gender revealed 63 specimens (40.6 %) were collected form males and 92 (59 %) from females (Figure 3-3).

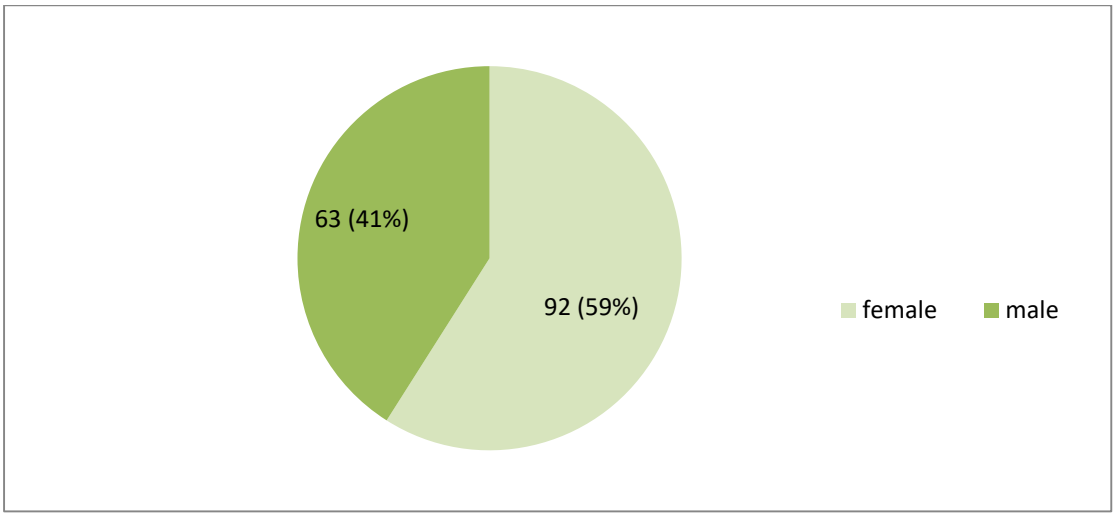


Figure (3-4) Distribution of *Klebsiella pneumoniae* isolates according to gender of patients

The Distribution of *Klebsiella pneumoniae* isolates according to number, gender was illustrated in table (3-3) . A total of thirty two isolates of *Klebsiella pneumoniae* were isolated from 155 clinical specimens (different cases) . Of these, 9 isolates(25%) were isolated from (urine) , 7 isolates(22%) were isolated from (sputum) , 5 isolates(16%) were isolated from (stool) , 3 isolates (9%) from (blood) and 4 isolates (13%) from (wound).

Female was more than male in general; in urine was (5), blood (3), sputum (5), only in stool male was more than female (4).

Table (3-3): Distribution of *Klebsiella pneumoniae* isolates according to number, gender of patient.

Sample	Gender		Number of isolate	Percentage (%)
	Female	Male		
Blood	3	0	3	9
Sputum	5	2	7	22
Stool	2	3	5	16
Urine	5	4	9	25
CSF	4	0	4	13
Wound	2	2	4	13
Total	21(65.6%)	11(34.4%)	32	100

3-3: Age Factor :

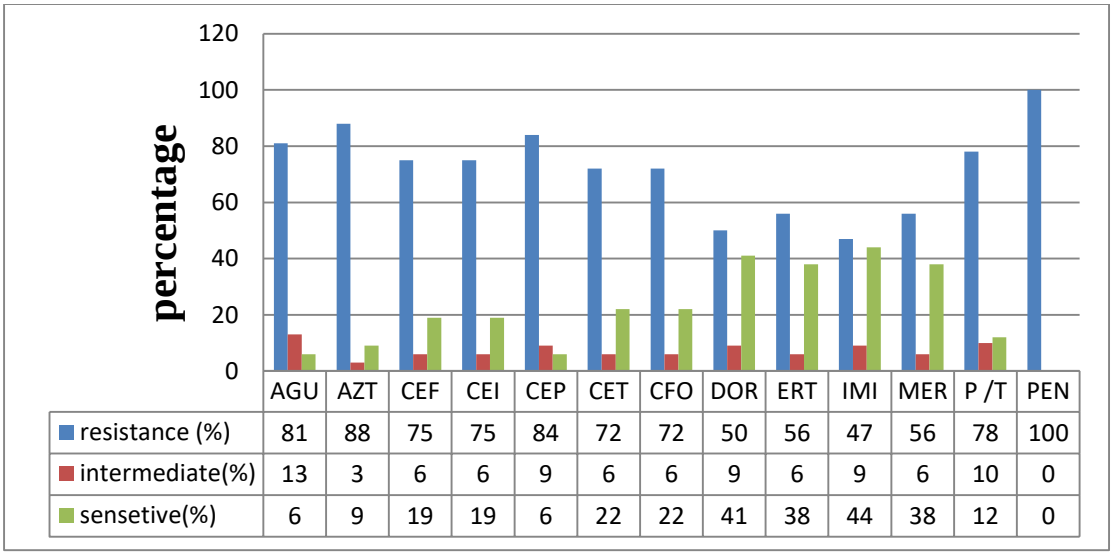
The age of patients was ranged from 4-70 years (mean, 39.86 ± SD 15.7). There are significant differences between age groups ($p < 0.05$), the patients were divided into four groups according to their age, as in Table (3-4).

Table (3-4): distribution of *Klebsiella pneumoniae* isolates according to age groups of patients.

Age group	Number of isolate	Percentage (%)	*P* value
4-20	4	12.5	**0.000**
21-37	10	31.25	
38-53	11	34..30	
54-69	7	21.80	
Total	32	100	

3.4. Antibiotic resistance pattern among *Klebsiella pneumoniae*.

Thirty two isolates (KP1 to KP32) of *Klebsiella pneumoniae.* were tested against 13 common antibiotics. According to the results in Fig (3.4), the antibiotics susceptibility test results showed that the percentages of the resistant toward these antibiotics were as follow: agumentin 26 (٨١%), aztreonam 28 (٨7.5%),ceftazidime 22(68.7%), cefixime 24 (٧٥%), cephalotin 27 (٨٤.3%), ceftriaxone 23 (٧٢%), cefotaxim 23 (٧1.8%), doripenem 16 (٥٠%), ertapenem 18 (٥٦%), imipinem 14 (٤٧%), meropenem 18 (٥٦%), pipercillin./tazobactam. 25 (٧٨%), Pencillin 32 (100%).



	AGU	AZT	CEF	CEI	CEP	CET	CFO	DOR	ERT	IMI	MER	P /T	PEN
resistance (%)	81	88	75	75	84	72	72	50	56	47	56	78	100
intermediate(%)	13	3	6	6	9	6	6	9	6	9	6	10	0
sensetive(%)	6	9	19	19	6	22	22	41	38	44	38	12	0

Figure (3-5): Antibiotic profile of *Klebsiella pneumoniae* to 13 different antibiotics used in this study detected by (DDT).

Abbreviations : AUG, Augmentin; AZT, Aztreonam; CEF, Ceftriaxone; CEI, Cefixime; CEP, Cephalotin; CET, Ceftazidim; CFO, Cefotaxime; DOR, Doripenem; ERT, Ertapenem; IMI,Imipenem; MER, Meropenem; P /T, Pipercillin./Tazobactam.; PEN, Penicillin.

The Table (3-6) shows the resistance rate of the isolated *K. pneumoniae* from various clinical samples to common antibiotics. In all clinical samples, , *K. pneumoniae* was observed to be highly resistant to

penicillin 32 (100%), In the urine samples, *K. pneumoniae* was observed to be highly resistant to Agumentin, Aztreonam, Cephalotin , and Piper./Tazo. 28 (88%). *K. pneumoniae* isolated from the blood samples was extremely effective against Ceftazidim, Cefixime , , cefotaxim and Ertapenem 32 (100%)., whereas , in the sputum samples, *K. pneumoniae* was observed to be highly resistant to Agumentin, Aztreonam, Cephalotin 27 (85%) , and Piper./Tazo. 32 (100%). *K. pneumoniae* isolated from the wound samples was extremely effective against Ceftazidim, Cefixime , Ceftriaxone, cefotaxim and Ertapenem 32 (100%). while , in the vagina samples, *K. pneumoniae* was observed to be highly resistant to Aztreonam 32 (100%).

Table (3-5): Antibiotic Resistance Rate of *K. pneumoniae* isolate Isolated From Clinical Samples

Antibiotic	Blood (N=3)		sputum (N=7)		stool (N=5)		urine (N=9)		CSF (N=4)		wound (N=4)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Augmentin	2	66	6	85	4	80	8	88	3	75	3	75
Aztreonam	2	66	6	85	4	80	8	88	4	100	4	100
Ceftazidime	3	100	6	85	2	40	5	55	3	75	3	75
Cefixime	3	100	6	85	2	40	6	66	3	75	4	100
Cephalotin	2	66	6	85	4	80	8	88	3	75	4	100
Ceftriaxone	3	100	6	85	2	40	6	66	2	50	4	100
Cefotaxime	3	100	6	85	2	40	6	66	3	75	3	75
Doripenem	1	33	4	57	1	20	7	77	2	50	1	25
Ertapenem	3	100	4	57	2	40	7	77	1	25	1	25
Imipenem	1	33	4	57	1	20	6	66	1	25	1	25
Meropenem	3	100	4	57	2	40	7	77	1	25	1	25
Piper./Tazo.	2	66	7	100	3	60	8	88	2	50	3	75
Penicillin	3	100	7	100	5	100	9	100	4	100	4	100

Table 3-6 showed that most isolates were resistant to the antibiotics, especially to penicillin . *Klebsiella pneumoniae* isolates *(*KP2 , KP3, KP4 , KP11, KP12 , KP16, KP18 , KP23, KP32) were resistant to all

antibiotics used in this study. The isolates KP1 was resistant to all antibiotics used in this study except for ceftazidim، whereas isolates KP17 was resistant to all antibiotics used in this study except for aztreonam and cephalotin. whereas isolates KP22 was resistant to all antibiotics used in this study except for doripenem and imipinem. In the present study, the highest resistant percentages toward the antibiotics were found with penicillin and aztreonam (100 %, 88% respectively as in figure 3-5), while the lowest resistant percentages were found with doripenem, imipinem (50 %, 47% respectively).

Table (3-6): Phenotypic of antibiotic susceptibility of *Klebsiella pneumoniae* isolates

Isolate name	AGU	AZT	CEF	CEI	CEP	CET	CFO	DOR	ERT	IMI	MER	P /T	PEN
Kp1	R	R	R	R	R	S	R	R	R	R	R	R	R
Kp2	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp3	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp4	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp5	I	R	R	R	R	R	S	S	S	S	S	I	R
Kp6	R	R	R	R	R	R	R	R	R	S	R	R	R
Kp7	R	R	R	R	R	R	S	R	R	R	R	R	R
Kp8	I	S	R	R	R	S	R	S	S	S	S	I	R
Kp9	R	R	S	R	R	R	R	I	I	I	I	R	R
Kp10	R	R	R	S	R	R	S	S	S	S	S	R	R
Kp11	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp12	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp13	I	R	R	R	S	R	R	S	S	S	S	I	R
Kp14	I	R	S	S	S	S	S	S	S	S	S	S	R
Kp15	R	S	S	S	R	S	R	S	S	S	S	R	R

Kp16	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp17	R	I	R	R	I	R	R	R	R	R	R	R	R
Kp18	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp19	S	R	R	R	R	R	R	S	R	S	R	S	R
Kp20	R	R	I	I	R	I	I	R	R	R	R	R	R
Kp21	R	R	R	R	R	R	R	S	S	S	S	R	R
Kp22	R	R	R	R	R	R	R	S	R	S	R	R	R
Kp23	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp24	R	S	S	S	R	S	S	S	S	S	S	R	R
Kp25	R	R	R	R	R	R	R	I	I	I	I	S	R
Kp26	R	R	R	R	R	R	R	S	S	S	S	R	R
Kp27	R	R	S	S	I	S	S	R	R	R	R	R	R
Kp28	R	R	S	S	R	S	S	I	R	I	R	S	R
Kp29	R	R	R	R	R	R	R	S	S	S	S	R	R
Kp30	S	R	R	R	I	R	R	S	S	S	S	R	R
Kp31	R	R	I	I	R	I	I	R	S	S	S	R	R
Kp32	R	R	R	R	R	R	R	R	R	R	R	R	R

Abbreviations : AUG, Augmentin; AZT, Aztreonam; CEF, Ceftriaxone; CEI, Cefixime; CEP, Cephalotin; CET, Ceftazidim; CFO, CEFOTAXIM; DOR, Doripenem; ERT, Ertapenem; IMI,Imipenem; MER, Meropenem; P /T, Piper./Tazo.; PEN, Penicillin.

In table (3-7), all of the isolates (100%) in the existing study were found to be resistant to Pencillin, 28 % of isolates were resistant to all antibiotics . 9 % of isolates were resistant to 13 antibiotics, 6% of isolates were resistant to 11 antibiotics.

This study also showed that most of the isolates of *K. pneumoniae* were 100 % multi-drug resistant (3 different antibiotic resistance). The

details of the distribution of the MDR phenotype among *Klebsiella pneumoniae* and percentages of the isolates were summarized in Table (3.7).

Table (3-7): Frequency of multidrug-resistant isolates of *Klebsiella pneumoniae isolates*

Antibiotics	No.of resistant isolate	%
AGU./AZT./CEF./CEI./CEP./CET./CFO./DOR./ERT. /IMI./MER./P /T./PEN	9	28
AGU./AZT./CEF./CEI./CEP./CFO./DOR./ERT./IMI. /MER./P /T./PEN	1	3
AGU./AZT./CEF./CEI./CEP./CET./DOR./ERT./IMI. /MER./P /T./PEN	1	3
AGU./AZT./CEF./CEI./CEP./CET./CFO./DOR./ERT./ MER./P /T./PEN	1	3
AGU./AZT./CEF./CEI./CEP./CET./CFO./ERT./ MER./P /T./PEN	1	3
AGU./CEF./CEI./CET./CFO./DOR./ERT./IMI./MER. /P /T./PEN	1	3
AGU./AZT./CEF./CEI./CEP./CET./CFO./PEN	4	13
AGU./AZT./CEF./CEI./CEP./CET./CFO./P /T./PEN	2	6
AGU./AZT./CEF./CEI./CEP./CET./CFO./PEN	1	3
AZT./CEF./CEI./CEP./CET./CFO./ ERT./ MER./PEN	1	3
AZT./CEF./CEI./CEP./CET./PEN	2	6
AZT./CEF./CEI. /CET./PEN	2	6
AZT./CEF./CEI./ CET./CFO./PEN	3	9
AGU./AZT./DOR./ERT./IMI./MER./P /T./PEN	2	6
AGU./AZT./CEP./ PEN	2	6

Abbreviations : AUG, Augmentin; AZT, Aztreonam; CEF, Ceftriaxone; CEI, Cefixime; CEP, Cephalotin; CET, Ceftazidim; CFO, Cefotaxime; DOR, Doripenem; ERT, Ertapenem; IMI,Imipenem; MER, Meropenem; P /T, Piper./Tazo.; PEN, Penicillin.

3.5. Molecular Characterization of Antibiotic Resistance gene(blaNDM , blaKPC , blaOXA-48 for *K. pneumoniae* isolates :

Three selected genes were investigated in this study to explore their role in the antibiotic resistance of *Klebsiella pneumoniae* associated diseases. These genes were blaNDM gene (New Delhi metallo-beta-lactamase 1), blaKPC gene (*Klebsiella pneumoniae* carbapenemase), and blaOXA-48 gene by PCR technique. Among a total of 32 local isolates of *Klebsiella pneumoniae*, the molecular detection for gene by PCR revealed that gene was positive in 87% (28/32) of the *Klebsiella pneumoniae* strains , **including blaNDM** in 47% was identified in 15 isolates of *Klebsiella pneumoniae* (47%) and absent in 17 (53%) isolates. **Regarding blaKPC** gene it was identified in 12 isolates of *Klebsiella pneumoniae* (37%) and absent in 20 (63%) isolates. **While blaOXA-48** gene it was identified in 19 isolates of *Klebsiella pneumoniae* (59%) and absent in 13 (41%) isolates as shown in table (3-9), figure (3-6), figure (3-7) and figure (3-8)

Table (3-8) : Distribution of the antibiotic resistance gene (blaNDM, blaKPC and blaOXA-48) among *Klebsiella pneumoniae* isolates (N=32) detected by PCR.

Genes	blaNDM		blaKPC		blaOXA-48		P value
	No.	%	No.	%	No.	%	
Positive	15	47	12	37	19	59	**0.631**
Negative	17	53	20	63	13	41	
P value	**0.070**		**0.001**		**0.002**		

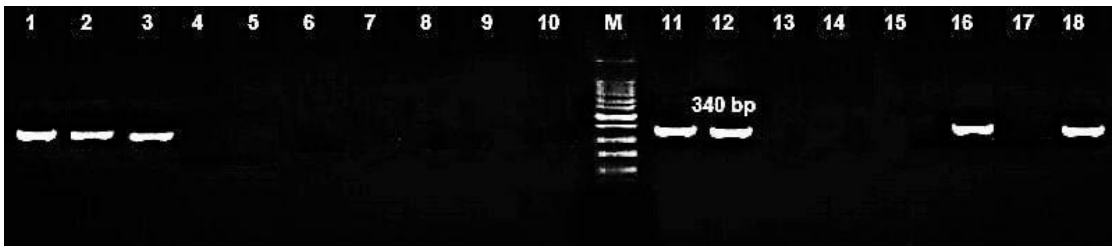


Figure (3-6) : Gel electrophoresis of PCR product for detection of blaKPC (340bp) using 1 % agarose for 80 min. at 75 V\Cm. Lane M: Marker DNA ladder size (100 bp). Lanes 1, 2, 3, 11, 12, 16 and 18 positive for blaKPC (340bp)

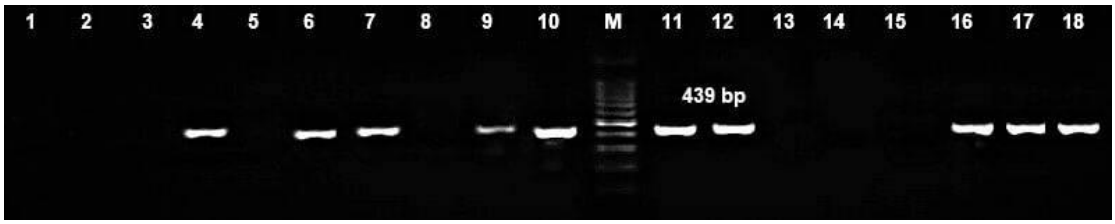


Figure (3-7) : Gel electrophoresis of PCR product for detection of blaNDM gene (439bp) using 1 % agarose for 80 min. at 75 V\Cm. Lane M: Marker DNA ladder size (100 bp). Lanes 4, 6, 7, 9, 10, 11,12,16,17 and 18 positive for blaNDM gene (439bp)

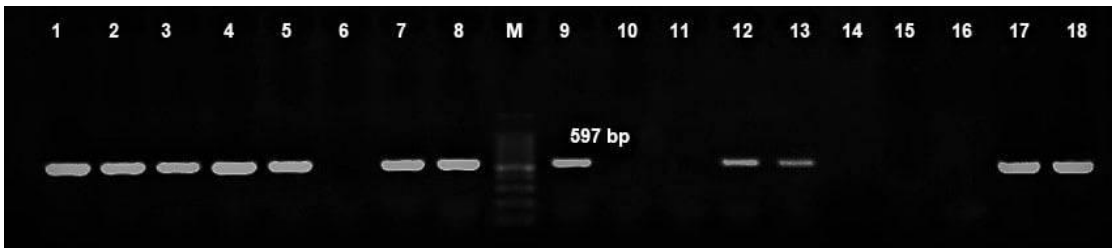


Figure (3-8) : Gel electrophoresis of PCR product for detection of blaOXA-48 (597bp) using 1 % agarose for 80 min. at 75 V\Cm. Lane M: Marker DNA ladder size (100 bp). Lanes 1, 2, 3,,4,5,7,8,9,12,13,17 and 18 positive for blaOXA-48 (597bp)

The agarose gel electrophoresis of amplification products of PCR showed that the isolates KP4, KP6,KP7 ,KP9,KP10, KP11, KP12, KP16, KP17, KP18,KP19,KP23,KP24,KP29, and KP32 were specific to blaNDM (Figure 3.7) and 13 isolatesKP1,KP2,KP3,KP21,KP22,KP31 KP11, KP12 , KP16,KP18, KP29, KP32 were specific to blaKPC, (Figure 3.6) and 19 isolates KP5,KP8,KP13,KP25,KP27,KP28 ,KP30,KP4,KP7, KP9,KP17,KP19,KP23,KP1,KP2, KP3, KP22, KP12, and KP18 were specific to blaOXA-48 (Figure 3. 8) Detecting the genes by PCR,three (9%) isolates carry the gene blaNDM only , 2 (6%) isolates carry blaKPC gene , 7 (22 %) isolates carry blaOXA-48 , 2 (6%) isolates carry (blaNDM, blaKPC, and blaOXA-48) , only 4 (6%) isolates carry (blaNDM and blaKPC) , 6 (19%) isolates carry (blaNDM and bla OXA-48) , 4 (13%) isolates carry (blaKPC , and blaOXA-48) , 4 (13%) isolates don't carry any genes as shown in as in table (3-9).

Table (3-9): Distribution of the gene groups of *Klebsiella pneumoniae isolates*.

Isolate No.	Genotype	No.	%
KP31 ,KP21	blaKPC	2	6
KP1, KP2 ,KP3, KP22	blaKPC and blaOXA-48	4	12.5
KP10, KP24 ,KP6	blaNDM	3	9
KP16 ,KP29 ,KP32	blaNDM , and blaKPC	4	13
KP17,KP11,KP4,KP7,KP9,KP19,KP 23	blaNDM and blaOXA-48	7	19
KP12, KP18	blaNDM , blaKPC, and blaOXA-48	2	6
KP30 ,KP13,KP5 ,KP8 ,KP25 ,KP28,KP27	blaOXA-48	7	22
KP15 ,KP20 ,KP14 ,KP26	None	4	12.5

In the current study, antibiotics resistance profile and molecular detection of *blaNDM, blaKPC, and blaOXA-48* genes in *Klebsiella pneumoniae* isolates revealed that there was a harmony between results of antibiotic resistance and positive molecular detection of these genes. The highest resistance rate to most antibiotics was observed in the isolates of blaKPC then blaNDM gene, the isolates that have blaNDM, blaKPC, and blaOXA-48 genes was resistance to all antibiotiacs (100%) , the isolates that have blaNDM, and blaKPC genes was resistance to 6 antibiotiacs (100%) , the isolates that have blaNDM, and blaOXA-48 genes was resistance to 3 antibiotiacs (100%) , the isolates that have blaKPC, and blaOXA-48 genes was resistance to 10 antibiotiacs (100%) , the isolates that haven't any genes was resistance only to penicillin (100%) , as shown in Table (3-10).

Table (3-10): Pattern of resistance according to *blaNDM, blaKPC, and blaOXA-48* gene carriage rate in *Klebsiella pneumoniae isolates*

Antibiotic	blaNDM		blaKPC		blaOXA-48		blaNDM, blaKPC, and blaOXA-48		blaNDM and blaKPC		blaNDM and blaOXA-48		blaKPC, and blaOXA-48		None	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
AGU	3	100	2	100	3	٤٣	2	100	4	100	5	83	4	100	3	75
AZT	2	67	2	100	6	٨٥	2	100	4	100	5	83	4	100	3	75
CEF	2	67	1	50	5	٧٠	2	100	4	100	5	83	4	100	1	25
CEI	1	33	1	50	5	٧٠	2	100	4	100	6	100	4	100	1	25
CEP	3	100	2	100	4	٥٦	2	100	4	100	5	83	4	100	3	75
CET	2	67	1	50	4	٥٦	2	100	4	100	6	100	3	75	1	25

CFO	1	33	1	50	1	١٤	2	100	4	100	5	83	4	100	2	50
DOR	1	33	1	50	2	٢٨	2	100	3	75	4	67	3	75	1	25
ERT	1	33	0	0	1	١٤	2	100	3	75	5	83	4	100	1	25
IMI	0	0	0	0	2	٢٨	2	100	3	75	4	67	3	75	1	25
MER	1	33	0	0	1	١٤	2	100	3	75	5	83	4	100	1	25
P /T	3	100	2	100	2	٢٨	2	100	4	100	5	83	4	100	3	75
PEN	3	100	2	100	7	١٠٠	2	100	4	100	6	100	4	100	4	100

Abbreviations : AUG, Augmentin; AZT, Aztreonam; CEF, Ceftriaxone; CEI, Cefixime; CEP, Cephalotin; CET, Ceftazidim; CFO, Cefotaxime; DOR, Doripenem; ERT, Ertapenem; IMI,Imipenem; MER, Meropenem; P /T, Piper./Tazo.; PEN, Penicillin

The distribution of antibiotic resistance genes (*blaNDM, blaKPC, and blaOXA-48*) according to the site of collection among *Klebsiella pneumoniae* isolates had been studied, results presented in Tables (3-11) , (3-12) and (3-13). As shown in Table (3-11), the highest percentage of blaNDM was found in urine (16%) followed by Sputum (9%),blood,stool,CSF(6%), wound (3%).

Table (3-11):Distribution of *blaNDM* gene according to source of isolates (N=32).

Specimens	**blaNDM gene**				**Total**	
	Positive		**Negative**			
	Number	**%**	**Number**	**%**	**Number**	**%**
Blood	**2**	**6**	**1**	**3**	**3**	**9**
Sputum	**3**	**9**	**4**	**13**	**7**	**22**
Stool	**2**	**6**	**3**	**9**	**5**	**16**
Urine	**5**	**16**	**4**	**13**	**9**	**28**
CSF	**2**	**6**	**2**	**6**	**4**	**13**
Wound	**1**	**3**	**3**	**9**	**4**	**13**
Total	**15**	**47**	**17**	**53**	**32**	**100**

The highest percentage of blaKPC was found in Sputum (13) followed by urine (9%), CSF (6%), blood, wound (3%) , stool (0%), as shown in Table (3-13).

Table (3-12): Distribution of blaKPC gene according to source of isolates (N=32).

Specimens	blaKPC gene				Total	
	Positive		Negative			
	Number	%	Number	%	Number	%
Blood	1	3	2	6	3	9
Sputum	4	13	3	9	7	21
Stool	0	0	5	17	5	17
Urine	3	9	6	19	9	27
CSF	2	6	2	6	4	13
Wound	1	3	3	9	4	13
Total	12	34	20	66	32	100

The highest percentage of blaOXA-48 gene was found in Urine (27%) followed by wound , sputum (9%), CSF (6%), blood and stool (3%) as shown in Table (3-13).

Table (3-13):Distribution of *blaOXA-48* gene according to source of isolates (N=32).

Specimens	blaOXA-48 gene				Total	
	Positive		Negative			
	Number	%	Number	%	Number	%
Blood	1	3	2	7	3	10
Sputum	3	9	4	13	7	22
Stool	1	3	4	13	5	16
Urine	9	27	0	0	9	27
CSF	2	6	2	7	4	13
Wound	3	9	1	3	4	12
Total	19	57	13	43	32	100

4.1 Isolation and Identification of *K. pneumoniae* isolate:-

K. pneumoniae is capable of causing a wide variety of clinical disorders, some of which include an infection of the urinary tract, pneumonia, an infection of the skin and soft tissues, and bacteremia or septicemia. These infections are all serious and can even be fatal (Riaz *et al*.,2012 : Azar and Ebadi, 2015). According to the findings of Naqid *et al*. (2020), 130 *K. pneumoniae* isolates originated from a variety of clinical sample collections. These findings were verified by the incidence distribution of patient specimens. *K. pneumoniae* infection was found in all patient samples tested, including urine-66.2%, blood-12.3%, and wound-10%). Multiple Iraqi studies have found that urine is a major distribution point for K.P. (Assafi *et al*., 2015; Hussein *et al*.,2018). Riaz *et al*. (2012) and Akter *et al*. (2014) found *Klebsiella* species most often in urine.

4.1.1 Identification of *Klebsiella pneumoniae* on different media.

Klebsiella was effectively discovered and described by using a selective approach that included culturing materials on MacConkey agar. This was essential to the process. In this medium, the inclusion of bile salts and crystal violet inhibits the growth of Gram-positive bacteria . On the other hand, they stimulate the growth of Enterobacteriaceae and related enteric Gram-negative rods. Since lactose is the sole source of carbon in this medium, only bacteria that are capable of fermenting lactose will be able to thrive there. As the pH falls lower than 6.8, the neutral red indicator dye will convert, resulting in pink colonies. The non-

lactose bacterial growth, on the other hand, may have a transparent or colorless appearance (Holt *et al*., 1994). The aniline dyes (eosin and methylene blue) in EMB agar mix to create a precipitate at acidic pH, which manifests as a metallic green sheen. As a result, these aniline dyes are utilized as markers for acid generation, which allows for simple separation between *Klebsiella* and *E. coli*. As a result of this, colonies of *Klebsiella* seem pink, but colonies of *E. coli* look dark and are surrounded by a green metallic sheen. This is because *E. coli* colonies are dark owing to the significant quantity of acid created during fermentation (Atlas *et al*., 1995). In addition, every single *K. pneumoniae* isolate produced a positive result for the string test, which is indicative of the hypermucoviscosity phenotype. According to Nadasy *et al*. (2007), this trait was not seen in the noninvasive *K. pneumoniae* isolates that were isolated from individuals with noninvasive infections.

4.1.2. Identification of *Klebsiella pneumoniae* using biochemical methods

Many investigations have shown that conventional biochemical tests are not sufficient for detecting *K. pneumoniae* if the assays are not appropriately chosen (Hansen *et al*.,2004; Alves *et al*2006). These tests need to be investigated further to see whether or not they are able to differentiate between different species (Claus , 1993). 36 clinical isolates had a similar biochemical response pattern that was equivalent to that which was described for *Klebsiella* species. These particular isolates were positive for glucose, lactose, sucrose, and mannitol, however they tested negative for oxidase and indole. The pattern was comparable to the one reported with *Klebsiella* species (Sikarwar and Batra, 2011).

4.2. Gender factor:-

Researchers have found that female patients were more likely to have *K. pneumoniae* (76.2%) isolated from clinical samples than male patients (23.8%). It has been found that *K. pneumoniae* was more common in female patients than male patients when isolated from urine, blood, wound, and oral samples, but more common in male patients when isolated from sputum samples (Naqid *et al*., 2020).

In contrast to the findings of(Shilpa *et al*. 2016), who found that *K. pneumoniae* was more commonly isolated from female patients than from male patients, a study conducted in Bangladesh found that male patients were more susceptible to *Klebsiella* infection isolated from urine and wound samples than female patients(Shilpa *et al*. 2016),. This was the case despite the fact that Shilpa *et al*. (2016) discovered that *K. pneumoniae* was isolated from female patients at a higher rate than from male patients. *K. pneumoniae* was remained more often isolated from female patients than male patients, notwithstanding what Shilpa *et al*. found (Akter *et al*.,2016). It is difficult to identify the causes of this variation; nevertheless, variations in sample collection methods, research designs, populations, patient inclusion criteria, and environmental variables are all possible contributors. *K. pneumoniae* was responsible for large percentages of an overall infection in the human body, which is a little higher than the findings found in this research. It's possible that this is because of the thick polysaccharide capsule that this bacterium has, which allows it to live in environments that aren't ideal for its growth. It's possible that this is owing to the fact that *K. pneumoniae* is responsible for a significant portion of the overall infection in the human body (Iroha *et al*, 2008).

Namratha *et al*. (2015) found that *Klebsiella* spp. were more commonly colonized by immunocompromised patients, supporting the percentage isolation rates found in the current study; diabetes was found to be the most common risk factor associated with *Klebsiella* infections, followed by alcoholism and previous surgeries. In keeping with these findings, another investigation also indicated that between 8 and 9 percent of *Klebsiella pneumoniae* was recovered from clinical specimens (Martinz *et al*.,1987).

According to the results of this specific inquiry, *K. pneumoniae* was the pathogen that was shown to be the cause of urinary tract infections in female patients the most of the time. This was the case in a total of five out of six cases. This may be due to a number of factors, including the fact that the urethra is shorter and closer to the anus in women, both of which can increase the risk of infection. Also, women may be more likely to get inoculated with the virus into their bladders when engaging in sexual activities (Magliano *et al*.,2012). According to the results of a study that was directed in Iran, K. pneumonia was proven to be the infection that led to urinary-tract infections in female patients more often than any other kind of infection (Mirsoleymani *et al*2014). Other Iranian studies have reached the same conclusion: UTIs and *K. pneumoniae* infections are more common in women than in men (Azar and Ebadi,201). According to Lina *et al*. (2007), older citizens, both men and women, are at an increased risk of acquiring a urinary tract infection (UTI) caused by *Klebsiella* pneumoniae. These findings were supported by the discovery of a number of risk factors associated with a *Klebsiella* infection. These risk factors included diabetes mellitus, hypertension, the use of steroid medication, intoxication, and smoking.

4.3 Age Factor:-

Osazuwa *et al*. (2010) observed that people between the ages of 16 and 30 have a greater risk of contracting the virus. Yet, other research has shown that those aged 45–60 had an especially high prevalence of *Klebsiella pneumoniae* (Russo and Johnson,2003). another study indicated that *Klebsiella pneumoniae* prevalence was highest in people aged 31–45, whereas it was lowest in those aged 18–25. (Hussein *et al*.,2018).

4.4. Antibiotic resistance pattern among *Klebsiella pneumoniae*.

Globally, antimicrobial resistance (AMR) is a prominent cause of treatment failure in infectious diseases (Agrawal *et al*., 2008; Akter *et al*., 2014), leading to higher rates of illness and death as well as a larger cost burden on the healthcare system (Bouza *et al*.,2002). Somewhere around 700,000 people every year lose their lives because of AMR. Hence, if antimicrobial resistance is not brought under control and avoided, it will become the primary cause of mortality among persons who are hospitalized as well as those who are not hospitalized in developing and wealthy countries (Shilpa *et al*.,2016). Multiple-drug-resistant *K. pneumoniae* is a global health threat (Saleem,2016). Hospitalized patients receive broad-spectrum antibiotics, which increases *K. pneumoniae* carriage and multidrug-resistant germs (Chakraborty, 2016). *K. pneumoniae* has evolved resistance to first-, second-, and third-generation cephalosporins in many countries (Nawaz *et al*.,2009).

This study has shown that *K. pneumoniae* is the most prevalent pathogen that may acquire ESBL-mediated resistance to broad-spectrum beta-lactam medications. This is an extra plus (Agrawal *et al*.,2008). Resistance to penicillin in *K. pneumoniae* is due to the bacteria's capacity to transport the plasmid generating beta-lactamase variations, which is a typical mechanism of gram-negative infections (Aktas *et al*.,2002). Previous studies were confirmed, and it was revealed that all *Klebsiella* spp. strains were resistant to penicillin (Brisse and Duijkeren,2005; AL-Taai,2015). Several studies have documented the various mechanisms by which *Klebsiella* develops resistance to antibiotics. Maybe *Klebsiella* spp. were exposed to antibiotics by chance due to the haphazard use of antibiotics in the treatment of infectious diseases. In contrast to the results of the current investigation, Gales *et al*. (2000) found that *Klebsiella* resistance to Beta-lactams varied from 63.3% to 70%. Ghorashi *et al*. (2011) indicated that *Klebsiella* may resist cephalosporin, penicillin, and other Beta-lactams by altering the permeability of the plasma membrane. The various extended-spectrum beta-lactamase enzymes produced by *Klebsiella* spp., as reported by Iroha *et al*., render -lactam antibiotics ineffective and enable *Klebsiella* spp. to persist in the face of -lactam therapy. As the latter mentioned, nearly three-quarters of *K. pneumoniae* isolates have been found to be resistant to cefotaxime. Infections caused by *K. pneumoniae* can be treated with imipenem and amikacin because these drugs are effective against sensitive isolates of the bacterium (Kumar *et al*., 2006).

Over 81 percent of *K. pneumoniae* isolates showed struggle to at least one of four antibiotic classes: cefoxitin, ceftriaxone and cefepime. Similar results were found in studies done in developing countries, where *Klebsiella* isolates were reported to be 100% resistant to ampicillin

(Aktas *et al*., 2002; Susethira and Uma, 2016). Because of its inexpensive price and simple dosage instructions, this antibiotic has seen widespread usage. Doripenem, ertapenem, imipenem, meropenem, and piperacillin/tazobactam were all effective against *Klebsiella pneumoniae* isolated from wound swabs, but ceftazidim and penicillin were ineffective against all of the bacteria in the research. Among the antibiotics tested, *K. pneumoniae* showed the greatest susceptibility to imipenem in an Iraqi study, while showing the greatest resistance to ceftriaxone and tetracycline (Sarathbabu,2014).

All of the *K. pneumoniae* strains that were isolated from sputum samples exhibited resistance to ampicillin but sensitivity to gentamicin (Naqid *et al*., 2020). Of the antibiotics tested, *K. pneumoniae* in the urine samples was shown to be highly resistant to ampicillin (97.7%), ceftriaxone (62.8%), and cefepime (60.5%), but very susceptible to ertapenem (97.7%) and imipenem (96.5%). This was the case even though both of these antibiotics (ertapenem and imipenem) were used.

Traditional treatments for Gram-negative bacteria like K. pneumoniae, such as third-generation cephalosporins, are becoming less and less effective as the number of bacteria that produce ESBL continues to climb (Pistella and Santini, 2016). Studies have reported alarmingly high rates of resistance to these antimicrobials in recent Ethiopian studies: 97.6% to ceftazidime, 94.1% to cefepime, and 88.2% to ceftriaxone in Bahir Dar (Teklu *et al*.,2019); and 86.4% to cefotaxime, 85.4% to cefzidime, and cefpime in Addis Ababa (Moges *et al*.,2019).Our results for ceftriaxone resistance are lower than those reported by Badamchi *et al*. from Iran (60.2%, 32.6%, and 54.8%, respectively) (Badamchi *et al*.,2018). Carbapenems showed the highest susceptibility among *K. pneumoniae* isolates, followed by amikacin (29.6% overall non-

susceptibility). Overall, susceptibility to all three antibiotics was high, although the rate was greatest for imipenem (81%), followed by meropenem (73%), and finally ertapenem (70%).

Similar findings were obtained in a prior research conducted in Egypt, when 75% of the samples tested susceptible to both meropenem and imipenem (El-Badawy *et al*.,2017). In line with our findings, a research conducted in Addis Ababa, Ethiopia, found that 10.7% of the *K. pneumoniae* isolates examined were resistant to meropenem (Teklu *et al*.,2019). The susceptibility profile of *K. pneumoniae* isolates collected from different clinical specimen types is summarized in this paper. Results showed that 96.9% of the isolated *K. pneumoniae* strains were resistant to ampicillin, 65.8% were resistant to ceftriaxone, and 60.8% were resistant to cefepime. The highest rates of sensitivity were seen against ertapenem (93.8%) and imipenem (82.3%). (Naqid *et al*.,2020).

Recently, researchers tested 132 *K. pneumoniae* isolates for susceptibility to 21 different antimicrobials representing 12 different classes of antibiotics. Cefotaxime (128/132, 97%), ceftriaxone (128/132, 97%), and erythromycin (128/132, 97%) all showed extremely high rates of resistance. In addition, the frequency of intermediate resistance to ceftazidime-21.2%, piperacillin-tazobactam-20.5%, aztreonam-22.7%, and ciprofloxacin-18.9% was shown. After amikacin, the carbapenems imipenem (107/126, 81.1%), meropenem (96/126, 72.7%), and ertapenem (93/126, 70.5%) were shown to be the most operative antimicrobials against *K. pneumoniae* isolates. amikacin was the second most operative antimicrobial, with a success rate of 93.2%. This is supported by a number of studies (Awoke, *et al*., 2021).

In comparison, Eshetie *et al*. (2015) found that 95.6% of *K. pneumoniae* isolates were MDR from patients with urinary tract

infections in Gondar, Ethiopia, and that 100% of *K. pneumoniae* isolates were MDR from patients with urinary tract infections in Baghdad, Iraq. These findings were based on the observation of patients with urinary tract infections in both of these locations (Aljanaby and Alhasnawi, 2017). As compared to studies done in Addis Abeba (Teklu *et al*., 2019) and Bahir Dar (citing 87.6%), ours was the highest (Moges *et al*.,2019). Most of the K. pneumonia isolates (90.91%) came from patients in hospitals, where there is a high concentration of antimicrobials and thus a selective pressure for drug resistance. In addition, the study expanded its coverage of antimicrobial drugs in light of the recommendations made by Magiorakos *et al*., allowing for a higher rate of MDR detection (Magiorakos *et al*.,2012).

Alarmingly, our research showed that *K. pneumoniae* was highly resistant to the majority of the antimicrobials we tested it against. There has been an increase in antimicrobial resistance in *K. pneumoniae* in five African and two Vietnamese urban regions due to factors including self-medication with antibiotics, incorrect antibiotic prescription, poor quality medications, and a lack of effective methods to prevent nosocomial infections (Breurec *et al*.,2013). MDR bacteria were resistant to multiple classes of antibiotics, and only two (1.5%) of the isolates tested positive for resistance. The MDR spectrum in our case was quite broad, spanning from resistance to three (R3) to twelve (R12) different classes of antimicrobials. For this reason, it was important to detail how many isolates fell into each MDR category. As a result, among all the isolates, 15.2% were resistant to no antimicrobials at all. There were two isolates that were completely resistant to all antibiotics tested. Also, among the isolates tested, 12.1% were resistant to multiple classes of antibiotics (Awoke *et al*.,2021).

4.5. Molecular characterization of *K. pneumoniae* isolates :

4.5.1. Detection of carbapenemase genes (blaNDM, blaKPC, and blaOXA-48).

It has been shown that the most important machinery of carbapenem resistance amongst CRE strains in China is the presence of carbapenemase genes. Some examples of these genes are blaKPC-2 and blaNDM. It is of the utmost importance to avoid swallowing microorganisms such as *Klebsiella pneumoniae* and *E. coli* at all costs (Zhang *et al*., 2017; Wang *et al*., 2018). OXA-48-like generating Enterobacteriaceae were shown to be 38 percent prevalent in Europe, second only to KPC-producing-Enterobacteriaceae (42 percent), and higher than NDM-producing-Enterobacteriaceae (12 percent) (Grundmann *et al*., 2017). KPC-2 -51.6%, NDM-35.7% and OXA-48-like -7.4% were shown to be the most common types of carbapenemases generated by CRE strains. All blaOXA-232-positive carbapenem-resistant *Klebsiella pneumoniae* were detected in youngsters. In the majority of cases, carbapenemase genes were discovered in *Klebsiella* pneumonia-64%, Escherichia coli-96.0%, and CRE strains that were recovered from adults (70.3%) and children (49%).

Nineteen different strains were able to manufacture several carbapenemases, while 16 different bacteria were blaIMP positive (Han *et al*.,2020). Of the 173 *Klebsiella* spp. isolates tested, blaNDM-1 (42%), blaKPC (10%), and blaOXA-48-like(6%) were shown to be the most prevalent carbapenem-resistance genes (Ebomah *et al*.,2020). Most of the people with positive genotypes had the blaNDM allele (80.5%), followed

by those with blaVIM (36.4%), blaKPC (28.6%), blaOXA-48 (26.6%), and blaIMP (6.5%). (Abdelaziz,2022).

Among the isolates, blaNDM-1, blaKPC, blaOXA-48-like (0%), blaNDM-1, blaKPC (2%), blaKPC, blaOXA-48-like (1%), and blaNDM-1, blaOXA-48-like(0%) were the most common combinations of carbapenem-resistance genes (Ebomah *et al*.,2020). There were 1735 strains of bacteria that were resistant to carbapenems, but only 54 of those were blaNDM-1 positive (3.1%). Forty-four distinct *K. pneumoniae* strains were represented here (Xiang *et al*.,2020). The majority of blaNDM-1 carriers are found to be K. pneumoniae, which is consistent with the results of our investigation and those of previous studies (Rolain *et al*., 2010;Deshpande *et al*.,2010;Zarfel *et al*.,2011;Dongmei *et al*., 2017). In spite of the fact that earlier studies suggested that Acinetobacter and *Klebsiella* were the most often found blaNDM-1-positive bacteria, the majority of blaNDM-1-positive hospital strains were found to be *K. pneumoniae* (59%). (Zhang *et al*., 2014;Chen *et al*., 2014;Shengshu *et al*., 2015).

Carbapenem-resistant *Klebsiella pneumoniae* spread clonally through a tertiary hospital in China. Both blaKPC-2 and blaNDM-1 were driven by distinct transposons that possessed the same core structure but showed wide variation in incidence and distribution across countries (Zhang *et al*.,2020).

Due to its increasing resistance, especially to carbapenems and other "last resort" antibiotics, *K. pneumoniae* is now seen as a major international health concern (Ripabelli *et al*.,2018). In 2017, the World Health Organization (WHO) designated carbapenem-resistant *Klebsiella pneumoniae* (CRKP) as a critical priority pathogen and the top priority in the discovery of new antibiotics (Tacconelli *et al*.,2018). *Klebsiella*

pneumoniae has evolved a wide variety of resistance mechanisms to carbapenems, some of which include the formation of carbapenemases, changes in the permeability of the outer membrane, and the overexpression of efflux systems (Pitout *et al*.,2015). In *Klebsiella* pneumoniae, the varieties of carbapenemase that belong to Ambler molecular class A (KPC), class B (VIM, IMP, and NDM), and class D (OXA-48-like) are the ones that are found the most often (Lee *et al*.,2016).

While the data demonstrate that a small fraction of carbapenem-resistant bacterial isolates harbor the blaOXA-48-like gene (6%), this resistance pattern is not seen frequently (Ebomah *et al*.,2020). The findings of the antimicrobial susceptibility tests revealed that the bacteria that were resistant to NDM-1 showed a high level of resistance to the majority of antibiotics (excluding amikacin).

NDM-1 displays a high level of resistance to aminoglycosides, beta-lactam antibiotics, and beta-lactamase inhibitors, as well as carbapenems (Shengshu *et al*., 2015). Dolipenem-ampicillin-furadantin-cefazolin-cefuroxime-cefotaxime ceftriaxone sodium-ceftazidime, and cefoxitin are some of the medicines that certain strains of NDM-1 might become resistant to. Other drugs that could be affected include cefotaxime, ceftazidime, and ceftazidime. The percentage of patients resistant to the carbapenem drugs meropenem, imipenem, and ertapenem is as follows: 97.30%, 97.30%, and 92.00%, respectively. Since several drug resistance genes may be transmitted from blaNDM-1 plasmids, it is reasonable to assume that NDM-1-resistant bacteria are also likely to be resistant to multiple drugs (Barguigua *et al*., 2015;Liu *et al*., 2016). Among the most recently identified and published carbapenemases, NDM stands out as a novel MBL enzyme. Urine from a Swedish patient of

Indian descent who had visited New Delhi yielded the first NDM-1 detection in a strain of carbapenem-resistant *K. pneumoniae* (Yong *et al*.,2009). Almost forty nations have since reported isolating NDM-causing bacteria (Seija *et al*.,2015). Nonetheless, most isolates come from healthcare settings. NDM-1 was first reported in Turkey in 2011 in a *K. pneumoniae* isolate from an Iraqi leukemia patient (Poirel *et al*.,2007). Subsequently, another group probed NDM-1 in several types of carbapenemase-resistant gram-negative bacilli, but they came up empty (Rasheed *et al*.,2013). Blood was used as the source for isolating all NDM-1 generating strains.

In addition, both carbapenemases (OXA-48 and NDM-1) were present in all blood isolates, and two of them (13% of the total) were present in both. Seventy-six percent of bacteria were found to have the -lactamase combination OXA-48, SHV-1-like, TEM-1-like, and CTX-M-type -lactamase. Antibiotics such as tigecycline, colistin, trimethoprim/sulfamethoxazole, gentamicin, tobramycin, and amikacin are able to kill them (Iraz *et al*.,2015). After those from the respiratory tract (17.6%), pus and wound swabs (11.76%), and blood cultures (5.6%), impenem-resistant isolates found in urine had the highest proportion of NDM producers. This was followed by those from the respiratory tract (17.6%).

This finding was reached by many studies (Chandola *et al*., 2018). Eighty-three of the isolates (80.9%) were resistant to carbapenems, while 57.5% of the isolates obtained from the outpatient setting were resistant to carbapenems. The most common CPase, as determined by molecular characterisation, was blaNDM (80.5%), found in 54 isolates, followed by blaVIM (36.4%), found in 24 isolates. 28.6% of 20 *K. pneumoniae* tested positive for blaKPC, while 26.0% of 17 *K. pneumoniae* tested positive for

blaOXA-48. Interestingly, more than half of the Enterobacterales isolates (54.5%) included multiple copies of the CPase-encoding gene. The most common combination of bla genes was blaNDM and blaVIM (31.2%), followed by blaNDM and blaKPC (24.7%), and finally blaVIM and blaKPC (13%). (Abdelaziz,2022). The overall phenotypic resistance to carbapenem was found to be 23.3% (53/227) while the frequency of genotypic resistance was determined to be 43.1% (98/227).

The blaOXA-48-like gene was found in 36.4% of the population, followed by the blaIMP gene in 19.4%, the blaVIM gene in 17.1%, the blaKPC gene in 14.0%, and the blaNDM gene in 13.2%. (Ssekatawa *et al*., 2021). We discovered that a larger percentage of carbapenemase-producing *K. pneumoniae* was isolated from urine samples (9/32, or 27%), which is consistent with research by Hashemizadeh *et al*. (2020). Ten of the twenty-eight (35.7%) carbapenemase-producing *K. pneumoniae* isolates were obtained from urine, whereas just one was obtained from sputum (3.6%). (Awoke *et al*.,2022).

Conclusions:

From the finding of the current study conclude the following

1. All carbapenems antibiotic (doripenem , ertapenem , imipinem meropenem) are the most effective against *Klebsiella pneumonia* in Beta-lactam antibiotics but also presence of some resistance isolate

2. Penicillin is never effective against *Klebsiella pneumonia* with 100% resistance

٣. Bacterial isolates that contain blaNDM, blaKPC, and blaOXA-48 were resistance to all antibiotics.

Recommendations:

١. It is need to carry out comprehensive study for carbapenemase gene sequences and detect the type and the site of mutation on these genes.

٢. It is important to use the sensitivity test for checking the ability of isolated *klebsiella pneumoniae* toward the antibiotics in the hospitals.

٣. Studying other genes related with β-lactam resistance.

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جمهورية العراق
وزاره التعليم العالي والبحث العلمي
جامعة بابل - كليه الطب

دراسة المقاومة المتعددة لـ الكلبسيلا الرئوية المعزولة من المرضى الراقدين في المستشفى في مدينة الحلة

رسالة مقدمة الى مجلس كلية الطب / جامعه بابل

كجزء من متطلبات نيل درجة الماجستير

في الأدوية / الأدوية والسموم

من قبل

مصطفى محمد غني حمود

بكالوريوس صيدلة (٢٠١٦)

كليه الصيدلة / جامعة بابل

بإشراف

الاستاذ المساعد الدكتور

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دكتوراه في علم الادوية

الاستاذ الدكتور

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دكتوراه في الاحياء المجهرية

٢٠٢٣ م

١٤٤٤ هـ

الخلاصة

المضاد الحيوي هو نوع من المواد المضادة للميكروبات و فعالة ضد البكتيريا. وهو أهم نوع من العوامل المضادة لمكافحة الالتهابات البكتيرية ، وتستخدم أدوية المضادات الحيوية على نطاق واسع في علاج العدوى والوقاية منها. المضادات الحيوية (بيتا لاكتام) هي مضادات حيوية تحتوي على حلقة بيتا لاكتام في تركيبتها الكيميائية. وهذا يشمل مشتقات البنسلين ، والسيفالوسبورينات ، والمونوباكتام ، والكاربابينيمات

توجد بكتريا الكلبسيلا الرئوية في كل مكان في الطبيعة بما في ذلك الماء والتربة والحيوانات ، ويمكنها استعمار الأجهزة الطبية وبيئة الرعاية الصحية. تعتبر أنواع بكتريا الكليبسيلا من مسببات الأمراض الانتهازية التي تستعمر الأسطح المخاطية دون التسبب في أمراض ؛ ومع ذلك ، قد ينتشر من الغشاء المخاطي إلى أنسجة أخرى مسببة التهابات مهددة للحياة بما في ذلك الالتهاب الرئوي والتهابات المسالك البولية والتهابات مجرى الدم والإنتان. عدوى بكتريا الرئوية هي مشكلة خاصة بين حديثي الولادة وكبار السن والأفراد الذين يعانون من نقص المناعة في بيئة الرعاية الصحية.

هدفت الدراسة إلى تحديد أنماط مقاومة المضادات الحيوية في بكتريا الكليبسيلا الرئوية و تحديد المضاد الحيوي الأكثر فعالية في علاج العدوى

جمعت ١٥٥ عينة من مستشفيات بابل (مستشفى الحلة الجراحي، مستشفى مرجان التعليمي ، مشتشفى بابل للولادة والاطفال) و من مصادر مختلفة للمرضى والتي تشمل (البلغم ، الدم ، الحروق ، البراز والبول) ومن كلا الجنسين خلال الفترة من سبتمبر ٢٠٢١ إلى ديسمبر ٢٠٢٢. فقط اثنان وثلاثون عزلة (٢١٪) *من بكتريا الكليبسيلا الرئوية* تم تحديدها وفقًا للصفات المظهرية والاختبارات البيوكيميائية: ٧ (٢٢٪) عزلات من البلغم ، ٩ (٢٨٪) عزلات من البول ، ٥ (١٦٪) عزلات من البراز ، ٣ (٩٪) عزلات من الدم ، ٥ (١٦٪)) من الجرح وكذلك ٣ (٩٪) عزلات من المهبل. وتبين ان ٩٢ عينة (٤١٪) لذكور و ٦٣ (٥٩٪) إناث ، وتراوحت الفئات العمرية للمرضى المشاركين في الدراسة من سنة إلى سبعين سنة. تراوح عمر المرضى بين ٤-٧٠ سنة (المتوسط ، ٣٩,٨٦ ± ١٥,٧ درجة مئوية). توجد فروق ذات دلالة إحصائية بين الفئات العمرية ($p< 0.05$) ، حيث تم تقسيم المرضى إلى أربع مجموعات حسب أعمارهم. كانت الأناث أكثر من الذكور بشكل عام ؛

تم اجراء اختبارات الحساسية للمضادات الحيوية للعزلات البكتيرية ل ١٣ نوع من المضادات الحيوية ، باستخدام طريقة الانتشار القرصي وفقًا لـ CLSI-2022. أظهرت النتائج أن نسب المقاومة لهذه المضادات الحيوية كانت كالتالي: Agumentin 81%، Aztreonam 88%، Ceftazidim 75%، Cefixime 75%، Cephalotin 84%، Ceftriaxone 72%، Cefotaxime 72%، Doripenem 50%، Ertapenem 56%، Imipinem 47%، Meropenem 56%، Piper. / Tazo. 78%، Pencillin 100% . تبين أن جميع العزلات (١٠٠٪) في الدراسة الحالية مقاومة للبنسلين ، و ٢٨٪ من العزلات كانت مقاومة لجميع المضادات الحيوية. ٩٪ من العزلات كانت مقاومة لـ ١٢ مضاد حيوي ، ٦٪ من العزلات كانت مقاومة لـ ١١ مضاد حيوي. أظهرت هذه الدراسة أيضًا أن معظم عزلات *بكتريا الكليبسيلا الرئوية* . مقاومة ١٠٠٪ للأدوية المتعددة.

كان جين Carbapenemase موجبًا في ٨٧٪ (٣٢/٢٨) من بكتريا الكليبسيلا الرئوية ، بما في ذلك blaNDM في ٤٧٪ تم التعرف عليه في ١٥ عزلة من *بكتريا الكليبسيلا الرئوية* وغير موجود في ١٧ (٥٣٪) عزلة. لوحظ أعلى معدل مقاومة لمعظم المضادات الحيوية في عزلات blaKPC ثم جين blaNDM ، والعزلات التي تحتوي على جينات blaNDM و blaKPC و blaOXA-48 كانت مقاومة لجميع المضادات الحيوية (١٠٠٪) والعزلات التي تحتوي على جينات blaNDM و blaKPC كانت مقاومة ٦ مضادات حيوية (١٠٠٪) ، والعزلات التي تحتوي على blaNDM ، وجينات blaOXA-48 كانت مقاومة لـ ٣ مضادات حيوية (١٠٠٪) ، والعزلات التي تحتوي على جينات blaKPC ، و blaOXA-48 كانت مقاومة لـ ١٠ مضادات حيوية (١٠٠%) ، العزلات التي ليس بها أي جينات كانت مقاومة للبنسلين فقط (١٠٠٪). أما جين blaKPC فقد تم تحديده في (37)٪ 12 عزلة من البكتريا الرئوية وغائب في ٢٠ عزلة (٦٣٪). بينما تم التعرف على جين blaOXA-48 في ١٩ عزلة من بكتريا الكليبسيلا الرئوية (٥٩٪) وغائب في ١٣ (٤١٪) عزلة.

تم العثور على أعلى نسبة من blaKPC في البلغم (١٣%) يليه البول (٩٪) ، CSF (6٪) ، الدم ، الجرح (٣٪) ، البراز (٠٪) ، أعلى نسبة للجين blaOXA-48 وجدت. في البول (٢٧٪) يليه الجرح ، البلغم (٩٪) ، CSF (6٪) ، الدم والبراز (٣٪) ، تم العثور على أعلى نسبة من blaNDM في البول (١٦٪) يليه البلغم (٩٪). ، دم ، براز ، CSF (٦٪) ، جرح (٣٪).

في الاستنتاجات ، أظهرت النتائج أن جميع المضادات الحيوية من نوع carbapenems هي الأكثر فعالية ضد الكليبسيلا الرئوية في مجموعة Beta-lactam بينما كانت المقاومة للبنسلين ١٠٠٪. كانت العزلات البكتيرية المحتوية على blaNDM و blaKPC و blaOXA-48 مقاومة لجميع المضادات الحيوية.