Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Diyala

College of Veterinary Medicine

Department of Internal and Preventive Medicine

Study Hematological and Histopathological Changes in Albino Male Rabbits Infected Experimentally by *Klebsiella pneumonia* Isolated from Human

A Thesis

Submitted to the Council of the College of Veterinary Medicine/ University of Diyala in partial Fulfillments of the Requirement for degree of Master of Science in Veterinary medicine/ Zoonotic diseases.

By

Hiba Yassen Abbass

Supervised By

Prof. Nazar Jabar (Ph.D) **Assist.Prof. Anas A.Humadi (Ph.D)**

2025 A.D **1446 A.H**

Abstract

The study was conducted on 65 urine samples, collected from patients suffering from urinary tract infection, 125 blood samples collected from pediatric patients suffering from unexplained fever and 100 stool samples collected from patients suffering from persistent diarrhea, from both Genders with different ages, during the period (September–December 2023), at AL- Batool Hospital, and Ba'quba Teaching Hospital- Iraq, Diyala of Ba'qubah City. The samples of urine, stool and blood were submitted to bacterial examinations in order to isolate and identify *Klebsiella pneumoniae*. The samples were cultured on selective media (MacConkey and EMB agar) for morphological examination then identified by biochemical test and confirmed by Vitek-2 test. Results of total bacterial isolates which diagnosed as *Klebsiella pneumoniae sp. pneumoniae* was only six isolates out of 290 (2.30%). Two positive results out of (65) urine samples (3.07%), three positive results out of (100) stool samples (3%), and only one positive result from 125 blood samples (0.8%).

An experimental study was conducted in 30 albino male rabbits, After the two-weeks of adaption period, in the Animal House of College of Veterinary Medicine. A total of 30 male rabbits were divided into three groups: First group (GI): was given (1CC /animal) an oral dose of phosphate buffer saline (PBS) by a stomach tube as a control group for 60 days, second group (GII), were given one dose weekly (1CC) of viable *K. pneumoniae* (1 X 10^{6} CFU/ml) orally by stomach tube for 60 days, third group (GIII): were given twice dose weekly were given (1CC) viable *K. pneumonia* (1 X 10^{6} CFU/ml) orally by stomach tube for 60 days.

During the (14 days) Albino male rabbits exposure to viable *K. pneumoniae sp. pneumoniae*, the main clinical signs were observed in the

second and third group, including fever, dyspnea, diarrhea, cramps, and difficult urination have also been seen. After (60) days of experiment, under general anaesthesia the whole blood samples were collected from the heart and then put in EDTA tube to use of hematological analysis and DNA comet assay.

Then Hematological analysis done at the Veterinary Hospital of Baghdad and DNA comet assay done on AL-Nahrin University and measure the damage of DNA in head and tail in lymphocytes.The animals were euthanized and collection of tissue samples about one centimeter in length were obtained from internal organ (Intestine, Liver, spleen, lung and kidney) for observe grossly and histopathologically. *Klebsiella pneumoniae* caused severe pathological changes in different organs, particularly lung tissue, kidneys, and gastro intestinal tract (GIT).

The result of blood test showed that there was a significant decrease of RBCs, Hb and PCV, while significant increase in WBCs, lymphocytes, monocytes and neutrophil in third and second group in compare to control group.DNA comet assay: The lymphocytes were isolated by blood centrifugation and collected the buffy coat then diluted with 5ml Phosphate Buffer Saline (PBS) and centrifuged, after a number of steps which done until lymphocytes cells precipitated and collected, then DNA comet process done and examine slide under fluorescent microscope, Pictures of DNA damage quantification by use image analysis software comet score program. which show severe damage in lymphocytes and the parameter modification tail length, tail intensity, tail moment and percentage of DNA in tail measured, a comparison between the three groups was done, observing severe damage in lymphocytes in the third group and slightly damage in the second group in comparison to no damage in control group.

Finally, the pathological changes of three groups for samples were obtained from the intestine, liver spleen, lung and kidney, after histology prcedure and staining by hematoxylin and eosin stain then microscopic examination, the gross appearance of first group (control) showed no significant of any pathological changes in all organs (Intestine, liver, spleen, lung and kidney), while in second and third group, intestine histopathological changes included: edema in sub muscular layer with hemorrhage and infiltration of mononuclear cells in mucosa and sub-mucosa, increased in crypt goblet cells with mucin and congestion of blood vessels, elongated villi, and muscle vacuoles.

Liver histopathological changes represented by causing liver abscesses, hemorrhage also showed edema and congested vein, necrotic hepatocytes with vacuoles, and finally acute cellular swelling, and mononuclear cells surrounded the bile duct, severe mononuclear cells pre portal area with increase in kupffer cells, also showed width sinusoid with fibrin, also showed liver cirrhosis, hemorrhage, with mononuclear cells infiltration.

Spleen histopathological changes included thickening of capsule with severe hemosiderosis, hemorrhage with severe spleenitis, congested central atrophy, depleted pulp with hemorrhage, depleted follicle, severe edema, also showed macrophage laden hemosiderin, free hemosiderin.

Lung histopathological changes included: interstitial pneumonia with hemorrhage with ballooning emphysema, and dilated blood vessels, mononuclear cells infiltration with thickening artery, also showed increase in epithelial columnar cells of bronchus, hyperemia of artery, in other section showed edema, congested of blood vessels with slight thickening in the alveolar cells, thickening in interlobular septa, also thrombus in pulmonary artery also showed fibrosis of bronchus, hyperplasia of epithelium with arteriosclerosis, alveoli atelectasis.

Histopathological changes of Kidney included cortex with acute cellular swelling, edema with interstitial nephritis, dilated of blood vessels, infiltration of inflammatory cells (mononuclear cells), congested of blood vessels for glomeruli tuft, interstitial hemorrhage, narrowing of bowman space with acute cellular swelling and atrophied glomeruli.

Chapter

one

Introduction

1.1 Introduction

Klebsiella pneumoniae (*K. pneumoniae*) bacteria is a non-motile, Gram negative, encapsulated, rod about (0.6- 6μm) in length and (0.3-1μm) in width, facultative anaerobic, possessing a prominent polysaccharide capsule, ubiquitous endemic pathogen. It belongs to the family Enterobacteriaceae and is considered one of the most significant species of the genus Klebsiella. It exists as normal flora in human and animal gastrointestinal tracts and causes recurrent infections in humans as well as immune-compromised patients and animals. Most infections caused by Klebsiella species result from contaminated food consumption, such as musty fish and/or water **(**Lau *et al.*, 2007 and Priyanka *et al.*, 2020). In addition *Klebsiella pneumoniae* isolated from the botanical environment, soil and water, on which they are found as normally inhabitants (Grimont and Grimont 2006 and Abbas *et al.*, 2024).

Human beings are considered the primary reservoir and carrier for *K. pneumoniae*, it is carried in the community about (1-6%) in the nasopharynx and (5-38%) in stool samples, species of Klebsiella are rarely found on the skin (Podschun and Ullmann, 1998). Upon entering the body, the bacteria may exhibit significant levels of pathogenicity and resistance to antibiotics, and they're responsible for severe infections in the respiratory system (pneumonia), urinary tract infection, meningitis, and blood stream infection (bacteremia), in addition to septicemia and suppurative abscesses at different sites (Paczosa and Mecsas., 2016 and Ashurst and Dawson, 2018).

Klebsiella has been present as a significant nosocomial pathogen in neonatal care units. Nosocomial Klebsiella infections are also noticeably troublesome, particularly in premature babies and intensive care units (ICUs) due to easily colonized pediatric patients by *Klebsiella spp.* The oropharyngeal and intestinal tracts act as the reservoirs for nosocomial

outbreaks. In fact, *Klebsiella pneumoniae* has been reported as a notable cause of infections in patients with indwelling urinary catheters (Haryani *et al.*, 2007 and Gorrie *et al.*, 2017).

The mortality rate of bloodstream infections generated by *K. pneumoniae* varied between 20 and 30%. The population death rate was around 1.3 per 100,000 persons, with especially hypervirulent variant strains induced by *K. pneumoniae* linked to 50% mortality rates (Li *et al.*, 2023). Pneumonia can colonize the colon of a patient with ulcerative colitis and can be isolated from them, pathological analyses showed that but without given clinical treatment. Pneumonia from healthy persons was collected by Tianjin Union Medical Center (Zhang *et al.*, 2023). *Klebsiella pneumoniae* is considered an opportunistic pathogen of hospitalized patients. particularly those who are immune compromised, leading to increased morbidity and death because of its many resistance mechanisms (Wang *et al.*, 2020).

Comet assays used to identify DNA damage and applications in genotoxicity testing, molecular epidemiology, human bio-monitoring, ecogenotoxicology, and DNA damage and repair research (Møller *et al.*, 2020 and Jiang *et al.*, 2023). DNA damage in mammalian cells can be detected by DNA comet assay, the degree of damage is determined in peripheral blood lymphocytes. It's very accurate at estimating low-level damage, and it has the ability to limited detection of the structural organization of the DNA (Kuchařová *et al.*, 2019; Collins *et al.*, 2023).

Many studies have been done on the genus Klebsiella in both animals and humans, but little research has been done about the complications and pathogenicity of *K. pneumoniae* by DNA comet assay procedures being done on blood to get DNA modifications that can be analyzed by using a comet score program.

According to that, we decided to study DNA damage in lymphocytes of Albino male Rabbits which are infected experimentally through oral with *K. pneumoniae sp. pneumoniae* isolates from patients of hospitals in Ba'qubah City, Center of Diyala Governorate, Iraq by DNA comet assay technique.

1.2 Aims of study

The study aimed to:

1. Isolation and identification of *Klebsiella pneumoniae* from urine of patient with UTI, stool and blood of patients suffering from certain disturbances by conventional methods and Vitek-2.
2. Experimentally study the DNA damage by DNA comet assay, hematological parameters and the histopathological changes of the internal organs of rabbits experimentally infected with *K. pneumoniae* (Intestine, Liver, spleen, lung and kidney).

Chapter Two

Literatures Review

2. Literatures Review

2.1 *Klebsiella spp*

The genus Klebsiella belongs to the family Enterobacteriaceae, The organisms are named by Trevisan (1885), honer to German microbiologist Edwin Klebs, a 19th century (1834–1913) (Oliveira and Reygaert, 2020). First time described of *Klebsiella pneumonia* as an encapsulated bacillus by Carl Friedlander in 1882, that isolated them from the lung of patients with pneumonia, especially those with immune compromised individuals such as those suffering from chronic diseases or alcoholics, and named them Friedlander's bacillus (Friedlaender, 1882 and Calfee, 2017).

K. pneumoniae is a Gram-negative bacillus that is encapsulated, non-motile, thick and short, in compare with other Enterobacteriaceae families, with diameters ranging from (0.3–1μm) micrometers and lengths of (0.6–6μm) micrometers (Keynan and Rubinstein, 2007). They occur as rods with rounded ends, and they can found in shortened chains, pairs or in one cell. Lactose, sucrose, and glucose fermenting, behind which grow facultative anaerobic or aerobically growing pathogens, has the ability to consume urea, and its colonies are large, pink, smooth on the center and mucoid on MacConkey agar. It also produces gas from lactose fermenting at 44.5°C, is positive in the utilization of malonate, Voges-Proskauer test (Vi-test), is also capable of reducing nitrates to nitrite, and does not produce H2S and is non-hemolytic on blood agar (Ryan *et al.*, 2004 and Goldman and Green., 2009).

K. pneumoniae is a significant member of the Klebsiella genus in the Enterobacteriaceae family. It is a type of bacteria that can survive with or without oxygen, is not motile, has a rod-like shape, and is classified as gram-

negative. It is lactose-fermenting and has a noticeable polysaccharide capsule that covers its entire surface. This capsule contributes to its large size when observed under a microscope using gram stain and also helps the bacteria resist the various defense mechanisms of the host. *Klebsiella pneumoniae* was identified more than a century ago as a causative agent (Puspanadan *et al.,* 2012). Also it is cause infections in the lower biliary tracts and urinary (Arnold *et al.*, 2011).

The group ESKAPE, which includes six types of bacteria (*Enterobacter species, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterococcus faecium*). Recently, these bacteria have been susceptible to blocking the effects of common antibiotics like carbapnems, methicillin, vancomycin … *etc.* (Rice, 2010; Santajit and Indrawattana, 2016 and De Oliverira *et al.,* 2020).

According to Brisse *et al., (*2006) who mentioned in the global ranking the taxonomic history of the Klebsiella species in the family Enterobacteriaceae,.

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

The Klebsiella species and subspecies are classified using a variety of criteria. Klebsiella is divided into five types based on differences in biochemical activity, including *K. aerogenes, K. pneumonia, K. ozaenae, K. edwardsii, and K. rhinoscleromati* (Cowan *et al.*, 1960).

2.2 Genus: Klebsiella

The Klebsiella genus includes number of species, which includes species that are part of the *K. pneumoniae* species complex (KpSC) as well as other Klebsiella species such as *K. terrigena, K. indica, K. spallanzanii, K. oxytoca, K. huaxiensis, K. pasteurii, K. grimontii, and K. michiganensis*, planticola, ozaenae, ornithinolytica, variicola, mobilis, granulomatis, singaporensis, and rhinoscleromatis (Wyres *et al.*, 2020).

Klebsiella pneumonia can remain as not contagious in the human respiratory tract and intestines. Complex and changeable relation between human hosts and *K. Pneumoniae*, it may be commensal, opportunistic or pathogenic normally (Togawa *et al.*, 2015 and Ku *et al.*, 2017). Skin, gut and respiratory tract are common site of commensal colonization. Commensal colonization in the gut, the skin, and the respiratory tract is common, but many factors interact with prevalence estimates include: recent health-care contact, geographical location and age (Gorrie *et al.*, 2017 and Martin and Bachman., 2018).

In addition, nosocomial and community-acquired infections caused by *K. pneumoniae*, which normally colonizes the human intestine, are not contagious, but if it enters other parts of the body, Klebsiella can cause different illnesses, including: septicemia, pneumonia, urinary tract infections, and meningitis in the nervous system (Li and Huang., 2019). *K. pneumoniae* has three diversity that each constitute a subspecies according to taxonomy of *Klebsiella spp.*

- *K. pneumoniae sub sp. pneumoniae*;
- *K. pneumoniae sub sp. ozaenae; and*
- *K. pneumoniae sub sp. rhinoscleromatis* (Ørskov and Ørskov., 1984 and Dong *et al.*, 2022).

In 2006, the Genome Sequencing Center measured the genome size of the *Klebsiella pneumoniae* bacterium, which has one chromosome of about 5.5 megabase pairs (Mbp) in size and contains around 5,500 genes (Holt *et al.*, 2015 and Wyres and Holt, 2016 and Sethuvel *et al.*, 2019).

2.3 Sources of Infection in Human.

Vegetables may be a source of infection with *K. pneumoniae*, raw vegetables are often eaten in salads and other meals. *Klebsiella pneumoniae* is often present in the oral cavity, skin, and intestines and is also prevalent in healthcare environments and medical equipment (Abu-Zaid *et al.*, 2016).

Klebsiella pneumoniae biofilms on medical equipment, such as endotracheal tubes and catheters, are a major cause of infection in catheterized patients (Guerra *et al.*, 2022). Transmission and spread modes in hospital patient to patient following contacts with positive colonizers, also transmission via medical travel (Navon-Venezia *et al.*, 2017).

Opportunistic *Klebsiella pneumoniae* primarily impacts those with low immune systems or those debilitated by prior diseases. *Klebsiella pneumoniae* often colonizes the gastrointestinal system before causing nosocomial infections. It may also be detected in the urinary tract, respiratory tract, and blood. Klebsiella transmission between patients occurs through contaminated medical equipment, medical personnel's hands, and blood products. The entry points for Klebsiella infections are: respiratory, surgical wounds, catheter sites, peritoneum, urinary, and biliary tracts (Paczosa and Mecsas., 2016). Pyogenic liver abscesses are an emerging worldwide disease caused by serotypes K1/K2 (KLA), often leading to metastatic infections of the central nervous system, and they have become more prevalent globally in the last twenty years. Also, metastatic spread is a distinctive feature of hypermucoviscous *K. pneumoniae* and is rare among enteric Gram-negative

bacteria when the host immunity is active. *Klebsiella pneumoniae* was identified as a significant foodborne pathogen in fresh vegetables (Hamilton *et al.,* 2006 and Aguilar-Zapata *et al.*, 2022).

In hospitals, *K. pneumoniae* spreads from person to person. Interestingly, the ability to spread *Klebsiella pneumonia*, Carbapenem-resistant (CRKP), varies between different carriers; only a few of them, probably those with a higher rectal *K. pneumoniae* concentration, are significantly responsible for environmental contamination (Liao *et al.*, 2021). Gorrie *et al.* (2017) conclude that colonization of *K. pneumoniae* is an important risk factor for infection in the intensive care unit (ICU) and indicate ~50% of *K. pneumoniae* infections result from microbiota of patients' own. colonization Screening for admission could limit the risk of infection for the colonized patient and others.

2.4 Pathogenicity, Occurrence of *Klebsiella pneumoniae.*

2.4.1 Antibiotic Resistant of *Klebsiella pneumoniae.*

As a member of the Enterobacteriaceae family, *Klebsiella pneumoniae* is a serious public health problem that has high economic costs in addition to high morbidity and mortality, it is found in soil, plants, and water and is a normal component of the gastrointestinal tract's microbiota, as well as the nasopharynx, so the rise of pathogens that are resistant to antibiotics, which associated with nosocomial infections (Ahmed and Alaa, 2016 and Martin and Bachman., 2018).

In some cases, the occurrence of a reduction in the concentration of antibiotics and the limited or nonexistent effectiveness of antibiotics in inhibiting bacterial growth result from affected bacteria by external factors that may show a response by activating efflux pump proteins encoded within

their genomes. This activation subsequently leads to the elimination of antimicrobial or chemical agents from the bacterial cell (Huang *et al.*, 2022).

One essential characteristic of *K. pneumoniae* that has facilitated its continuous development its capacity to acquire new genetic material that makes this bacterium exhibit resistance to many antibiotics, hence posing a challenge in the treatment of human infections, it is particularly prevalent in hospital settings and is linked with a high incidence of illness and high mortality rates owing to the limited availability of effective treatment choices (Nirwati *et al.,* 2019).

Klebsiella pneumoniae has many extra genomes made up of plasmids and gene sites on chromosomes. *K. pneumoniae* strains can be classified into three groups based on their extra genomes: opportunistic, hypervirulent, and multidrug-resistant (MDR) (Martin and Bachman, 2018).

Most ESKAPE pathogens, *Klebsiella pneumoniae being* one of them, are multidrug resistant because they have resistance genes that are carried on the bacterial chromosome, the plasmid, mechanisms of drug resistance include drug inactivation or alteration, modification of drug binding sites or targets, changes in cell permeability that result in reduced intracellular drug accumulation, biofilm formation, and the ability to escape the biocide action of antimicrobial agents (Santajit and Indrawattana., 2016).Therefore, the infection become more difficult or impossible to treat and can lead to serious infection and death (Ontong *et al.,* 2021).

Subsequently, it has been acknowledged as an additional circulating pathotype that induces elevated pathogenicity and mortality in association with carbapenemase-producing *Klebsiella pneumoniae* (cKP) strains (Zhu *et al.,* 2021 and Spadar *et al.,* 2023). Most strains of *K. pneumoniae* isolated from hospitalized patients have the capacity to produce biofilm. Also, the MDR-*Kp* strains tend to form stronger biofilms than the non-MDR strains (Shadkam, *et al.,* 2021). The ability to produce the beta

lactmase (ESBL) enzyme exceeds 50%–60% non-susceptibility for fluoroquinolones, third-generation cephalosporins, and aminoglycosides in 2015 carbapenem-resistant *K. pneumoniae* (CRKP) presenting in several countries, such as Italy, Romania, and Greece, while non-susceptible rates of 40%–60%. The percentage of carbapenem-non-susceptible *K. pneumoniae* is extremely high in endemic countries (Navon-Venezia *et al.,* 2017).There are five antibiotics classes used to treated *K. pneumonia* infections, including aminoglycosides, β-lactams, quinolones, polymyxins and tigecycline (Munita and Arias, 2016).

2.4.2 Virulence factors classification

The key determinants contributing to the pathogenicity of *K. pneumoniae* include lipopolysaccharide and polysaccharide capsules. The virulence factors fimbriae types 1 and 3 of capsule polysaccharides are essential for the development of biofilm in *K. pneumoniae*, which has a variety of virulence characteristics, including antibiotic resistance, which affects its ability to cause infectious illnesses. Siderophores and Capsules are main virulence factors that play a vital role in the hypermucoviscosit phenotype of hypervirulent *K. pneumoniae* (Russo and Marr, 2019 and Riwu *et al.,* 2022).

Many research which compare between type of Klebsiella virulence factors showed that hvKp strains have the ability to produce bigger and very active iron-absorbing molecules in comparison to non-virulent strains, the different in iron-absorbing capabilities may contribute to enhance virulence and pathogenicity exhibited by hvKp strains (Paczosa and Mecsas, 2016).

Pneumonia virulence factors, which include: adhesion factors, capsule antigens, enterotoxin production like lipopolysaccharide, as resistance killer effects for system and serum, increase iron (Siderophore),

and antibiotic multi-resistance are considered the main reasons for spread of hospital acquired infections. There are seven key groups of virulence factors that are primarily responsible for the virulence of *K. pneumoniae,* which is crucial in pathogenicity to elude the host's innate immune responses (Jasim *et al.,* 2020) and include:

- The capsule (CPS)
- Lipopolysaccharide (LPS).
- Siderophores and fimbrial adhesions
- Hypermucoviscous phenotype (HMP)
- Outer membrane proteins
- B-Lactam antibiotics

The most important virulence elements are biofilm production, which shields or inhibits *K. pneumoniae* from phagocytic infection by engulfing cells through phagocytosis (Lan *et al.,* 2020).

2.4.2.1 The capsule (CPS):

The capsule that surrounds the surface of *K. pneumoniae* acts as the primary virulence factor associated with the viscous phenotype, and it protects the bacteria from phagocytosis and prevents bactericidal serum factors effects. *K. pneumoniae* escaped complement, antimicrobial peptides, phagocytosis, and specific antibodies by it is surrounded capsules make it difficult for bacteria to be bound, while active suppression and attack of immune cells induced by capsules are very infrequently seen (Paczosa and Mecsas, 2016). The bacteria's capacity to move through the blood and induce sepsis depends on both CPS and lipopolysaccharide (LPS), and CPS type K1 is a key virulence factor in *K. pneumoniae* that causes pyogenic liver abscess (PLA) (Hsieh *et al.,* 2012 and Alcántar-Curiel and Girón, 2015).

Genes that produce capsules are located in the chromosome region of capsular polysaccharide synthesis (cps) (Ernst *et al.*, 2020). Some *Klebsiella pneumoniae* strains polysaccharide can activate lectine complement pathway throught interaction with manose binding lectine (MBL) and this occurs through capsular polysaccharides containing rhamnobiose or mannobiose recognition. Strains without capsules show a high level of C3b on their surface and are phagocytosed by epithelial cells of the lung (Sahly *et al.,* 2009 and de Astorza *et al.*, 2004).

2.4.2.2 Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS) 4O antigen (O polysaccharide (OPS)) and Capsular polysaccharide (K antigen) source of main virulence factors that protect the bacteria from the host immune system components. The O-antigen acts to prevent complement protein deposition. All gram-negative bacteria produce endotoxin Lipopolysaccharide, is made up of an oligosaccharide core, lipid A, and an O antigen (Clarke *et al.,* 2018).

The lipopolysaccharide (LPS) plays an important role, it can both activate immune responses and contribute to immune evasion. LPS O-antigens have the ability to bind to the complement component C3b, which enhances antigen presentation to T cells. In addition, LPS weakens the killing by complement-mediated means and facilitates the survival of bacteria (Bulati *et al.*, 2021).

2.4.2.3 Secration of Siderophores.

Siderophores are compounds characterized by their low molecular weight, which helps in the consumption process of iron in addition to their high affinity for ferric iron when bacteria are challenged with the host. Many

strains of bacteria that produce siderophores show higher mucoviscosity levels. Multidrug-resistant lineages and hypervirulents had distinguished predominant siderophore loci. Synergy of hypermucoviscosity reacts to high siderophore production during bacterial infection (Wei *et al.,* 2023). *K. pneumoniae* secreted siderophores which consider very necessary for reproduction of bacteria and proverbial virulence, and enhanced consumption of iron from the host which increase bacterial growth which lead to enhance invasion (Holden *et al.*, 2016).

2.4.2.4 Fimbria

Defined as multi-subunit structures acting as adherence factors, fimbria or pili, are filamentous organelles protruded outward on the surface of the bacterial cell, *K. pneumoniae* can produce two fimbrial adhesins (Costa *et al.,* 2015). *Klebsiella pneumoniae* have pilli on their surface, its primary role is the adherence of bacteria to the surfaces of host epithelial cells and it is important in the production of biofilms on biotic and abiotic surfaces (Guerra *et al.,* 2022). Interaction of bacteria with macrophages and immune cells occur with the presence or absence of a cell surface receptor. Other function of protein associated with type 1 fimbriae (Cano *et al.*, 2015).

2.4.2.5 Formation of Biofilm.

Biofilm are organized communities that resist human defense mechanisms and antimicrobial factors such as complement systems, phagocytosis, and antimicrobial peptides, bacterial Biofilms, characterized by their complex and varied architecture, surround bacteria within an extracellular matrix comprising proteins, carbohydrates, and genetic material arising from bacteria and host, it is act as maintains microorganisms during colonizing stage of host and it interferes with the pathogenesis of many

bacterial species by adhering to abiotic surfaces (Marks *et al.,* 2014 and Rabin *et al.,* 2015).

In the human being host, most bacterial infection biofilms are produced by more than one species of bacterial, most common bacterial organisms present in the communities include: *K. pneumoniae*, *Pseudomonas protegens and Pseudomonas aeruginosa* (Joshi *et al.,* 2021). 65–80% of bacterial illnesses caused by biofilms (Fux *et al.,* 2005 and Parvatkar and Majik, 2014 and Periasamy *et al.,* 2015).

2.4.2.6. B-Lactam Antibiotics

Beta-lactamases and carbapenemases are broad spread globally, which responsible the presence of antibiotic resistance genes, and play an important role in bacterial resistance to many types of antibiotics poses a big constraint on the choice of effective treatment for infections caused by *K. pneumonia*, at present, there are strains of *K. pneumoniae* that are capable of generating extended Spectrum (Saki *et al.,* 2022; Hawkey and Jones, 2009). The enzymes are responsible for lactam antibiotics deactivation, which are necessary group of antimicrobial agents used for the treatment success of patients infected with *K. pneumoniae*.

Consequently, the antimicrobial peptide colistin (AMP), also referred to as polymyxin E, has been used for the purpose of treating infections caused by multidrug-resistant *K. pneumoniae* (Olaitan *et al.,* 2014 and Uruén *et al.,* 2020). Several clinical isolates of *K. pneumoniae* are resistant to all available antibiotics, the colistin effectiveness considered as last resort antibiotics infection treated for multi-drug-resistant Klebsiella infections (Elemam *et al.,* 2009). Rises of multi-drug-resistant strains warrant the development of new classes of antimicrobial agents or following alternative strategies for treatment (Akpaka *et al.,* 2021).

2.4.2.7 Serum Complement System Resistance

The serum complement system plays a crucial role in enhancing the activity of phagocytic cells and/or directly destroying bacteria by membrane attack complex formation, hence aiding in the elimination of microbial pathogens. when there is complement system defects occur this allow for *K. pneumoniae* survival in serum (Bain *et al.,* 2020).

One of the most significant virulence factors of *K. pneumoniae* is resistance to the complement system because the vital role which played by this system in stimulating of humoral immunity against bacterial infection. As a result, various surface antigens of *K. pneumonia* are used, such as CPS, LPS and outer membrane protein-A (OmpA), to escape from the host complement immune system (Doorduijn *et al.,* 2016).

In addition, the ability of *K. pneumoniae* to resist reactive oxygen species (ROS) generation is considered one of the important virulence factors interfering with the *K. pneumoniae* pathogenesis and associated with resistance to respiratory burst and Ca2+ dependent killing. *K. pneumoniae* completed resistance by ability to regulate intracellular levels of Ca2+ ions within host cells. This process mediated by *K. pneumonia* surface-associated proteins especially those involved in adhesion (biofilm formation), such as fimbriae (pili) (Ahn *et al.,* 2016 and Mohammed *et al.,* 2020).

2.5 Target Organ and Infected Sites of *K. pneumonia*

The sites of infection and target organs infected by *Klebsiella pneumoniae* depend on the site of bacterial colonization. This colonization mostly occurs on the mucosal surface of tissue organs, particularly within the respiratory system.

In the nasopharynx, the colonization rate ranges from around 1% to 6%, while in the lower respiratory tract, it ranges from approximately 3% to 15% (Paczosa and Mecsas 2016).

On the other hand, *K. pneumoniae* causes a spectrum of destructive changes and severe inflammation during lung infection, typically affecting middle-aged as well as older men with debilitating symptoms demonstrated by producing a bloody, thick mucoid sputum, described as currant jelly sputum; occasionally necrosis, associated with hemorrhage, and interstitial pneumonia, which occurs with chronic bronchopneumonia within pulmonary tissue (Luan *et al.,* 2018 and Venkataraman *et al.,* 2018).

However, the primary reservoir for *Klebsiella pneumoniae* infection is in the gastrointestinal tract and hands of patients. It is causes of bacteremia, septicemia, and infections in immune-compromised individuals, such as those with diabetes mellitus, particularly in a hospital setting (Ling *et al.,* 2015).

Additionally, *Klebsiella pneumoniae* is frequently colonizing different body sites, including the urinary tract, surgical wounds, and biliary tract. This colonization can lead to the development of several clinical syndromes, such as urinary tract infections (UTIs), bacteremia, thrombophilia, cholecystitis with diarrhea, and wound infections with osteomyelitis and meningitis. (Li *et al.,* 2019).

Pneumonia can colonize the colon, and it is isolated from colon of patients with ulcerative colitis (UC), contribute to intestinal inflammation through activation caspase-11 inflammasomes, which was shown by pathologic examination without given clinical treatment. Collection of *K. Pneumoniae* from healthful individuals was collecting by Tianjin Union Medical Center (Zhang *et al.,* 2023).

2.6 Immune response against *Klebsiella pneumonia*

Klebsiella pneumoniae considered important bacterial pathogen that is responsible for causing both community-acquired infection and nosocomial diseases in many areas (Ashurst and Dawson, 2018). *K. pneumonia*e can spread from animal to animal and from person to person because it can colonize the respiratory tract, pharynx, gastrointestinal tract, and urinary tract. Because it causes food-borne disease, it can also spread from animal to human (Martin and Bachman, 2018).

Animal feces contain these pathogens, and Klebsiella shedding is spread by breathing in bacteria from the ground after being exposed to them through the respiratory tract. the bacteria then get into the urinary tract and cause infections there. When the bacteria get into the udder through feces, they cause mastitis, which causes significant economic losses in dairy herds (Abadullah and Zghair, 2016).

However, the virulence factors identified in the pulmonary sources of patients with pneumonia caused by *Klebsiella pneumoniae* exhibit distinct characteristics compared to strains isolated from UTI-causing.To initiate the process of infection, *Klebsiella pneumoniae* must successfully overcome the mechanical barriers. The first process of *K. pneumoniae* infection must successfully overcome the mechanical barriers (Riwu *et al.,* 2022).

Furthermore, *Klebsiella pneumoniae* evades both cellular innate immunity and humoral defenses through the utilization of its virulence factors. It is important to note that the immune response against *K. pneumoniae* clearance exhibits variability depending on the bacterial strain. Consequently, it is necessary to consider the interaction between different humoral and mechanical defenses, as well as the various types of cellular immune responses (Kaspar *et al.,* 2015and Xiong *et al.,* 2016).

Pneumonia infection in the respiratory system exhibits distinct characteristics compared to other types of pneumonia affecting lung tissue. These properties are associated with bacterial concentration factor, changes in plasma zinc levels, the condition of the affected organism or organ, concentrations of alpha-2-macroglobulin (also known as alpha-2-macrofetoprotein), and seromucoid levels (Berendt *et al.*, 1977).

During urinary tract infection the primary defense mechanism of the host cell against *K. pneumoniae* is chemical defense by decreasing the pH level of urine with increase urine excretion, which acts as a strong mechanical force, effectively eliminates bacterial infections, and act as a preventive measure against the introduction of *K. pneumoniae* into the urinary bladder (Groisman, 2001and Chapelle *et al*., 2021).

K. pneumoniae infected the respiratory tract, the host's defense mechanisms are initiated by the reaction of the pseudo-stratified epithelial lining in the trachea and bronchi. This lining is composed of goblet cells, cilia cells, and basal cells. The first line of defense is the mucociliary movement, which involves the accumulation of a mucus blanket. This mucus acts as a barrier against microbes and particles, capturing them. The ciliary lining then facilitates the movement of these trapped substances upwards, helping in their removal from the respiratory tract (Paczosa and Mecsas., 2016).

In addition, *K. pneumoniae* biofilm protects bacteria colonizing the GIT from physical and chemical obstacles and humoral and cellular immune defenses, especially the complement system, by preventing mediate bacterial killing by preventing complement cascade activation, which led to membrane attack complexes (MAT) and no pores in the bacterial surfaces (Sebghati and Clegg, 1999 and Rosen *et al.,* 2018).

On the other hand, reported that *K. pneumoniae* is resistant to the complement system because it has several surface antigens like CPS and

LPS, which allow it to evade host complement (C3 fragment) formed by alveolar pneumocyte type-2, which act as fixative macrophages and synthesize lung alveolar surfactant (Doorduijn *et al.*, 2016)

The mechanical defense mechanisms observed in the respiratory tissue during *K. pneumoniae* infection are mediated by the production of surfactants, specifically surfactant proteins A and B (SP-A and SP-B), as well as transferrin associated with immunoglobulins. These components play a crucial role in enhancing bacterial eradication by promoting the recruitment of neutrophils (Coya *et al.,* 2015).

Klebsiella pneumoniae is recognized as a pathogen that may exist both extracellularly and intracellularly. Consequently, both the extracellular and intracellular immune responses are of the greatest significance in effectively eliminating this pathogen and reducing the impact of infectious diseases caused by it (Belon *et al.,* 2015).

2.7 Pathogenesis.

It was shown by Jia *et al.* (2021) that damage in mitochondria caused by *K. pneumonia* plays an important role in the cellular damage it produces, especially in the death of epithelial cells. Damage to cells was caused by a process that triggered mitochondrial malfunction and dis-regulation in calcium (Hussain, 2019; Cheng *et al.,* 2021). Damage in mitochondria is usually characterized by a decrease in mitochondrial membrane potential (MMP), increases in reactive oxygen species (ROS), calcium, and morphological damage (Wang *et al.*, 2015).

In addition, after 24 hours of infection, a minor tissue degradation with inflammatory cell infiltration occurred in the peribronchial regions; however, by 48 hours post-infection, the lung injury was very severe, with extensive alveolar rupture, hemorrhage, vascular leakage, varied lesions, and

inflammatory cell infiltration. By 72 hours after treatment started, there was a decrease in lung damage and a reduction of inflammatory cell counts in the peribronchial and perivascular spaces (Liu *et al.*, 2020). Perlee *et al*. (2020) reported that the respiratory system immune response against Klebsiella infection occurs by stimulation of lung tissue to release caspase-11 and is an important thing for limiting the overgrowth of bacteria in the parenchyma of the lung. Primarily, the immune response was characterized by an increase in TNF, pro-inflammatory cytokines, as an important and primary signal in granuloma formation, a result of chronic inflammation against bacterial infection, which increased macrophage activation and cells recruited to the site of infection (Bai and Guo, 2023).

In the liver, bile accumulates in the parenchyma as a consequence of the creation of liver abscesses; this leads to extra-hepatic cholestasis owing to the dysfunction of fatty acids that are engulfed by macrophages (Chu *et al*., 2019). Similar findings were also published by Lefkowitch (2010) hepatobiliary loss occurs during *K. pneumoniae* infection because monocytes play a crucial role in preventing bacterial invasion of the liver, which may result in a life-threatening condition known as liver abscess.

Researchers have discovered a correlation between the incidence of infections that caused by hypervirulent *Klebsiella pneumoniae* (hvKp) and the development of Klebsiella-induced liver abscesses (KLAs). The virulence factor of *Klebsiella pneumoniae* has also been associated with the development of pyogenic liver abscess (PLA) in the host (Kamal *et al*., 2017).

In the intestinal tract and stomach, the isolation of *K. pneumoniae* from these organs may be stimulate the overproduction of polysaccharide capsule, excessive production cause weakens the immune system's resistance and leads to the elimination of several beneficial microorganisms. Kaur *et al.* (2018) and Raffelsberger *et al.* (2021) showed that disease-specific changes

to the gut microbiota may account for the higher incidence of *K. pnemoniae* and carriage in Crohn's disease and ulcerative colitis; they observed that GIT infection with severe *K. pneumoniae* colitis was caused by the establishment of resistant strains of *K. pneumoniae.* As a defense mechanism against *K. pneumonia* invasion, the natural defense mechanisms caused goblet cell hyperplasia, which results in the production of mucus, antimicrobial peptides, pattern recognition molecules, and immunoglobulin A (IgA).
Consistent with our findings. Zhang *et al.* (2012) found that young mice infected with *Klebsiella pneumoniae* can cause necrotizing enterocolitis NEC-like damage.

The pathological changes in heart tissue occurred due to the invasion and distribution of *K. pneumoniae* through the bloodstream. This resulted in abnormal blood flow towards a previously damaged valve, leading to the focal deposition of platelets and fibrin, which provided a site for the growth and colonization of bacteria (Riangwiwat and Dworkin, 2019).

2.8 DNA Comet assay technique

The comet assay is direct, sensitive, and inexpensive technique. Ostling and Johanson in 1984 was first developed the comet assay by demonstrating the migration of DNA fragments from nuclei under a neutral condition (Ostling and Johanson., 1984). This technique was developed later by Singh *et al.*,1988 that an alkaline condition lead to largely increased the specificity and reproducibility of test. Breaks of double-stranded DNA detected by use the neutral comet assay, while the alkaline comet assay is more accurate for smaller amounts of DNA breakage, including double- and single-stranded DNA breaks, DNA-DNA or DNA-protein cross-linking, alkali-labile sites, and DNA single-strand breaks associated with incomplete

excision repair sites (Shah *et al.,* 2016). The two types of assays allow visualization of DNA fragments and provide a direct way to evaluate damage to DNA under an electric field principal, migration of DNA fragments out of the nucleoid body ("comet head") makes a DNA stain in the agarose gel ("comet tail"). Through staining of nucleotide, can be quantified of extend DNA damage by analyzing "comets" formed by single-cell electrophoresis. Tail moment calculation can help to compare DNA damage among different experimental groups with traditional methods of DNA damage detection.

Also this assay technique is used to detect genotoxicity or cytotoxicity in eukaryote cells. This assay has been known to be rapid, cost-effective, and adaptive for in vivo studies. Several parameters, interfere with quantitative analysis for DNA damage, including tailed length, tailed nuclei, %DNA in the tail, and tail moment in the comet assay (Singh *et al.,* 1988; Tice *et al.,* 2000; Neri *et al.,* 2015).

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب البيطري

فرع الطب الباطني والوقائي

دراسة الصورة الدموية والتغيرات ألمرضية في ذكور الأرانب المهقاء المصابة تجريبياً بالكليبسيلا الرئوية المعزولة من الانسان

رسالة

مقدمه الى مجلس كلية الطب البيطري/ جامعة ديالى وهي جزء من متطلبات نيل درجه الماجستير في علوم الطب البيطري /الامراض المشتركة

من قبل

هبة ياسين عباس

اشراف

أ.د نزار جبار مصلح

أ.م.د انس عبد المجيد حمادي

2025 ميلادي

1446 هجري