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**Identification of *Klebsiella spp.* From different ruminants
secretion in Thi-Qar province**

A thesis

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By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قل هل يستوي الذين يعلمون والذين لا يعلمون

انما يتفكر اولي الالباب))

صداق الله العظيم

سورة طه الاية (١١٤)

“Certification”

We certify that this theses (**Identification of Klebsiella spp. From different ruminants secretion in Thi-Qar province**) was prepared under our supervision by (Arjewan Mohson Ajmi) at the Department of Microbiology collage of Veterinary Medicine, University Shatra in partial requirement for Master degree in Veterinary Medicine / Microbiology.

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Dedication

My homeland my believed country Iraq.

My father, who did not complete the journey with me.

My mother ,may god prolong her life.

To my dear husband than you for your sporting to me in every step of the way

to my beloved family, thank you for believing in me and for being strongest supporters . your love and sacrifices have been my motivation

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Summary

The current study include detection of *Klebsiella* species from variation infected cattle discharge. 120 samples and swabs were collected divided in to (40 from nasal secretion, 40 from vaginal discharge and finally 40 from a milk samples). These samples cultured on different culture media and identified by using cultural characteristic and microscopic appearance. *Klebsiella pneumoniae* Identified using VitekII system and confirm the last diagnosed of those organisms by using polymerase chain reaction (PCR) assay (16S rRNA). Polymerase chain reaction used to detected some gene K1,K2(which is one of several genes responsible for capsule formation), and also blaSHV gene which is one of the genes related with multi drug resistance.

Results of VitekII showed the bacterial isolation rate from 120 of the different cow samples 58(48.3%) which included different negative bacteria . From the milk samples results, referred that 20 (50%) which contained bacterial isolates, on the other hand, 20 (50%) of samples were negative for bacterial isolates. From positive cultures of milk samples several Gram negative bacterial isolates were identified such as *E.coli* 5 (25%), *Enterobacter cloacae complex* 3(15%), *Leclerciaadecarboxylata* 2(10%), *Klebsiella pneumonia* 2(10%) *Acinetobacter* spp. 2(10%) *Stenotrophomonas maltophillicia* 2 (10%), while other bacterial isolates were *Pseudomonas alcaligenes*, , *Providencia rettgeri*, *Pseudomonas stutzeri*, and *Pseudomonas aeruginosa* 1(5%) respectively.

From nasal swabs the total numbers were 40 samples. no growth was 20 the other negative bacteria 20(50%) include *E.coli* 13(65%), *Burkholderia cepacia* 1(5%), *Coronobactersakazakii* 1(5%), *Achromobacterdenitrificans* 1(5%), *Pseudomonas alcaligenes* 1 (5%), *Burkholderia galdiioli* 1(5%) and *Klebsiella pneumoniae* 2(10%). From 40 vaginal swabs isolated the present study involved no growth were in 22 swabs, while the positive numbers were 18(45%) included

E.coli 13(72,5%), *Escherichia hermannii* 1(5,5%), *Achromobacter lowffii* 1(5,5%), *Aeromonas hydrophila* 1(5,5%), *Pseudomonas aeruginosa* 1(5,5%) and *Enterobacter cloacae* complex 1(5,5%).

Klebsiella pneumoniae were isolated from milk samples in rate 2/20 (10%) . On the other hand, its isolated from nasal discharge in rate 2/20(10%) Nevertheless, *Klebsiella pneumoniae* could not be isolated from all vaginal secretion 0/18(0%).

Isolated strain of *Klebsiella pneumoniae* tested against 14 anti-biotic with various mechanisms of action such as (Ampicilin/ sulbactam, Piperacillin/Tazobactam, Cefotaxime, Ceftazidime, Ceftazidime/Avibactam, Ceftazidime/Tazobactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline and Trimethoprim/sulfamethoxazole). The result of this study indicated that all isolated of *Klebsiella pneumoniae* exhibited sensitive to all this antibiotic in rate 100%.

The result involved that K1,K2 genes were not detected in all samples of *Klebsiella*. Also, completely isolates of *Klebsiella pneumoniae* harbored blaSHV gene (100%), Also the result included that *Klebsiella pneumoniae* can be consider as major pathogen responsible for different medical case affected bovine in Al-Shatrah district in Thi-Qar province .

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List of Abbreviations

Abbreviations	Meanings
BHI	Brain Heart Infusion
%	Percentage
CFU	Colony forming unit
C°	Celsius Degree
DNA	Deoxyribonucleic Acid
DW	Distilled water
E.coli	Escherichia Coli
ESBLs	Extended Spectrum B-Lactamase
GNB	Gram-Negative Bacteria
G	Gram
H	Hour
MDR	Multidrug Resistant
K	Klebsiella
NCBI	National Center for Biotechnology
Mg	Milligram
mL	Milliliter
Nm	Nanometer
PCR	Polymerase Chain Reaction
PH	Potential of Hydrogen
Spp.	Species
rRNA	Ribosomal Ribonucleic Acid
RNA	Ribonucleic Acid
Rpm	Round per minute
U	Unit
V	Volt
LPS	Lipopolysaccharide
µg	Micron Gram

Chapter One

Introduction

1.Introduction

A significant category of zoonotic organisms worldwide are *Klebsiella spp.*, and there is a chance that people and dairy cows might contract them. (Song *et al.*, 2023). *Klebsiella* species are opportunistic infections that colonize mucosal surfaces without producing disease, yet they have the potential to move from the mucosa to other tissues and endanger life. (Paczosa and Mecsas, 2016).

Gram-negative, bacilliform, non-sporulation, non-ciliated bacteria with a thick cell wall, *Klebsiella* species are the cause of the mucous appearance of colony in a solid-state cultures grown in vitro as well as the invasion of bacteria in vivo. *Klebsiella pneumoniae* is thought to be the most clinically significant of the several *Klebsiella* species and subspecies in animals as well as humans. *Klebsiella* species can be found in soil, wastewater, plants, surface water, and other habitats. *Klebsiella pneumoniae* is pathogen capable of causing a different infections. Classically, *Klebsiella pneumoniae* is known the main causes of pneumonia, Urinary tract infections and bacteremia (Choby, *et al.*,2020). Bacterial pneumonia causes very economic loss to the beef industry and that increase in stress and viral infection, taking antibiotics to at-risk calves is often to prevent the disease. The mass of administration of antibiotic lead to negative result in meat production and that perhaps lead to increase of resistant pathogen (Vulikh *et al.*, 2019).

The most pathogenic bacteria which affected bovine is Gram-negative bacteria which is an significant cause of bovine mastitis all over the world (Schukken, *et al.*, 2013).The Sub clinical mastitis in cattle is a main and silent problem causes greater economic losses to the farmers. One of the primary causes of decreased milk quality and output is mastitis, which is defined as inflammation of the

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mammary glands' parenchyma and is characterized by pathologic alterations to glandular tissues as well as physical, chemical, and typically bacteriological abnormalities in milk (Kumari *et al.*,2018).

Klebsiella pnemonieae is an important bacterium that can significantly impact livestock health. this bacterium is found in the environment and can causes range of diseases in animals, leading to negative impacts on productivity and economic returns in the agricultural sector, there are a few studies which involved the role of *Klebsiella* spp. as a causative agents affected bovine in Thi-Qar province, there for the study aimed to:

1.2 Aime of study:

The current study was involved identification and isolation of *Klebsiella* spp. from different infected bovine recreation by the following:

1. Detection and diagnosed *klebsiella* spp. from different secretion of cattle in Thi-Qar province.
2. Identification of *klebsiella pneumoniae* using culturing, morphological characteristic and confirmed diagnosis by ViteKII system
3. Detection anti-microbial susceptibility test of *Klebsiella pneumoniae*.
4. Confirm the final diagnosed of those organism by using polymerase chain reaction (PCR)assay to detection 16srRNA and molecular study of some gene such as (K1, K2) that responsible of capsule, and blaSHv gene which responsible of drug resistance.

Chapter Two

Literatures Review

2. Literatures Review

2.1 Diseases caused by *Klebsiella pneumoniae*

The bacteria *Klebsiella* spp. specially *Klebsiella pneumoniae* are significant opportunistic pathogens that can lead to variety of clinical infections in both human and animals. These bacteria are known for their ability to develop resistance to multiple drugs making treatment challenging. In domestic animals *Klebsiella* species can act as reservoirs for pathogenic strains, potentially transmitting multidrug-resistant infection to humans , despite the public health implications , researches on *Klebsiella* infection in domestic animals tend to be limited. Often focusing on isolated case reports or specific outbreaks rather than comprehensive studies(Ribeiro, 2022).

Also Ribeiro (2022) highlight on the need for more extensive surveillance and investigation into the prevalence, transmission dynamics, and resistance patterns of *Klebsiella* species in domestic animals, Comprehending these elements is essential to creating preventative and treatment plans that reduce human infection risk and enhance animal health.

2.1.1 Respiratory infection

Respiratory tract infections are the most common disease, it's severity was vary according to age and sex (Monto, 2002). There are two types of respiratory tract infections. The first type, known as a lower respiratory tract infection, is the one that causes the greatest number of illnesses. The results of these infections are determined by pulmonary immunity. The second type consisted of upper respiratory tract infections, which included tonsillitis, pharyngitis, nasopharyngitis, and otitis (Jain, 2001; Mizgerd , 2012). One frequent opportunistic infection of the upper respiratory tract is *Klebsiella pneumoniae*. The isolation of afflicted animals

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and the provision of a warm, drought-free environment are two ways to control infectious respiratory illnesses. Antibiotics are used to treat bacterial pneumonias and stop subsequent bacterial infections. Additionally, non-steroidal anti-inflammatory medications must be used to lessen pyrexia and lung congestion. (Fang *et al.*, 2004) *Klebsiella pneumoniae* is regarded as one of the most important infections in public health since it is an animal-borne bacterial pathogen (zoonose) (Cheng *et al.*, 2018). A number of diseases, including pneumonia, bacteremia, liver abscess, and urinary tract infections, have been linked to *Klebsiella pneumoniae*. (Jun, 2018). epidemiology the *Klebsiella pneumoniae* classified to classical *Klebsiella pneumoniae* (cKp) infections and hyper virulence *Klebsiella pneumoniae* (hvKp) causes tissue-invasive infections (Russo, 2018). Due to their capacity to acquire plasmids harboring several antibiotic resistance determinants, the majority of infections in North America and Europe are caused by classical *Klebsiella pneumoniae* (cKp) strains, making treatment challenging (Conlan , 2014). Although hvK.p. infections in cattle is often known to be the cause of mastitis, it has also been linked to pulmonary diseases in animals. However, there wasn't much proof linking *Klebsiella pneumoniae* to respiratory illnesses in cows,(Chehabi *et al.*, 2019). The pathological data has not been well examined, and there is currently little information available on the capsule serotypes of *Klebsiella pneumoniae* in cattle (Darniati *et al.*, 2021).

2.1.2: Mastitis

The inflammatory reaction that occurs when domestic dairy animals' udder tissue becomes infected is known as mastitis. In dairy cattle, mastitis is a particularly prevalent and potentially lethal condition. One of the most expensive diseases for the dairy industry, mastitis is linked to decreased milk production as well as changes in the composition and quality of milk. (Nee 2020), As a result, the majority of the field's research has concentrated on controlling bovine mastitis, and several attempts are being made to create novel and potent anti-mastitis

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medications. Although the use of antibiotics is a well-established part of mastitis management programs, ongoing research into novel therapeutic options that are efficient in treating and controlling bovine mastitis (Gomes and Henriques, 2016) . Researchers like Wilson (1997) demonstrated that the virulence of mastitis varies based on the pathogen that caused the infection. For instance, *Mycoplasma* species, *Arcanobacterium pyogenes*, *Pasteurella* species, *Klebsiella* species, and *Enterobacter* species have all been shown to significantly reduce milk production.

2.2 *Klebsiella*

2.2.1 History of *Klebsiella*

At first time *Klebsiella* was isolated from the lung of dead human was suffer from pneumonia by Friedlander in 1882, initially called Friedlander's bacillus (podschun *et al.*,1998). The German microbiology Edwin Klebsi (1834–1913) gave the genus *Klebsiella* its name in the late nineteenth century (Brisse *et al.*, 2006).

2.2.2 Classification of *Klebsiella pnemonuiae*

Based on evidence from DNA-DNA hybridization and biochemical findings, the second edition of Bergey's handbook presents the categorization of 12 species that share DNA homology (Brenner *et al.*, 2005). Taxonomically, *K. ozaenae* and *K. rhinoscleromatis* are regarded as subspecies of *K. pneumoniae* based on DNA-DNA hybridization evidence. *K. granulomatis*, *K. oxytoca*, *K. trevisanii*, *K. singaporensis*, *K. terrigena*, *K. ozaenae*, *K. ornithinolytica*, *K. planticola*, *K. variicola*, and *K. rhinoscleromatis* were also present (Al-obadi, 2014). *K.pneumoniae* related to *K. oxytaca* both of them indol- negative and they able to growth on melezitose and 3-hydroxybutyrate (Hansen *et al.*, 2004; Siu *et al.*, 2014).

Klebsiella pneumoniae was classified according to (George *et al.*, 2004) to the following:

Domain: Eubacteria

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Kingdom: Bacteria

Phylum: Proteobacteria

Order: Enterobacteriales

Class: Proteobacteria Gamma

Family: *Enterobacteriaceae*

Genus : *Klebsiella*

Species.: *Klebsiella pneumonia*

2.3 *Klebsiella pneumonia*

Klebsiella pneumoniae is Gram-negative an opportunistic pathogen from *enterobacterial* family, non-spore form, non- motile, highly opportunistic pathogen may be cause a wide different diseases and increasingly adept at developing resistance to antibiotics, This rod-shaped, facultative anaerobic bacteria ranges in width from 0.3 to 1.0 μm and in length from 0.6 to 6.0 μm . (Wang *et al.*, 2012; Effah *et al.*, 2020). *Klebsiella pneumonia* has a characteristic poly saccharide capsule, It envelops the entire cell surface. The bacteria's big appearance, particularly in gram stain, is attributed to it, and it is thought to be resistant to the host defensive system (Mansour *et al.*, 2014).

Most *Klebsiella* species thrive well at temperatures between 12 to 43°C , with 37 degrees being the ideal range. The majority of *Klebsiella* are killed at 55°C for 30 minutes (Talaro and Chess, 2017).The colonies of *Klebsiella* appear big and mucoid ,the bacteria consider as an aerobic bacteria but may be stay alive in oxygen-deficient environment the level of mucoidity changed from strain to

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another and also effect by the concentration of carbohydrates in the growth media (Yu *et al.*, 2007) .Fermentation of lactose and acid generation, can be seen by a pink color on MacConky agar(Todar, 2015). *Klebsiella* spp. always in humans may be found in mouth, gastrointestinal tract and nasopharynx (Gonzalez-Ferrer,2021). However, it is frequently found in soils, surface waterways, drinking water, sewage, water, and industrial effluents (Podschun *et al.*, 2001). *Klebsiella* has 2 kinds of cell surface antigens, O antigen and K antigen they take part in the pathogenicity of these species (Umeh and Berkowitz, 2009).

2.4 Biochemical characteristics and growth requirements

Following incubation at 30°C or 37°C, the *Klebsiella pneumoniae* colonies had a big, spherical shape. (Brisse *et al.*, 2006). *Klebsiella pneumoniae* exhibited mucoid characteristics and seldom viscosity, which varies according on the strain and medium composition, it grows simply on usual media that used to isolated *Enterobacteriaceae* family, like nutrient agar, bromo cresol purple lactose agar, tryptic casein soy agar, and blood agar (Venkatalaxmi, 2023). However, alternative differential plating medium including Bromo-thymol blue agar, which Eosin-Methylene Blue agar, Mac Conkey agar, and Drigalski agar can be utilized. (Sharma *et al.*, 2015). *Klebsiella pneumoniae* appear on MacConky agar as large mucoid and pink-red, short, plump and straight rod under microscope, beside that the red pigments that is commonly spreading in agar, this shows that lactose is fermented and that lysine decarboxylase is produced, but not ornithine decarboxylase, and that Voges is typically positive. -Proskauer examination (Pavan *et al.*, 2022). Additionally, it is unable to grow at 10°C, does not produce hydrogen sulfide and fails the Indole test (Yu W., *et al.*, 2008).

Many *Klebsiella pneumoniae* isolates require arginine or adenine, or both, as growth agents. *Klebsiella* species thrive in minimum media that contains just ions

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of ammonium or nitrate as a nitrogen source and a carbon source. (Arya *et al.*,2023).

2.5 Pathogenesis of *Klebsiella pneumoniae*:

Klebsiella pneumoniae consider as a category of bacteria can cause infection mainly in health care setting its pathogenicity is influenced by several factor such as its transmission *Klebsiella pneumoniae* spread through direct contact particularly via contaminated hands, it does not spread via the air. *Klebsiella* infections are most common in healthcare facilities patients at high risk for contracting this fatal disease including those with ventilators, catheter, or surgical incisions (Zhu *et al.*, a2021).

Klebsiella pneumonia prominent member of the *Klebsiella* genus which is responsible for approximately 86% of all *klebsiella* –related infection in humans making it the weightiest pathogen with in the genus (Sen and Demirdal, 2020). *Klebsiella oxytoc* follow as the second most common species accounting for around 26% of infection (Martin and achman, 2018). *Klebsiella* spp. are recognized as major hospital-acquired pathogens capable of infection soft tissue, wounds and causing sepsis as well as various respiratory and urinary tract infection (Bengoechea and Sa Pessoa, 2019). In case of nosocomial infection, the presence of virulence factors and drug resistance enhances the colonization potential of *Klebsiella*.

The key pathogenicity of *Klebsiella* include types 1and 3 fimbriae, iron acquisition mechanisms and cellular components like lipopolysaccharides. The presence of *Klebsiella* fimbriae has been linked to an increased susceptibility to urinary tract infections (Rosen *et al.*, 2016).

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The lipopolysaccharide and capsule make it more harmful and protect the bacteria from being destroyed by the immune system cells those helps prevent and septic shock (Fatima *et al.*, 2021).

2.6 Virulence factor of *Klebsiella pneumonia*

Klebsiella pneumonia. has a lot of virulence factor in common with other pathogens such as capsule antigens, adhesion factor, enterotoxin production such as lipopolysaccharide and multi resistance for antibiotics. All these factors were viewed as the primary cause of the spreading (Jasim *et al.*, 2020). The main virulence factors including :

2.6.1 : The pili

Structural proteins called pili aid bacteria in locating certain kerchief shells within the host (Riwu *et al.*, 2022). With a diameter of 1–11 nm and a length of 10 m, the pili that give the *Enterobacteriaceae* (also known as fimbriae) family its attachment characteristics are non-flagellar filamentous projections. They included about 1,000 copies of the skeleton component packed in expressively arranged appendages(Alcantar-Curiel *et al.*, 2013).

Through mannose-sensitive attachment, pili can strengthen the bacterial bond with epithelial cells, reducing the likelihood of host tissue invasion. Capacity to agglutinate tannic acid-treated erythrocytes (Loschko *et al.*,2011;Wu and Li, 2015). Bacteria must be have a strong adhesion to tolerate forces from thing like the water pipe in the urinary tract, contracting and relaxing or gaseous the force from the lung expanding and contracting. The lung properties such as elastic and stretchy that like wave movement, help bacteria stay binding the host cell by reducing the force of these agitations. *Klebsiella pneumoniae* have a diverse of characteristic hyphae for this type 1 and type 3 hyphae(Chen and Wen, 2011).The pili arrangement in

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form of three-dimensional Hemi Blindness to form a compact column of the pili , while the adhesive molecules are located at the distal end of the pili adjacent to the host cell (Mu and Bullitt, 2006). The flexible structure of pili may give to the bacterium's ability to survive from erosion. *Klebsiella* pili were mainly classified in to two groups based on whether or not their adhesive response was inhibited by D-mannose(Chen and Wen, 2011).

2.6.1.1:Fimbriae type1

This kind of pili are made of mannose sensitive, the placenta's epithelial cell are connected by glycoproteins containing mannose ,the invasive *Klebsiella pneumoniae* bacteria can help of producing of type 1 pili in order to event being link by glycoproteins which dependent on mannose in epithelial cell. The invasive *Klebsiella pneumoniae* bacteria can inhibit the formation of type 1 pili in order to evade being related by mannose-dependent epithelial cell glycoproteins (Said-Salman *et al.*,2021).

According to a recent study that looked at the relationship between *Klebsiella pneumoniae* and cells in the respiratory and urinary systems, type 1 pili is believed to make infection easier by permitting colonization of the upper respiratory and urine tracts. (Clegg and Sebghati, 2002). This is because the first kind can improve bacterial death and eradication by facilitating adhesion to phagocytes (Kuehn, 2009).

Klebsiella pneumoniae expresses the type 1 pili from a collection of 8 genes on the chromosome called fim. The fim A gene encodes one of the main proteins that give this gene their basic helical shape. Fim A expression is regulated by the E gene also the gene that produce fim B. The researcher believed that this protein which is

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generated by both fim *F* and G. control the length of the primary shaft. The fim *H* gene encodes the site found in the fim *A* gene(Gahlot, 2022).

The proteins that the genetics fimC and fimD produce, respectively, aid in bacterial surface assembly. The attachment factors of bacteria hold structure protein known as Type1 Fimbriae and Type3 Fimbriae including type1 of pili which has mannose-sensitive hem agglutinins, distinct molecular receptor and simplicities adherence to surface of a specific tissue in the gut *Klebsiella pneumoniae* produces the above two chaif necessary adhesion factors. Mannose-resistant hemagglutinins in pili 3 (Zadeh *et al.*, 2021).

Type1 pili were present in *E.coli* , the member which was most common of the *enerobacteriaceae* family, In terms of genetic organization and fundamental structure, *Klebsiella.pneumonia*'s type1 pili were analogical to those of *E .coli* (Struve *et al.*, 2008).

2.6.1.2: Fimbriae Type3

Fimbriae type 3 pili have lengths of 0.5–2 nm and widths of 2-4 nm. Finally, it was discovered. these strains of this type of *Klebsiella pneumoniae* bacteria were can adhere to respiratory tract's epithelial cell and vessel lining cell ,also mediate the agglutination of red blood cell (Ong, *et al.*,2010). A helix-like structure of(4.1 nm) in length makes up type 3 pili. The pili's shape is non-linear at first as they get larger, then finally they release and stretch to a little micrometers before turning in to the third stage. The type 3 pili so appear curved rather than straight(Chen and Wen, 2011).

The pili in type 3 not same in all genera of intestinal bacteria as serological studies have presented a great variety of in antigens (Rendueles,2020). This group is called *Mrk* gene (Ong *et al.*, 2010).

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Fimbriae type 3 showed that increase microbes to bind to renal epithelial cell, pulmonary endothelium, and epithelial cell, mannose with stand mannose resist (MR)(D-mannose) cannot inhibit it (Lau, 2007).

Also the type 3 pili showed to be generated on a solid substrate and tied together in bundle. Serological investigations have discovered a major variety of type3 pili in antigens, suggesting that they were not all the same in gut bacterial genera (Rendueles, 2020). The *Klebsiella pneumoniae* strains have the *mrk* gene collection on their chromosomes, while the other strains have both chromosomal and plasmid gene assemblies, and this gene expression was limited in sites when nutrients were few and ideal in *mrk* (Kuehn, 2009).

In recent times some studies shown that the gene pool encodes the probable regulation of the plasma *mrkE pneumonia*, that distributed along a central pillar permeating inside the *mrkA* polymer, but nothing is known about *mrkF's* function in the substructure. Since *Mrke* is situated directly downstream of the *MfrkA* gene. It is commonly not involved in the gene set of pili type 3 (Struve *et al.*, 2009). Confident information was reported in other studies, namely that a number of components, involved adhesion *mrkD*, chaperone *mrkB*, *mrkC*, regulatory gene *mrkG* and *E*, *mrkA* structure, *mrkF* constancy, and *mrkD*, were included in the expression of type 3 pili (Cleggand Murphy,2016).

Some studies are arrived to that, in addition to *Klebsiella* species, type 3 pili isolation of *Providencia*, *Enterobacter*, *Serratia*, and *Proteus* (Struve, *etal.*,2009). Moreover, the mainly species formerly stated, *Klebsiella* as well as owns the 28-KPF pili, that expressed by gene on the dR plasmid and have been appear to be crucial in binding to intestinal epithelial cell surfaces (Jasim,2021). The CF29K gene and the fimbria KFP-28 gene were both carried by the same plasmid, and they appeared to help the attachment process(Prada-Avella.,2021).

2.6.1.3: KPC fimbriae

The *Klebsiella pneumoniae* strains that caused general pyogenic diseases the fimbriae formed by the kpc ABCD gene cluster the most commonly associated with K1-positive strains of *Klebsiella pneumoniae*, Kpc fimbriae have a great linked to hyper-mucoviscous *Klebsiella pneumoniae*. Additionally, They are necessary to the development of *Klebsiella* biofilms and the transmission of infection(Zhu *et al.*,2021).

2.6.1.4 Non-fimbrial adhesins,CF29K

Klebsiella rods' adherence to protein matrix was confirmed by the in vitro study. Non-fimbrial adhesin CF29K is said to be the cause of *Klebsiella* strains' adherence to intestinal epithelium and the epithelial cells of the respiratory and urinary tract (Przondo-Mordarska *et al.*, 2003).

2.6.1.5: The role of pili in *Klebsiella* pathogenicity

The learn about the pathogen's skill to colonizes the right host tissue is what first allows it make illness. The first step in colonization is for gluey proteins to bind the bacteria to receptor expressed in the cell that line the mucous membrane starting series of event, counting communication between the bacteria and the host ,that ultimately affects how an infection turn out(Walker and Colledge, 2013; Siegel and Weiser, 2015). Bacteria pathogens encode the mostly of many pili and non-pili adhesion type that mediate an organism's adherence to a host cell, the idea of adhesion is crucial in bacteria to host adhesion because it have ability for the bacteria to get inside and make its effect on the host. Many requests of microorganisms causing an infection happen or keeping in touch in order for the microbe to exert its effects, regularly. A set of bacterial adhesion factor have been known in the kinds of bacteria positive and negative through a range of secretion

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processes. Gram-negative bacteria and the K-link are examples of negative bacteria (Jasim, 2021). The Bacteria used different ways to attachment, one of the various ways that bacteria can used attachment to fimbriae, also known as pili, which are characters on the cell surface (Chong and Khan, 2019). The pili types play an important role in infection, but in diagnosis is not totally understood, , modern investigation the function of type 3 pili in *Klebsiella pneumoniae* virulence(Struve *et al.*, 2009). Research has indicated that type 3 pili are crucial for the formation of biological membranes (Ong *et al.*, 2010).

2. 6.2: smooth lipopolysaccharide(LPS with O antigen)

This lipid may be found on the outer leaflet bacteria membranes such as linoleic acid phosphate sulfate (LPS) was named as endotoxin. In several serotypes can cause disease and have the ability of protection *Klebsiella pneumoniae* against the complement system and cationic antimicrobial peptide. Both CPS and LPS can be used by *Klebsiella pneumoniae* to combat the complement system (Adamo and Margarit, 2018). Lipid A, Core oligosaccharides, and O-antigen make up LPS from bacteria, with the composition of O-antigen being extremely variable between *Klebsiella* spp. strains (Joo, *et al.*, 2016).

The O- antigens differed significantly between strains of *Klebsiella* spp. Furthermore, *Klebsiella* species' O antigens involved the sugars N-acetyl, D-glucosamine, D-ribofuranose, D-galactose, D-galactofuranose, and D-mannose. Different O-antigine have variety degree of antigenicity based on the changes in their structure and changes in their composition, and have various sugar composition, glycoside link, repeating unit numbers, and empiric and enantionmeric forms, as the K-antigen dose(Patro and Rathinavelan, 2019). O-antigens were shown to have an acetyl group replacement, whereas K-antigens had some substitution (but only for one of the O-antigens). But *Klebsiella* strains with

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truncated or non-existent O-antigen (referred to as "rough LPS") are vulnerable to complement system-mediated death. Complement system-mediated killing is not effective against strains of *Klebsiella* spp. that have smooth LPS or the full-length O antigen. (Patro and Rathinavelan, 2019). The O-antigen shields the Kp surface molecules, thus even if the complement-resistant strains trigger the complement cascade, they cannot be killed (Shankar-Sinha *et al.*, 2004 ; Merle *et al.*, 2015).

2.6.3: Capsules(K antigen)

Klebsiella spp. are sheltered from their surrounding environment by a covering of a heavy layers of CPS fibers that cover their full surface (Abreu and Barbosa, 2017). *Klebsiella pneumoniae* is protected from its natural immune mechanisms by this shield, which also prevents the accumulation of complement and opsonization, decreases recognition and adhesion by phagocytes and epithelial cells, and stops lysis by complement cascades and antimicrobial peptides (Martin and Bachman, 2018).

Some environmental stressors were effected on bacteria such as dryness and detergents. However the polysaccharide capsule helps bacteria to survive. like the *E.coli* outer membrane. A high-molecular-weight CPS made up of linear or branching oligosaccharides serves as the complement's physical wall in *Klebsiella* species. Mechanism of *Klebsiella pneumoniae* (Abreu and Barbosa, 2017), the strains with weak outer membranes were easily ingested by the body's defense mechanisms (phagocytosis). Innate immune cells had an easier time engulfing and digesting capsular than non-capsular *Klebsiella.pneumoniae* strain, *Klebsiella's* pathogenic potential. Eliminating the genes required for the clinical strains of *pneumoniae* to produce capsules can significantly decrease the number of these strains, and it has been shown that it is the thickness of the CPS, rather than its chemical makeup, influences *Klebsiella* protection (Martin and Bachman, 2018). It

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may be categorized into 80 serological groups and is continuously expanding. It can have four to six sugars, complex acidic polysaccharides, repeated subunits, and frequently uronic acid. (Wu and Li, 2015). The serotype K1 and K2 plays major role in the infection of respiratory system (Brooks *et al.*, 2007). Because K2 *Klebsiella pneumoniae* becomes more deadly than K1 from PLA, providing an advantage in survival to maintain a steady share, it depends on the identical virulence factors backgrounds, with the exception of serotypes (Dong, 2024).

The dense capsules at the cell membrane prevents macrophages, neutrophils, epidermal cells, then dendritic cells from opsonizing and phagocytosing *Klebsiella pneumoniae*. Additionally, by preventing binding and internalization, it keeps the bacteria from being destroyed by bactericidal serum factors (Pan *et al.*, 2011). The capsule serves as a barrier to prevent host-derived antimicrobial peptides from entering, and antimicrobial polypeptides can be trapped by free capsular polysaccharide produced by bacterial cells, reducing the amount of antibacterial polypeptides that make it to the surface of bacteria (Campos *et al.*, 2004 ; Liob *et al.*, 2008).

2.6.4: Siderophores

The majority of harmful bacteria create siderophores, which are small molecules which bind iron and are subsequently released from their cells, after secretion, these molecules attach to iron in the environment and transport it back into the bacterial cell. The iron-siderophore complex are recognized by specific receptor in the outer membranes, facilitating their entry into periplasm. Within the periplasm, the siderophore interact with certain protein that help transport the iron to the inner membrane. Ultimately, iron is moved through an ABC transporter into the bacterial cytoplasm, where ferric iron is reduced to ferrous iron, making it accessible for the bacteria (Khan *et al.*, 2018).

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The iron. contributing to virulence by chelating processes that assist the bacterium compete with the host for iron absorption by removing iron from host iron-binding proteins (Izquierdo *et al* , 2003).

The genus *Klebsiella* primarily produces two different kinds of siderophores: hydroxamates, also known as "aerobactin," and phenolates, also known as "enterobactin" or "enterochelin." Enterochelin was shown to be produced by whole strains. Few are able to produce aerobactin. The third siderophore, "yersinyabactin," produced by *Klebsiella* species is fixed within the Yersinia elevated pathogenicity island, although its involvement in prevalence and disease is unknown (Brisse *et al.*, 2006).

2.6.5: A protein-tyrosine kinase

2.6.6: A protein phosphatase that phosphorylates tyrosine

The two proteins that are involved in the synthesis of capsular polysaccharides (Prenta *et al.*, 2002). In general, *Klebsiella pneumonia* exhibits multidrug-resistant phenotypes that are invariably brought on by the presence of carbapenemases or extended-spectrum β -lactamases, making it extremely challenging to select an appropriate antibiotic for therapy (Munoz *et al.*, 2013).

2.7 Haemolysins:

On sheep blood agar, *Klebsiella* species, especially the non-hemolytic forms, form gray-white mucoid colonies. On rabbit blood agar, they may show variable hemolytic activity.

Research conducted in 1980 by Smith, Ngui-Yen, and other researchers demonstrated that *Klebsiella* produces hemolysis, which is thought to be non-hemolytic for red blood cells from humans but becomes hemolytic in rabbit blood

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agar. The two effective hemolysis processes on red the blood cells were described (Sharma et al., 2015).

2.8 Epidemiology of *Klebsiella pneumoniae*:

Klebsiella are a kind of bacteria that are widely distributed in nature and are frequently found in soil, sewage, surface water, and plants. (Podschun *et al.*, 2001). Domestic animals' mucosal surfaces, including the skin and throat, can get contaminated with *Klebsiella pneumonia*, which is a frequent way for the infection to spread and facilitate cross-contamination of both food and water sources (Brooks *et al.*, 2007 ; Gyles 2008; Kim *et al.*, 2008). The both kind of *Klebsiella* spp. the common and opportunistic were an essential to balanced gut microbiome, these enteric bacteria are typically harmless and can even contribute positively to digestion and nutrient absorption .However, they can become pathogenic when they breach the intestinal barrier and infect other tissues ,*Klebsiella pneumoniae* , in particle ,is noteworthy in clinical settings , comprising a little portion of the population's feces and the natural flora within the respiratory system, and contributing to around 1% of cases of bacterial pneumonia. It ranks as the second most important species in the *Enterobacteriaceae* family, following *Escherichia coli*, highlighting its medical significance.(Greenwood *et al .*, 2012).

Age stress, dietary changes or hunger, transportation, castration, weaning, and dipping are some of the risk factors that enhance the likelihood of contracting *Klebsiella* spp. The immunological pack ground is mixed with environmental risk variables, such as shipping distance, humidity, dust particles, stock density, climate, ambient temperature, and ventilation. (Rahal *et al.*, 2014).

species of *Klebsiella*, specifically the opportunistic enterobacteria *Klebsiella pneumonia* is known to cause complicated clinical infections in both people and

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animals, and these infections frequently do not respond well to standard therapy. One possible source of the multidrug-resistant and pathogenic *Klebsiella* species that can infect people is domestic animals. Nevertheless, the majority of research on *Klebsiella*-caused illnesses in household animals is restricted to case studies or epidemic inquiries (Ribeiro *et al.*, 2022)

Klebsiella spp. were found to be very prevalent in rumen, water, and soil samples, which indicated that the farm was experiencing oral-fecal bacterial transmission (Zadoks *et al.*, 2011).

Some of genes which responsible of resistance can be coded for creation of enzymes (like lactamases) and can regulator the development of efflux pumps ,site changes and metabolic pathway alterations(Buiuc and Negut, 2008). Like other Gram-negative bacilli *Klebsiella Pneumonia* produce extended spectrum β -lactamases (ESBL) important resistance mechanism to β -lactam medicine found following the widespread implementation of extended spectrum cephalosporins in clinical practice(Shah *et al* ., 2004) .

2.9 Diagnosis of *klebsiella pneumoniae*:

The primary diagnosis for an illness caused by *Klebsiella pneumoniae* the organism must be isolated and identified based on the full clinical examination of the diseased animal. Gram staining, biochemical testing, and the Viteck II technique are nearly always used in laboratories for identification; Other biochemical tests have their own restrictions, such as the potential for 48-hour results presentation, and the isolation of bacteria takes 24 hours.At the genus level, however, it may not always be possible to correctly identify a species (Shon *et al.*, 2013).

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Assays such as the capsular irritation reaction and ELISA are examples of other serological examination tests used for *K. pneumoniae* detection that rely on antibody and antigen reaction. The issue with the majority of these tests is that they are expensive and have poor reproducibility, sensitivity, and specificity (Hackstein *et al.*, 2013). An accurate method for identifying *Klebsiella* is the Polymerase Chain Reaction technique sheep with pneumoniae after having pneumonia (Diancourt, *et al.*, 2005).

2.10 Treatment and control of *Klebsiella pneumoniae*:

Although clinical specimens of *Klebsiella pneumonia* acquire a β -lactamase that makes them resistant to ampicillin, which is amoxicillin, and other Penicillins, Clavulanic acid and other β -lactamase inhibitors found in pharmaceutical formulations usually work well (Wang *et al.*, 2011). While carbapenems are commonly used to treat diseases caused by Gram-negative bacteria which are resistant to a variety of medications, beginning with the first report of *K. pneumoniae* (Yigit *et al.*, 2001). *Klebsiella pneumonia* is vulnerable to Fluoroquinolones and Cephalosporins, especially β -lactamase-stable variants such as Cefuroxime and Cefotaxime. Resistance to Tetracycline and Chloramphenicol varies by strain, but they are frequently susceptible by Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, and Kanamycin (100%) each. However, they have convertible enzyme resistance to aminoglycosides as well as other antimicrobial agents (Greenwood *et al.*, 2002; Mansour *et al.*, 2014).

The use for suitable antibiotics, the selection of an sensitivity profile, which can be determined through laboratory tests specifically designed for antibiotic sensitivity (Balm, *et al.*, 2012). showed resistance to at least one class of antibiotics, as well as to third-generation fluoroquinolones, as submitted according to the European Centre to the Prevention and Control of Diseases. According to the European

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Center for Disease Control and Prevention, the most prevalent resistance phenotype is to cephalosporins and aminoglycosides. Resistance to antimicrobial agents (EARS-Net) 2018.

2.11: Emergence of Antibiotic Resistance in *Klebsiella* Species:

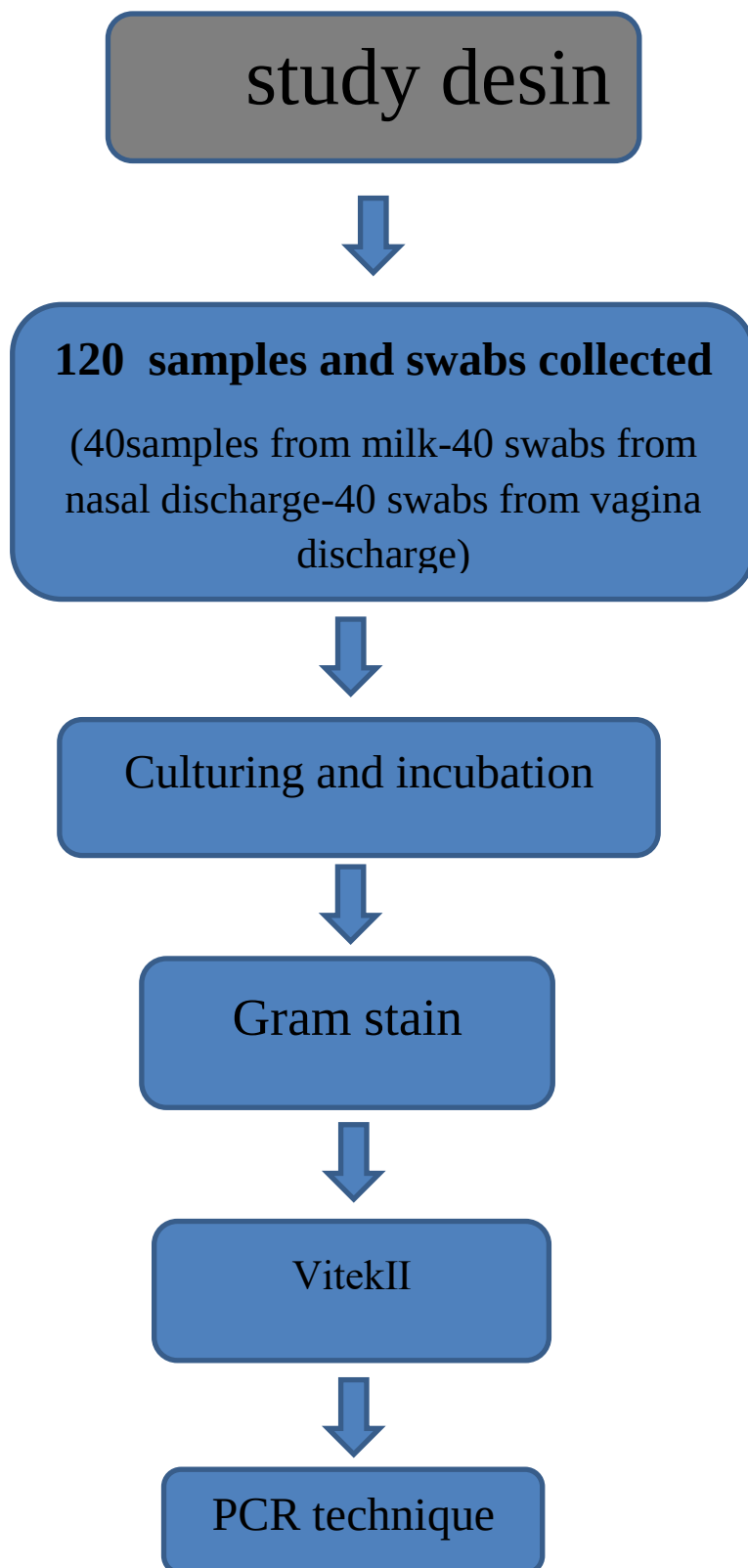
Microorganisms' resistance to antibiotic consider a serious public health concern (Naeem *et al.*, 2019). *K.pneumoniae* resistance to aminoglycosides ,fluoroquinolones and β -lactams had been documented. The susceptibility of *Klebsiella*. spp. to produce the β -lactamase enzyme give the resistance to a large class of antibiotics (Navon-Venezia *et al.*, 2017). The majority of specimens of *Klebsiella pneumoniae* hold SHV-1 gene, which encoded the β -lactmase(Liaqat *et al.*, 2019).

Gram-negative bacteria have exposed some plans to resist antibiotics multidrug resistance (MDR) is widely spread among antibiotic resistant bacteria by horizontal transmission (Munoz-price & Quinn, 2009). Moreover, transferring resistance between diverse bacterial pathogens is the capability of resistant strains to genetic elements (MGEs) provider. Vertical transmission of mobile elements, like transposons and plasmids is possible between distinct taxonomic grouping (Woodford *et al.*, 2011). Thus the transmission MDR isolates has been related to the fact that bacteria like *K.pneumonia* and *K.oxytoca* exchanged plasmid (Miro *et al.*, 2010).

One of the important key in the MDR profile is the efflux pump systems (Meleis *et al.*, 2012). AcrAB and mdtK are efflux pump systems discovered in *Klebsiella.pneumoniae* ,an outer-membrance channel (TolC). An inner-membrance *AcrB* secondary transporter, and a periplasmic component make up the *AcrAB* pump(*AcrA*) (Du *et al.*, 2014). Some studies in 1996 by the Okusu *et al* showed

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that numerous of multidrug-resistant (MDR) isolates showed resistance to quinolones drugs such as tetracyclines, and chloramphenicol due to the presence of this pump. Several of these antibiotics were carried by the mdtK system and other by MATE (Multidrug and Toxic Compound Extrusion) pumps (Sun *et al.*, 2014). Natural resistance to multiple β -lactams was demonstrated in *Klebsiella pneumoniae* due to the presence of the extended spectrum was demonstrated in *Klebsiella pneumoniae* due to the presence of the extended spectrum β -lactamase (ESBL) enzyme in the species core genome *Klebsiella pneumoniae* always has the SHV (species-specific enzyme) that is chromosomally encoded (Navon-Venezia *et al.*, 2017).



Chapter Three

Material & Methods

Materials & Methods**3.1 Materials****3.1.1 Equipment's and instruments**

The current study made use of a variety of tools.

Table (3-1) The tools, apparatus, and resources used in the laboratory.

No.	Instrument	Companies	Origins
1	Autoclave	DAIHAN LABTECH CO.LTD	Korea
2	Burner	Amal	Turkey
3	Deep freeze	GFL	Germany
4	Disposable loops	China	China
5	Dry micro tubes incubator		UK
6	Disposable petri dishes		China
7	DNA electrophoresis apparatus	Hettich	Germany
8	Electrophoresis system	Cleaver	UK
9	Eppendorf tube(2 ml)	BioBasic	Canada
10	Hood- cabinet	BIO Hazard	Korea
11	Incubator	Froilabo	France
12	Light microscope	Olympus	Japan
13	Microfuge IB Centrifuge	Beckman Coulter	Germany
14	Oven	Sacora	Japan
15	Pipettes	DARWELL	CHAIN
16	Refrigerator	Hettic	Japan
17	Sensitivity balance	Ohaus	Chain
18	Vitek2 system	Bio Merieux	France
19	Vortex	Bio Merieux	France
20	UVP	Velp	UK

3.1.2 Culturing media

The current study employed a variety of culturing medium, as the table below explains.

Table (3-2) Showed that the current study made advantage of culture media.

No	Media	Company	Origins
1	Aimes Trasport media	AFCO	Jordan
2	MacConkey agar	Oxoid	England
3	Brain heart infusion broth	Himedia	India

3.1.3 Chemical material and kits

The present study employed a variety of chemical compounds.

Table(3-3) shown the chemical components utilized in this investigation.

No	Material	Company	Origins
1	Saf-Green100bp Opti-DNA Marker	Abm	Canada
2	RedSafe nucleic acide staining solution	Intron	Korea
3	Primers	Macrogen	Korea

3.1.4 Chemical materials

The present study employed a variety of chemical materials.

Table (3-4): Utilizing chemical materials in molecular research

No.	Material	Company	Origin
1	Agaros	Bio basic	Canada
2	GeneRuler 100 bp DNA (ladder)	Bioneer	Korea
3	Loading dye	Geneaid	UK
4	Nuclease free water	Fisher	Garantie
5	Standard McFarland (0.5)	Bio Mérieux	Franc
6	TBE	Bio basic	Canada

3.1.5 kits

As indicated in the table, some kits were utilized in the present study.

Table (3-5) Showed the list of various kits utilized in the present study

No	Kits	Company	Origins
1	AST(Vitek2)	Bio Merieux	France
2	DNALoading dye	Bioneer	Korea Rep
4	Go Taq ® G2Green Master Mix	Promega	USA
5	ID(Vitek2)	BioMerieux	France
6	Presto™ Mini g DNA Bacteria Kit	Geneaid/	Taiwan

3.2-Methods:**3.2.1-Sample collection:**

One hundred and twenty swabs and samples was collecting from the cattle, from Thi-Qar province (Shatra dispesary, farms), during period From the November of the 2024 to January the of 2025, the information including age and location were fixed, milk samples putted in sterile container then in ice box and swabs (nasal and vaginal) putted in transport media then in ice box then the samples and swabs transport to microbiology laboratory in veterinary collage with period less than 24 hours .

3.2.2 Preparation of different media

Following the sterilization of MacConky agar on the petri dish plane and incubating at 37°C for 24 hours to confirm their sterility, the culture media used in the presented work were prepared in accordance with the manufacturer's instructions identified on the containers and sterilized in autoclave for 15 minutes at 121°C and 15 pounds per square inch (Brown & Smith, 2014). This ensured that the media were ready for culturing and could be stored in the refrigerator.

3.2.3 Maintenance of bacterial isolates

The following is how the bacterial isolates were maintained:

3.2.3.1-Short term storage

For several months, the pure isolates of bacteria were kept in screw-capped universal tubes filled with brain-heart agar and cultured for twenty-four hours at 37°C. The slants were then securely wrapped with Para film and stored for three months at 4°C (Brown & smith, 2014).

3.2.3.2 Long term storage

The next day, a loop of pure bacterial culture was used to inoculate a brain heart infusion broth, and after 18 hours of incubation at 37°C, The broth was prepared for incubation by adding glycerol at an ultimate concentration of 20% and keeping it at -20°C for two to eight months (Goldman & Green,2015).

3.2.4 Isolation and identification of bacteria

The samples and swabs were collected from cattle from different places from AL Shattra province. Then, the samples and swab transport and incubation in brain heart infusion on incubator in 37°C for 24 hours then growth on MacConky agar and put on incubation in 37°C for (24 to 48) hours in aerobic environment.

3.2.5 Gram stain method

A little piece of the colonies of bacteria is spread out on a sanitized slide and fixed with heat in addition to a drop of regular saline. and subsequently stained with safranine, alcohol, iodine, and crystal violet before being inspected under an oil immersion (100X) (leboffe and Pierce, 2021).

3.2.6 Identification by using automated methods [VitekII] system

There were 30 individual wells on the plastic reagent cards that made up the VitekII system each of which contain a microliter quantitative sample of various biochemical test medium needed for diagnosing a given organism, an inoculum was taken from cultured sample and put automatically on the card and the card's color changes as a result of the microbe's metabolism were periodically measured by a photometer. Following data entry into a computerized data analysis and storage system, Gram-negative (GN) and Gram-positive (GP) identity cards were produced (Maina & Kagotho, 2014).

3.2.7 Molecular studies

3.2.7.1 Extraction of bacterial DNA

All *Klebsiella pneumoniae* isolates had their genomic DNA extracted using the Presto™ Mini gDNA Microbial Kit in accordance with the manufacturer's instructions. This procedure can be summed up as follows:

A. Sample Preparation

After adding bacterial isolates (up to 1×10^9) from an overnight BHI culture in broth to a 1.5-milliliter micro centrifuge tube, the mixture was centrifuged for one minute in 14–16,000 rpm, Additionally, the supernatant was thrown away. After adding 180µl of GT buffers, the cell pellets were re-suspended using a pipette or vortex. 20 µl of proteinase K (PK) was added. Give it ten minutes or more to sit at 60°C. Flip the tube every three minutes while it's incubating.

B. Lysis

Following the addition of bacterial isolates (up to 1×10^9) within an overnight BHI culture a broth to a 1.5-milliliter micro centrifuge tube, 180 µl of GT Buffers that are was added, Using a pipette or vortex, the cell pellet was reconstituted, 20

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milliliters of Proteinase K (PK) were added, and the tube was placed in the incubator at 60°C for at least 10 minutes, rotating it every three minutes.

C. DNA Binding

Following the addition of 200 milliliters of 100% ethanol to the sample lysate, the mixture was vigorously shaken. After breaking up any potential precipitation with a pipette, put a GD Column in a two ml collection tube. Centrifuge the mixture at 14–16,000 x g for 2 minutes after transferring it, along with any insoluble form precipitates, to the GD Column. It is necessary to dispose of the 2 ml collecting tube that contained the flow-through and replace it with a fresh one that holds the GD Columns.

D. Wash

The flow-through was disposed of following the addition of 400 microliters of W1 Buffers to the GD Column and a 30-second centrifugation at 14–16,000 rpm. Reinstalled the GD Column after adding 600 µl of the buffer for washing (including ethanol) to the 2 ml collection tube. Centrifuge at 14–16,000 rpm for 30 seconds, then discard the flow-through. The GD Column should then be replaced in the 2-milliliter collection tube. Finally, the column matrix was centrifuged one more for 90 seconds at 14–16,000 rpm in order to dry it.

E. Elution

After drying, the GD column was moved to a sterile 1.5-milliliter micro centrifuged tube. The purified material was then eluted by centrifuging it for 30 seconds at 14–16,000 rpm after adding 100 milliliters of hot Elution Buffer¹ to the middle of the column matrix.

3.2.7.2 PCR detection of genes

The genes have been amplified using the primers specified in Table (3-5). A total of 12.5 µl of Mastering Mix, 1 µl of the specific reverse and forward primers for

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each gene, 3 µl of the DNA template as well as and water that lacks nuclease complete the last reaction in a tube volume of 20 µl.

3.2.7.3 Primers

Table (3-5) contains a list of all primers used in the present study.

Primer sequences utilized in gene amplification are shown in Table (3-5).

Gene	Primer Sequences (5' - 3')	Product size	Reference
16SrRNA	F*: AGAGTTTGATCCTGGCTCAG R*: GGTACCTTGTACGACTT	1500bp	Matter <i>et al</i> 2014
K1	F: 5'-AGATAGAGGTGTATTGTCGC R:GAGCTCTATATGTTGGATGC	352bp	Faizabad <i>et al</i> ,2013
K2	F: TCATACTTGACAGAGGGAGTAG R: ACGATCGTTACAGTGACAAG	321 bp	Faizabad <i>et al</i> ,2013
blaSHV	F: GGCCGCGTAGGCATGATAGA R: CCCGGCGATTGCTGATTTC	714bp	Paterson <i>et al</i> .,2003

A: Adanin, T: Thaymin, C: Cytocin, G: Guanin

Five microliters of DNA were combined with dye for loading and put into wells to electrophorese the separated DNA samples. After that, the wells were exposed to an electric field for 45–60 minutes at 70V. Tables listed the thermo cycling programs for the K1, K2, blaSHV, and 16SrRNA genes, respectively.

Table (3-6): Program of 16SrRNA gene .

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5 min	1
Denaturation	95	45 sec	35
Annealing	58	45 sec	
Extension	72	45 sec	
Final extension	72	7 min	1

Table (3-7): Program of K1, and K2 genes

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5 min	1
Denaturation	94	45 sec	35
Annealing	56	45 sec	
Extension	72	45 sec	
Final extension	72	5 min	1

Table (3-8): BalSHV gene program.

Step	Temperature, °C	Time	Cycle
Initial denaturation	96	5 min	1
Denaturation	96	60 sec	35
Annealing	60	60 sec	
Extension	72	60 sec	
Final extension	72	10 min	1

3.2.7.4 The electrophoresis of agarose gel

1- One gram or one gram to half gram(1% or 1.5%) of agarose were dissolved in 100 milliliters of 1X TBE buffer to create a 1% or 1.5% gel of agarose powder. The mixture containers were set on a heated surface along with a magnetic stirring device once the agarose had completely dissolved, and they were covered with foil of aluminum to prevent evaporation. Following PCR identification of the amplified the gene, 1.5% agarose was employed, and following DNA extraction, 1% agarose was utilized in electrophoresis.

2- After being removed from the hot plate, the flask was let to cool to between 45 and 55 °C.

3- The gel was then combined with 8 µl of safety dye and gently swirled to include it completely.

4- A 16-toothed comb that was appropriate was placed on the chamber's interior and dipped in agarose gel.

5- A micropipette tip was then used to quickly remove air bubbles from the molten gel after the molten agarose solution had been put into the chamber.

6- After the gel had set for half an hour at room temperature, the comb as well as tape were gently taken out.

7- The comb was gently removed upon final solidification, and the jar was then filled with TBE buffer and placed within the electrophoresis chamber.

8- Following the addition of 2 µl of the loading pigment (bromothymol blue) to 5 µl of the extracted DNA, the separated DNA samples or PCR product were electrophoresed and placed into the proper wells. They were then subjected to an electrical field (70V for 45–60 minutes).

9- Lastly, the PCR product or nucleic acid was analyzed using UV light (300 nm) that was passed across the gel (Podzorski *et al.*, 2006).

3.2.8 Statistical Analysis

Descriptive statistics were used for the statistical analysis, and percentages (%) were used to represent the nominal variables (source of isolation). Five age groups were created from the age variable, which was regarded as an ordinal variable" To investigate all of the relationships and quality of fit within the variables, the chi-square along with Fisher's accurate tests were employed. The correlation between numerical and interval variables was assessed using the Eta test. Values below 0.05 were deemed statistically significant, taking into account the Alpha significance threshold.

Chapter Four

Results

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Results

4.1: Isolation of *Klebsiella pneumoniae*

Results illustrated in table (4.1) summarize the bacterial isolation rate of the different cow samples. 120 samples, 58(48.3%) showed positive growth (20 milk, 20 nasal swabs, and 18 vaginal swabs). Only 4/58 (6.9%) isolates were identified as *Klebsiella pneumoniae*. The recovery rate of *Klebsiella pneumoniae* was equal in milk and nasal secretion 2/20 (10%) for each. Nevertheless, *Klebsiella pneumoniae* could not be isolated from all vaginal secretion 0/18(0%). The bacteria's recoveries from the isolation sources did not differ significantly ($P>0.05$), according to the statistical analysis.

Table (4.1): Isolation rate of different cow samples

No. of swabs	Sours	No.	Positive growth	Positive to <i>Klebsiella</i> and percent
120	Milk	40	20	2 (10%)
	Nasal secretion	40	20	2(10%)
	Vaginal secretion	40	18	0
Total		120	58(48.3%)	4(6.9%)
P-value				0.876

Chi-square (X^2) = 267

4.2: Identification of *Klebsiella pneumoniae*

To determine the identification of the bacterial isolation, a battery of morphological, physiological, 4 isolates were obtained from (120) samples. Culture, microscopy, then Vitek II system were also used to validate the isolates' bacteria identities.

4.3 Colony morphology

After 24-48 hours of incubation on agar from MacConkey at 37 °C, the first bacteria from the huge colony were identified, pink, regular edged, spherical colonies with a mucoid texture were produced by *Klebsiella* on MacConkey agar when the bacteria fermented the lactose.



Figure (4.1) *Klebsiella pneumoniae* on MacConkey agar

4.4: Staining reaction

Under a microscope, the *Klebsiella* isolates were confirmed to be non-motile, Gram-negative, tiny straight rods that were either single or in pairs, as previously described by (Garrityetal.,2005).

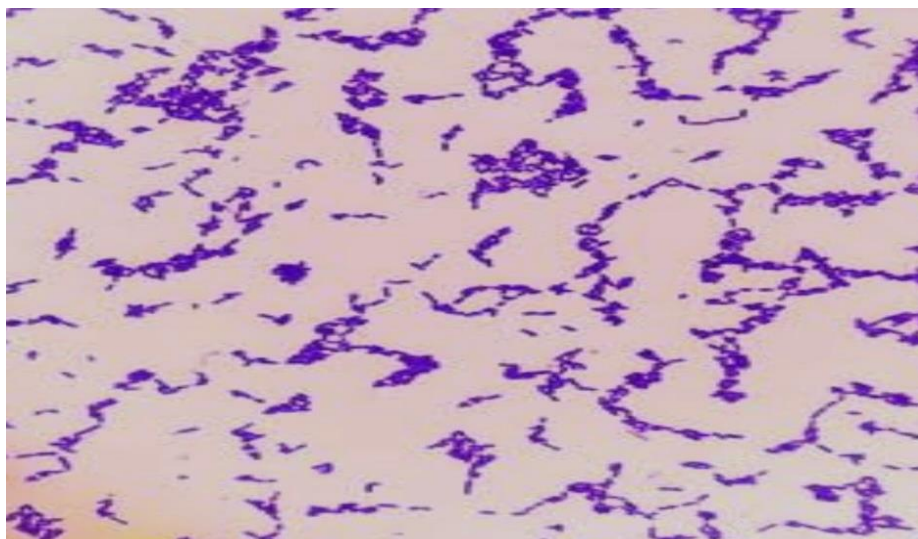


Figure (4-2) Gram stain of *Klebsiella pneumoniae*.

4.5: VitekII system for identification of *Klebsiella pneumoniae*:

Klebsiella pneumoniae identification by this method rapidly in this approach with environment free of kind of pollution that may have prevented the presence of the pathogen from being identified in any other way. The result from this system's testing corroborated those from the morphological and PCR test.

Table(4.2) confirmed vitekII result for *klebsiella pneumonia*.

Samples	Total number	Positive+	Percentage	Negative-p
Milk	20	2	10%	18
Nasal secretion	20	2	10%	18
Vagina secretion	18	0	0%	18

4.6: Result of mastitis according to age

Regarding the distribution of mastitis cases among the age groups, this study found that mastitis was significantly associated ($P < 0.05$) with the smaller age groups (1-2 years) table (4-3). An excellent statistical correlation (Eta value =1) was also found between mastitis and age. Mastitis was most prevalent in the 1-2 year age group (47.5%), followed by the 3 year age group (20%) and the 4 year age group (15%). In comparison, the age groups 5 and 6 years were recorded at 12.5% and 5%, respectively.

Table (4.3) Distribution of mastitis infection according to age

Age	Positive N0.	Percent	P-value (X^2)
1-2 years	19	47,5%%	000 (21.250)
2-3 years	8	20%	
3-4 years	6	15%	
4-5 years	5	12,5%	
6 years	2	5%	
Total	40	100%	

4.7 Result of bacterial vaginitis according to age .

The findings of the of vaginal infections by age group are shown in table (4-4). The age range was greater than one year to <4 years, with a mean of 1.95 ± 1.2 years. In this study, the prevalence of infections of the vagina by age group varied considerably ($P < 0.05$) . The age group over (1-2) years had the greatest infection rate (50%) while the age group between (2-3) years had the second-highest rate

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(25%). However, the vaginal infection in the age groups (3-4) and (4-5) years was equal (10%) for each. The lowest infection rate was found in the age group (5-6) years was (5%). Similarly, an excellent correlation was found between the age groups and the vaginal infection (Eta value = 98%).

Table (4.4): distribution of vaginal infection among different cow age groups

Age	Positive No.	Percent	P-value (X2)
1-2	20	50%	000 (27.00)
2-3	10	25%	
3 -4	4	10%	
4 -5	4	10%	
4-6	2	5%	
Total	40	100%	

X2 = Chi-square test, degree of freedom=4

4.8: Results of bacterial from nasal discharge according to age

In relation to nasal infections, this study discovered that there was a statistically significant ($P < 0.05$) distribution of nasal infections across the various age groups. The highest nasal infection rate (50%) was found in the smaller age group (<1 year old), followed by the 25%, which accounted for the age group (1-2 years old). On the other hand, the age groups (2-3) years and (3-4) years recorded 10% for each, and finally the lowest infection rate was found in the older age group (>4 years). Statistically, there is an excellent correlation (Eta value = 96%) between the age groups and the nasal infection rate.

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Table (4.5) distribution of nasal infection among different cow age groups

Age	Positive No.	Percent	P-value (X2)
<1	20	50%	000 (27.00)
1-2year	10	25%	
2-3 years	4	10%	
3-4 years	4	10%	
>4	2	5%	
Total	40	100%	

4.9: Comparative of vitekII result and PCR result.

The results in vitekII were similar for PCR results as explained in the table(4.6).

Table (4-6) positivity of VitekII and PCR in identification of *K. pneumonia* from different cow samples

Screening Techniques	Positive	Negative
VitekII	4	54
PCR	4	54
P-value	0.642	

Fisher exact test

4.10: Results of antimicrobial susceptibility test for *Klebsiella pneumoniae* .

All *Klebsiella pneumoniae* were sensitive in rate (100%)for the all antibiotics used in current study as show in table (4.6).

Anti-biotic	Isolation 1	Isolation 2	Isolation 3	Isolation4	Resistance	sensitive	percent
Ampicilin/sulbactam						sensitive	100%
Piperacillin/Tazobactam						sensitive	100%
Cefotaxime						sensitive	100%
Ceftazidime						sensitive	100%
Ceftazidime/Azobactam						sensitive	100%
Ceftazidime/Tazobactam						sensitive	100%
Cefepime						sensitive	100%
Imipenem						Sensitive	100%
Meropenem						sensitive	100%
Amikacin						sensitive	100%
Gentamicin						sensitive	100%
Ciprofloxacin						sensitive	100%
Tigecycline						sensitive	100%
Trimethoprim/sulfamethoxazole						sensitive	100%

Table(4.7) showed the bacteria resistant to anti-biotic

4.11: Results of PCR technique

The genomic DNA of every *Klebsiella pneumoniae* sample was displayed in Fig. (4-1) based on the findings of the PCR procedure.

The present results recorded that (4/4; 100%) of *K. pneumoniae* isolates giving positive results for 16SrRNA gene. The bands of this gene which determined the size of 16SrRNA gene, nearly 1500bp, as shown in Fig. (4-2). While, K1,K2 genes were not detected in all samples of *K. pneumoniae*. Also, completely isolates of *K. pneumoniae* harbored blaSHv gene (100%), as in Fig. (4-3).

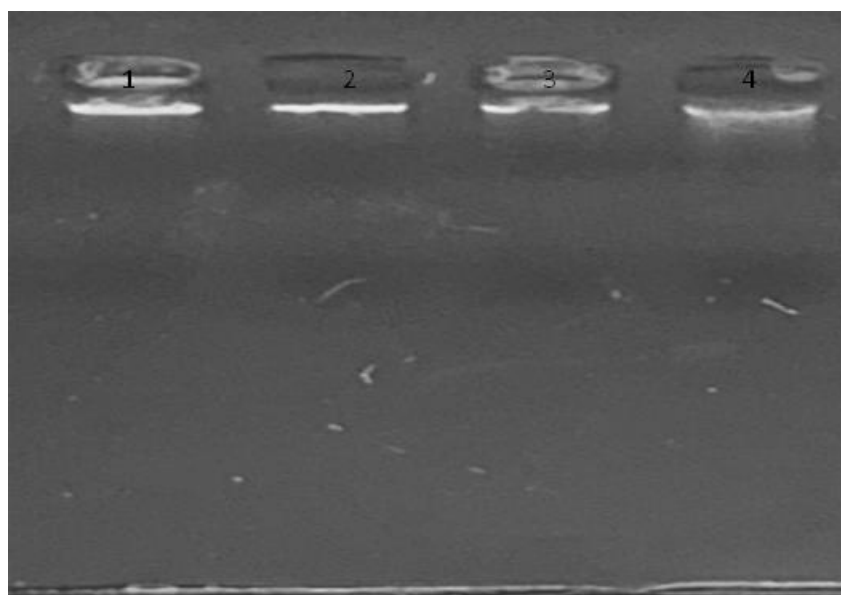


Fig (4-3): Electrophoresis Genomic DNA of *K. pneumoniae*

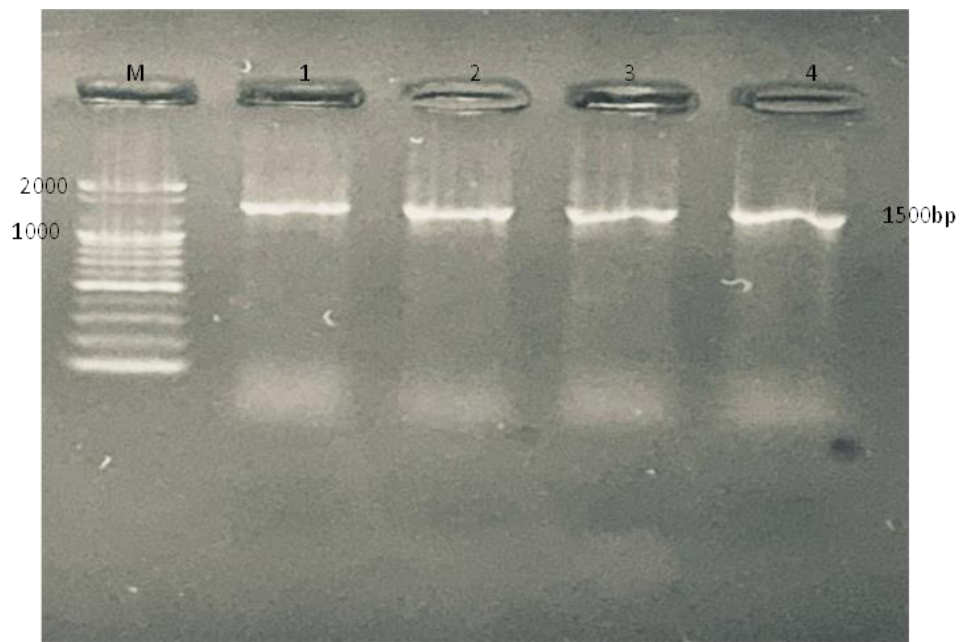


Fig. (4-4): The 16S rRNA gene was amplified using agarose gel electrophoresis, with M standing for ladder and 1-4 for positive findings.

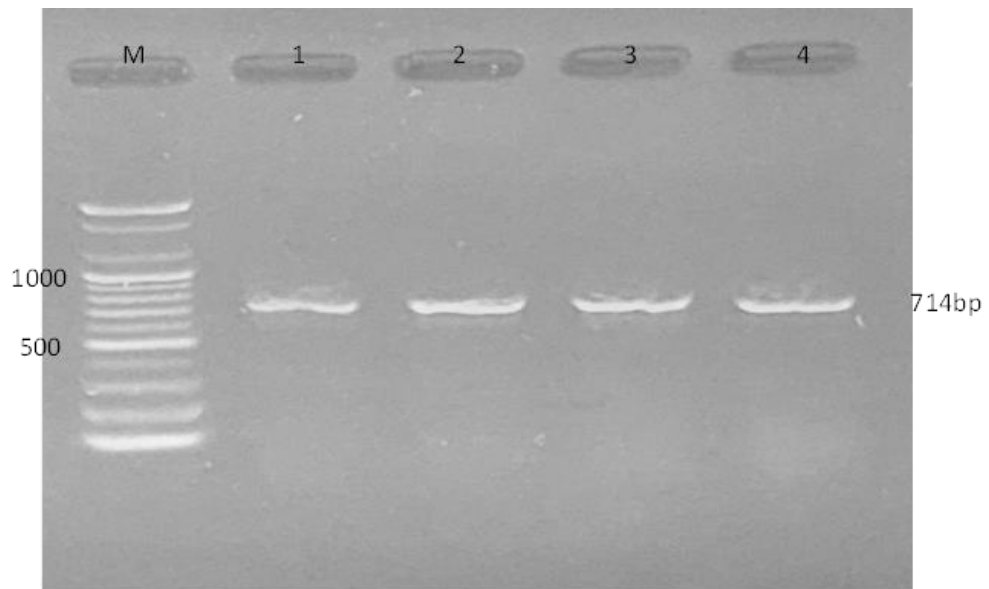


Fig. (4-3): Amplification of the blaSHV gene using agarose gel electrophoresis, where M stands for ladder, and 1-4 for positive findings.

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4.12: DNA Sequencing of 16S rRNA *K. pneumoniae* isolated from milk and nasal secretion of cattle

The main aim of the current study was to identify genetic variants and identify evolutionary relationships using the *Klebsiella pneumoniae* phylogenetic tree, which isolated from milk and nasal secretion of cattle. A 1500 bp genetic fragment found in bacteria samples was amplified, partially covering a coding region of the 16SrRNA gene.

Using a direct sequencing approach, the PCR amplicons found within the amplified genetic region were examined. The detected variations were then subjected to a phylogenetic tree in order to evaluate the precise phylogenetic distances in conjunction with other relevant bacterial genomes. According to the current findings, there is almost 99% homology between the *Klebsiella pneumonia* related samples under study. Numerous 16S rRNA gene genetic variants were found in isolates of *Klebsiella pneumoniae*. Subsequently, many variations were variably distributed in the (S2, and S3) samples such as (C7T, T103A, A122T, A130G, G365A, A373C, C375T, and others), while other nucleoids variations detected in all samples, as: (C377T, Gins378). Also, samples (S1, and S4) showed in current phylogenetic trees were closely relation, and have many nucleotide variations, like (C87T, Gins360, G361A). The *Klebsiella pneumonia* samples were submitted in NCBI with accession number: S1:LC860079,

S2:LC860080 , S3:LC860081, S4:LC860082.

4.13: DNA-Sequencing of PCR amplicons

The PCR amplified fragments were sequenced from the start according to the instructions provided by the sequencing the company (Macrogen Inc. Geumchen, Seoul, South Korea). To confirm that annotations and variations are free of PCR or

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sequencing artifacts, only clear chromatographs with ABI sequence files were analyzed. The virtual locations of the returned PCR fragments and other details were determined by comparing the spotted nucleic acid sequence data from microbiological samples with reference sequencing from the bacterial database.

4.14: Interpretation of sequencing data

Version 7.1 of the BioEdit Sequencing Alignment Editor software was used to edit, align, and evaluate the PCR products' sequencing findings with the corresponding sequences in the source database (DNASTAR, Madison, WI, USA). The PCR amplicons' and the reference genome's identified nucleic acid sites were both numbered.

4.15: Phylogenetic tree

Using the NCBI-BLASTn service, the changes found in the bacteria under study were compared to the nearby homologous reference sequences in a particular phylogeny (Zhang *et al.* 2000). In order to provide a standard tool in branch building, the iTOL suite was used to build an inclusive tree that included the discovered variation using the neighbor-joining approach (Letunic and Bork, 2019). The evolutionary history was estimated using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary investigations were conducted in MEGA11 (Tamura et al., 2021).

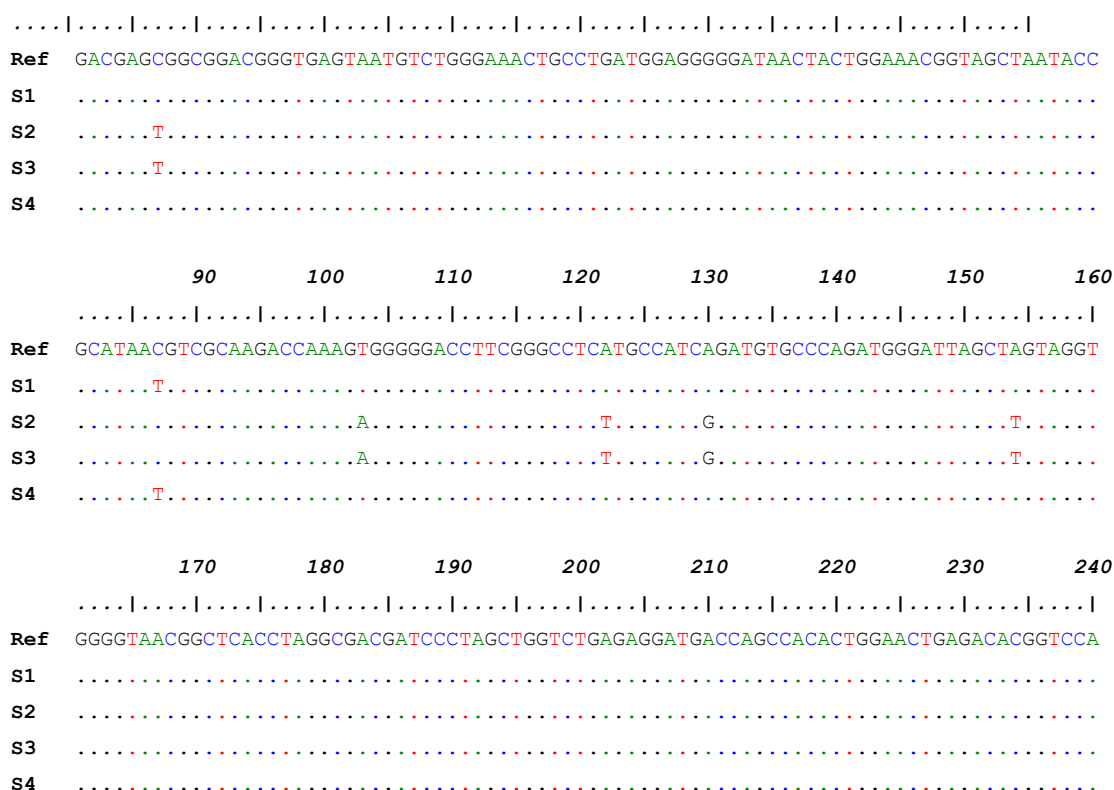
4.16: Results of 16S rRNA gene of *K. pneumoniae*

Four samples were utilized to examine the variance in the structure for the 16S rRNA genes, which are responsible for *Klebsiella pneumoniae*, and the amplicons were around 1500 bp long. The amplified amplicons were checked for clear, crisp, and distinct bands before being sent to sequencing.

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Using the NCBI blastn, the identification of the generated product based on the reaction sequence was confirmed. A significant amount of similarities in sequence between sequenced samples and those from the *Klebsiella pneumoniae* sequences was found using the NCBI BLASTn engine with respect to the 1500 bp amplicons produced by PCR of the current targeted 16SrRNA sequences. According to the NCBI BLASTn engine, the coding area of the 16S rRNA gene sequence genes was mostly encompassed by the around 99% match with the targeted target. by a comparison between the recoverable DNA sequences and the samples under study's published DNA sequencing (GenBank: NZ_KQ088287.1).

17 nucleic acid differences were found in the 1500 bp samples' alignment findings when compared to the respective *Klebsiella pneumoniae* reference sequences (Fig. 4-4). These sequences were produced by comparing the samples being studied with the most similar sequences that have been contributed to the database of the National Center for Information on Biotechnology (GenBank accession number NZ_KQ088287.1).



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250 260 270 280 290 300 310 320

Ref GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTTGAAGAAG

S1

S2 A.....

S3 A.....

S4

330 340 350 360 370 380 390 400

Ref GCCTTCGGGTGTAAAGCATTTCAGCGGGAGGAAGGC-GTAAGGTTAATAACCTC-TCGATTGACGTTACCCGCAGAA

S1 GA.....TG.....

S2 T.....GA.....A.....C.T.TGCTC.....

S3 T.....GA.....A.....C.T.TGCTC.....

S4 GA.....TG.....

410 420 430 440 450 460 470 480

Ref GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGTAAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTTAAAGC

S1

S2

S3

S4

490 500 510 520 530 540 550 560

Ref GCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACCTGCATTTCGAAACTGGCAGGCTAGAG

S1

S2 T..T.....A.....CT..T.....A..T.....

S3 T..T.....A.....CT..T.....A..T.....

S4

570 580 590 600 610 620 630 640

Ref TCTTGTAGAGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCC

S1

S2 ...C.....

S3 ...C.....

S4

650 660 670 680 690 700 710 720

Ref CCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCCTGGTAGTCCACGCCGTAAA

S1

S2 G.....C.....

S3 G.....C.....

S4

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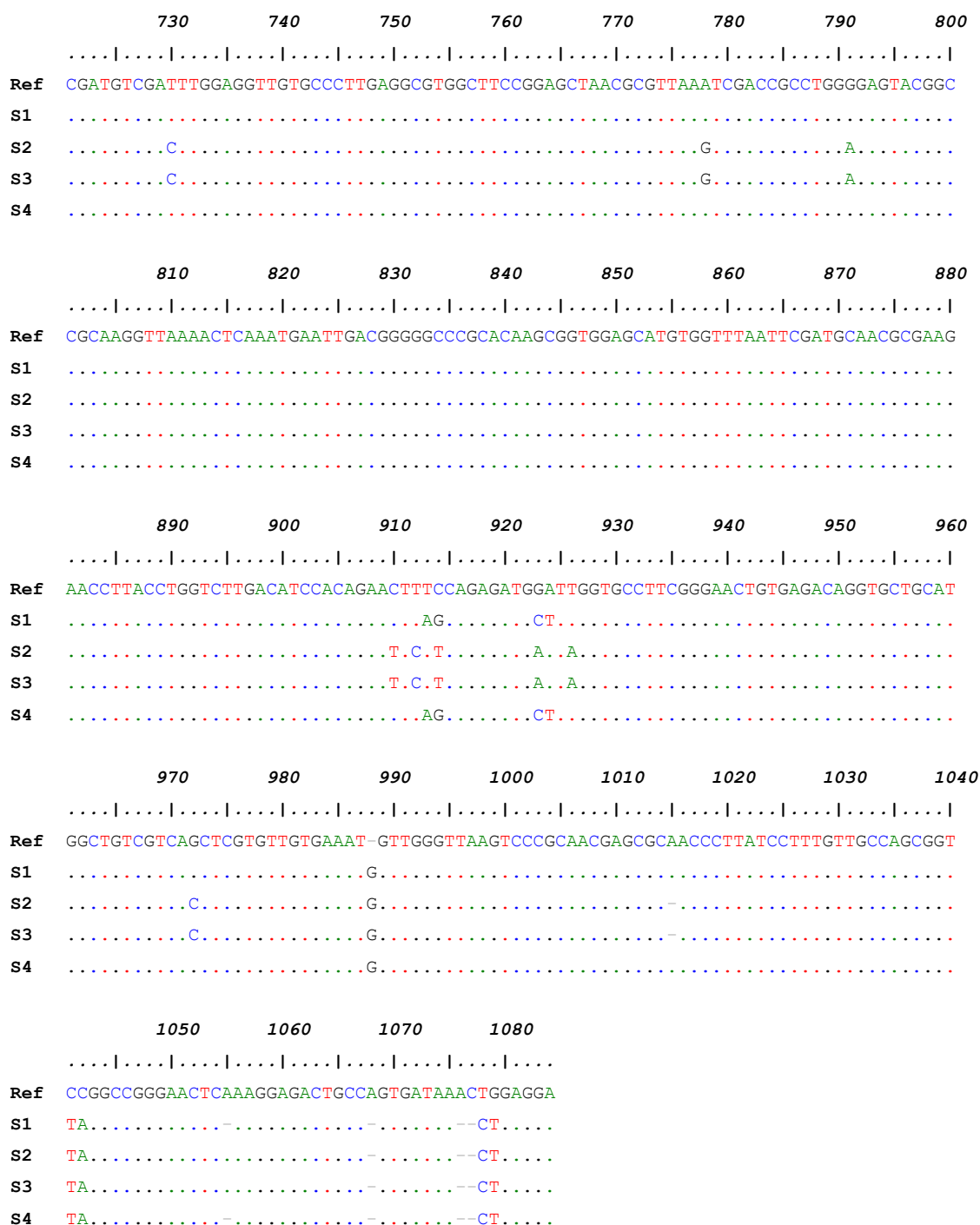


Figure (4-4) shows the alignment of four bacterial samples' nucleic acid sequences with the reference 16S rRNA sequences found in the genomic DNA sequences of *Klebsiella pneumoniae*.

The NCBI standard sequences are denoted by the notation ref:, whereas sample numbers are denoted by "S:."

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Table (4-8) provides a summary of all the findings derived from the 1500 bp segments that were sequenced. It details the precise locations of the variants that were identified.

Sample	Variant	Position in the PCR fragment
S2,S3	C7T	7
S1,S4	C87T	87
S2,S3	T103A	103
S2,S3	A122T	122
S2,S3	A130G	130
S2,S3	A154T	154
S2,S3	C338T	338
S2,S3	C359G	359
S1,S4	Gins360	360
S2,S3	Ains360	360
S1,S4	G361A	361
S2,S3	G365A	365
S2,S3	A373C	373
S2,S3	C375T	375
S1,S2,S3,S4	C377T	377
S1,S2,S3,S4	Gins378	378
S2,S3	T379C	379
S2,S3	T380C	380
S2,S3	G381C	381
S2,S3	C494T	494
S2,S3	C498T	498
S2,S3	G504A	504
S2,S3	T541C	541
S2,S3	C544T	544
S2,S3	G553A	553

S2,S3	A557T	557
S2,S3	T564C	564
S2,S3	A649G	649
S2,S3	A719C	719
S2,S3	T730C	730
S2,S3	A778G	778
S2,S3	G791A	791
S1,S2,S3,S4	T1078C	1078
S1,S2,S3,S4	G1079T	1079

4.17: Mutation result show most genetic variation in 16s rRNA

Table (4-8) shows the pattern of the detected changes in the 16S rRNA amplicons' 1500 bp when compared to the NCBI reference sequences (GenBank accession number NZ_KQ088287.1).

Sample	Variant	Position in the PCR fragment
--------	---------	------------------------------

The phylogenetic tree as shown in figure (4-5) described the most relative sequences to studied samples were found in very closed related with *Klebsiella pneumoniae* isolates. The samples (S1 and S4) were closely related in this phylogenetic tree with (KT588650.1, CP166017.1, PQ432922.1 *Klebsiella pneumoniae*), with adjacent branch; those samples were binding with other branch with ref sequence.

While, samples (S2,S3) were closely related in the current phylogenetic tree, and two samples separated from compared samples with separated branch. The results of phylogenetic tree and genetic variation detected by DNA sequencing improved the results of similarity and relation between (S2, and S3) sample.

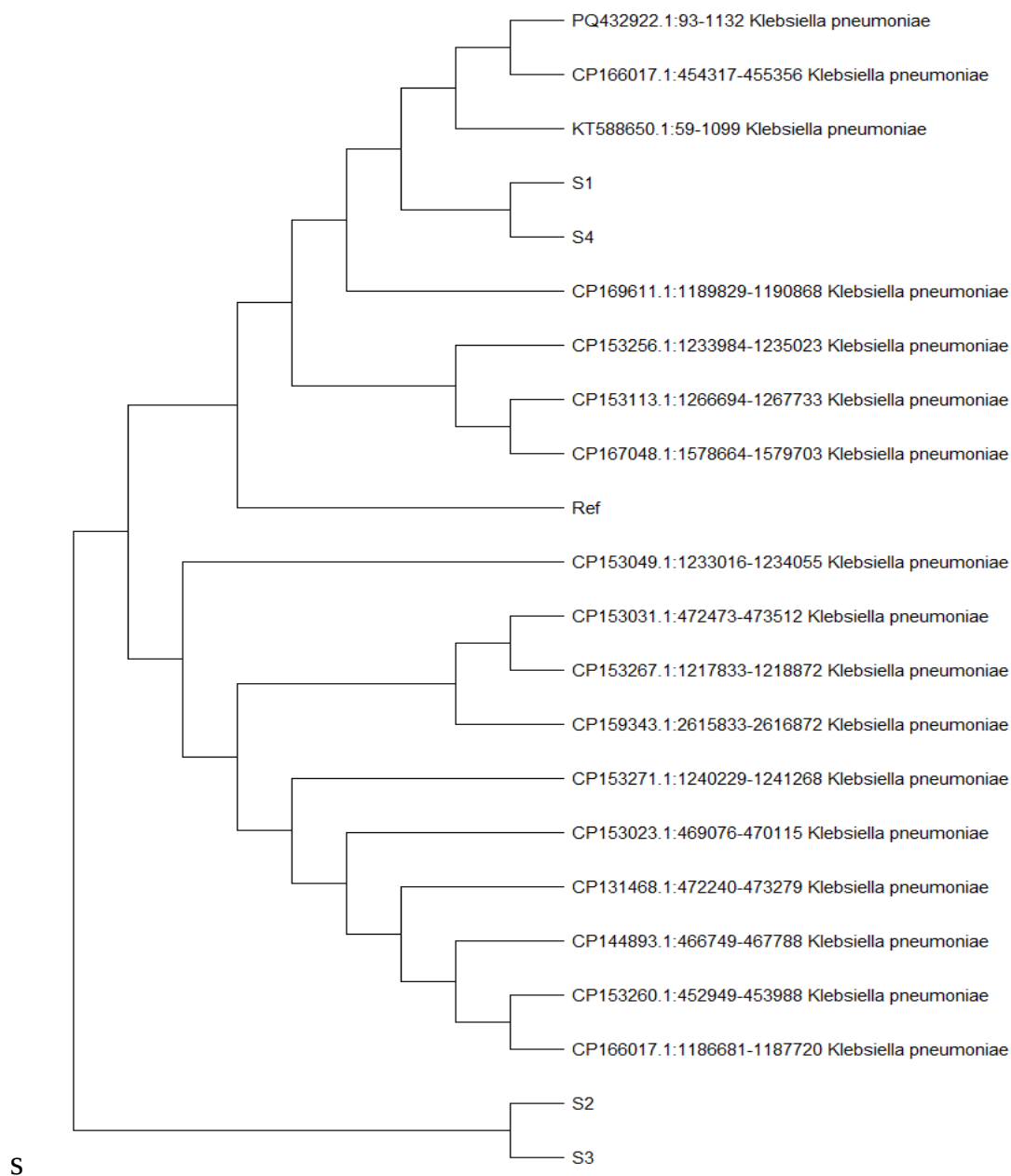


Fig. (4.5): phylogenetic tree of 16SrRNA gene in studied samples of *Klebsiella pneumoniae*.

Presently displayed is the tree having the greatest log probability (-1821.45). Neighbor-based heuristic search automatically produced the initial tree or trees. The Joining and BioNJ algorithms are then fed a bilateral distance matrix computed using the Tamura-Nei model, and the topologies with the highest log probability

value are chosen. 22 nucleotides sequences were researched in this study. First, the second, the third, and noncoding codon positions were contained. In total, there were 1041 locations in the completed dataset.

4.18 Submission in NCBI

Completely bacterial samples of *K. pneumonia* were sub-mitted in NCBI with accession number:

S1:LC860079

S2:LC860080

S3:LC860081

S4:LC860082

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Discussion

5.1:Isolation of *Klebsiella* isolates:

One of the most significant opportunistic pathogens in animals, *Klebsiella pneumoniae* mostly causes mastitis and respiratory infections. In recent research. The samples were examined for presence of *Klebsiella* isolates. A total of 120 clinical samples and swabs were included (40 from milk, 40 from nasal discharge and 40 from vagina discharge) about 58(48%) of bacteria culture belong to different Gram-negative bacteria and about 62 of these samples and swabs are no growth.

From the milk samples were examined and the results, referred that 20 (50%) which contained bacterial isolates, on the other hand, 20 (50%) of samples were negative for bacterial isolates. From positive cultures of milk samples several Gram negative bacterial isolates were identified such as *E.coli* 5 (25%), *Enterobacter cloacae complex* 3(15%), *Leclercia decarboxylata* 2(10%), *Klebsiella pneumonia* 2(10%) *Acinetobacter spp.* 2(10%) *Stenotrophomonas maltophilia* 2 (10%), while other bacterial isolates were *Pseudomonas alcaligenes*, , *Providencia rettgeri*, *Pseudomonas stutzeri*, and *Pseudomonas aeruginosa* 1(5%) respectively .

From nasal swabs the total numbers were 40 samples. no growth was 20 the other negative bacteria 20(50%) include *E.coli* 13(65%), *Burkholderia cepacia* 1(5%), *Coronobactersakazakii* 1(5%), *Achromobacterdenitrificans* 1(5%), *Pseudomonas alcaligenes* 1 (5%), *Burkholderia galdii* 1(5%) and *Klebsiella pneumoniae* 2 (10%). From 40 vaginal swabs isolated the present study involved no growth were in 22 swabs, while the positive numbers were 18 (45%) included *E.coli* 13(72,5%), *Escherichia hermannii* 1(5,5%), *Achromobacter lowffii* 1(5,5%), *Aeromonas hydrophila*1(5,5%), *Pseudomonas aeruginosa* 1(5,5%) and *Enterobaxtercloacae complex* 1(5,5%).

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Klebsiella pneumoniae were isolated from milk samples in rate 2/20 (10%) . On the other hand, its isolated from nasal discharge in rate 2/20(10%) Nevertheless, *Klebsiella pneumoniae* could not be isolated from all vaginal secretion 0/18(0%).

This result conceder nearly similar to Ramadan, M. M. (2023) which was examined samples and swaps from cattle and showed that *Klebsiella pneumoniae* number was in nasal swaps 24/233(10.3%) and 5/60(8.33%) milk and no recorded of *Klebsiell apneumoniae* from vagina secretion . The current study which agreement to other study done by Chiaverini and his group (2022), who examined samples from wild animals and the *Klebsiella pneumoniae* isolate recorded 13/130 in rate 13%. In addition to that, he detected the same cultural characters of *klebsiella* species and recorded that the isolated bacteria appeared as pink, large , mucoid colonies on McConkey agar for 24 h at 37 C.

Higher isolation rate of *klebsiella* species from different sources most of them were from pig, cattle, milk and the rest of isolates were isolated from vegetables, pets, livestock and farm animals (Klaper *et al.*, 2021).

5.2: Identification of *Klebsiella pneumoniae* by culturing and colony morphology and staining reaction

The bacteria were originally identified from the colony, which is a big, spherical, regular-edged colony with a mucous membrane texture and pink due to the acids generated by *Klebsiella* on the agar from MacConkey when the microbes fermented the lactose, after being incubated on the agar at 37 °C for 24 to Identification of *Klebsiella* spp. by using selective enriched media involved cultivating materials on MacConkey agar with crystal violet and bile salts which is favor to growth of *Enterobacteriaceae* and similar enteric Gram-negative rod while discouraging the development of Gram-positive bacteria and other very fussy Gram-negative

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bacteria. in this media the present of lactose can be differentiation between lactose-fermenting bacteria from non- fermenting bacteria. Fermenting bacteria when the pH. is low enough, turns colonies pink from the breakdown of neutral red indicator dye. *Klebsiella pneumoniae* under a microscope were confirmed to be non-motile, Gram-negative, tiny straight rods that were either single or in pairs, as previously described by (Garrityetal., 2005) which similar to Dubey et al (2013).

5.3: VitekII system for identification of *Klebsiella* isolates

The Vitek technology is utilized to screen the four isolates via the standard protocol demanding 0.5 McFarland inoculum and based on the opinion of the manufacturer's directions and interpretations delivered by the software version. This technique occurs by loop culture of stock agar and cultured on agar overnight then the bacterial suspension was put in normal saline ($1-2 \times 10^8$) CFU/ML.

In vitek II only 4/58 (6.9%) isolates were identified as *Klebsiella pneumoniae*. This result which similar to the results of Yang 2021when he isolated (4/66, 6.06%)

5.4: Antibiotic resistance of *Klebsiella pneumoniae* isolates

In this study the isolated strain of *Klebsiella pneumoniae* tested against 14 antibiotic with various mechanisms of action such as (Ampicilin/ Sulbactam, Piperacillin/Tazobactam, Cefotaxime, Ceftazidime, Ceftazidine/Avibactam, Ceftazidine/Tazobactam, Cefepime, Imipenem, Meropenem, Amekacin, Gentamicin , Ciprofloxacin, Tigecycline and Trimethoprim/sulfamethoxazole). The result of this study relevant that all isolated of *Klebsiella pneumoniae* exhibited sensitive to all this antibiotic in rate 100%. The other studies showed that *Klebsiella pneumoniae*'s resistance rate to some antibiotic as the following (ampicillin98,1%,

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ciprofloxacin 35.9%, Trimethoprim sulfamethoxazole 35%, Cefotaxime 34%, Gentamicin 31.1%, Tetracycline 31.1%) according to Haradet *et al* (2016).

Moreover, Gomaa (2021) verified that all *Klebsiella pneumonia* isolates were susceptible to the drugs a antibiotic such as Trimethoprim/Sulphathiazole and Trimethoprim (54.54% each), but resistant to the antibiotic Ampicillin and Amoxicillin-clavulanic acid (100%) following Cefepime (72.72%), Imipenem (82%) followed by Aztreonam (55%), and Amikacin and Azithromycin (45%).

Montso, *et al.*, (2019) showed that The highest multi drug resistance rate were found among *klebsiella* isolates from cattle in rate reached to (85–100%). Brisse and Duijkeren (2005) found that from 78 isolates, resistance was common against Ampicillin (99%) and Cephalexin (43%), but not against Tetracycline, ceftazidime, , Enrofloxacin, ceftiofur, Gentamicin and Trimethoprim sulfamethoxazole.

Ramadan, M. M. (2023) study showed that the biofim formation module indicated that all strains were biofilm formed strains. It was shown that 81.81% of the *Klebsiella pneumoniae* isolates were high (33.33%), moderate (22.22%), or weakly (44.44%) polymeric producers, compared to 18.18% of the total number of isolates that did not form biofilms (Gomaa, 2021). This result supports the findings of Massé *et al.* (2020).

5.5:Molecular discussion

The objective of the current study was to identify the evolutionary relationships by phylogenetic tree of all samples of *Klebsiella pneumoniae* isolated from cattle's milk and nasal secretions, as well as to identify genetic variants of the 16SrRNA genes by DNA sequencing. Life-threatening illnesses are caused by *Klebsiella pneumoniae*. Nonetheless, the organisms have been linked to the onset of sickness (Villegas *et al.*, 2006). Reports that bacteria that cause *Klebsiella*

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pneumoniae can spread swiftly across wide geographic areas are especially worrisome (Navon-Venezia *et al.*, 2009).

The 16SrRNA gene sequencing used to identified the *Klebsiella pneumoniae* that isolated from various sources as samples obtained from animal, or from clinical isolates such as urine, blood, feces, sputum, which were aligned and compared to the sequence of strains that deposited in Gen Bank data base (Budiarso *et al.*, 2021).

Despite their potential health risks, street meals are popular in nations that are developing due to their low prices and preference among the lower and middle classes. According to Rowbotham and Ruegg (2016) and Fuenzalida and Ruegg (2019), *Klebsiella pneumoniae* and *Klebsiella oxytoca* are commonly found in farm surroundings, cow skins and the milk, teet-end cotton swabs and clinical mastitis. Additionally, the *Klebsiella pneumoniae* species was a significant pathogen linked to the health of both humans and animals. Urinary tract infections, liver abscesses, and pneumonia have all been linked to *Klebsiella pneumoniae* (Bengoechea and Pessoa 2019; Fuenzalida and Ruegg 2019).

There were many nucleoids variations of 16SrRNA gene in *Klebsiella pneumoniae* in (S2, and S3) samples that closely relation such as (T380C, G381C, C494T, C498T, G504A, T541C, C544T, G553A, A557T, T564C, A649G, and others), while some nucleoids variations detected in all samples, like: (C377T, Gins378). The fact that the 16SrRNA gene differs among bacterial species must be emphasized. The chosen hyper variable portions of the 16S gene were used for the 16SrRNA gene sequencing; nevertheless, shorter sequences can yield findings that are similar to those of the entire genome. Unfortunately, no one hyper variable area that can distinguish among all identified bacterial species has been found to yet

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(Liu *et al.*, 2007). Also, samples (S1, and S4) showed in current phylogenetic trees were closely relation, and detected many of genetic variation in those samples.

Mutations caused by their surroundings can affect bacteria. However, this is a normal aspect of their growth, enabling them to regularly alter their genetic code (Chandran and A. Mazumder, 2023).

According to the Klaif *et al.* (2019) study, the genetic analysis shows overlap between the clinical and meat source The primary strain of *Klebsiella pneumoniae* found in human and animal isolates, as well as those recovered from urine Humans were closer to (MF429117.1), which was isolated from Chinese buffalo milk. This indicates a stronger correlation between human strains and cattle strains as well as zoonotic The potential danger of the bacteria *Klebsiella pneumoniae*, which could infect people through foodborne goods, was made clear by their relationship (Davis *et al.*, 2016).

The present results documented that high genetic variation in nucleotide positions of 16SrRNA gene in *Klebsiella pneumoniae* isolated from milk and nasal secretion of cattle that may be related isolated source of this microorganism. Budiarmo *et al.*, (2021) recorded 13 nucleotide polymorphic sites among *Klebsiella pneumoniae* group, Some isolates contained two distinct nucleotide polymorphisms that differed from all other isolates in nucleotide locations. Because of the relatively high genetic distance value of those isolates' sequences, nucleotide blast was done to determine how similar the isolates were to *Citrobacter* spp.

Conclusions & Recommendations

Conclusions & Recommendations

Conclusion

The present study concluded that :

- 1) *Klebsiella pneumonia* were considered as an important bacterial causes that effect different ruminants especially cattle
- 2) *Klebsiella pneumoniae* were isolated from nasal discharge , mastitis cases while not isolated from vagina.
- 3) *Klebsiella pneumoniae* can be identified directly by vitekII system and conformed by PCR to detect 16s rRNA.
- 4) All *Klebsiella pneumoniae* isolated obtained to be sensitive to most antibiotic tested.
- 5) All *Klebsiella pneumoniae* isolates contain (100%) 16SrRAN gene and bla SHV genes.
- 6) *Klebsiella pneumoniae* isolated does not have K1,K2.
- 7) Recorded four *Klebsiella pneumoniae* strains with in NCBI.

Conclusions & Recommendations

Recommendation

According to present results can be recommended the following :

- 1) Isolated *Klebsiella pneumoniae* from other domestic animals such as sheep and goat or from small animals (dogs and cats).
- 2) Study the other genes which responsible of antibiotic or other virulence .
- 3) Study pathogenesis of *Klebsiella pneumoniae* using experimental animals as model.
- 4) Study the ability of different *Klebsiella pneumoniae* antigen to produce cellular immunology.

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Appendices

Results of vitekII system to four isolate of *Klebsiella pneumoniae* and it's antibiotic sensivity

bioMérieux Customer:Microbiology Chart ReportPrinted October 5, 2024 8:26:06 AM CDT

Patient Name: N 6, .Patient ID: 1
Location:Physician:
Lab ID: 1Isolate Number: 1

Organism Quantity:
Selected Organism : *Klebsiella pneumoniae ssp pneumoniae*

Source: Nasal SwabCollected: Oct 4, 2024

Comments:	

Identification Information	Analysis Time: 7.77 hours	Status: Final
Selected Organism	96% Probability	<i>Klebsiella pneumoniae ssp pneumoniae</i>
	Bionumber:	6607735753565153
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	(-)	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	IHISa	+	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	+	64	ILATa	+			

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:27:49 AM CDT

Patient Name: N2, .

Patient ID: 24

Location:

Physician:

Lab ID: 24

Isolate Number: 2

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae* ssp *pneumoniae*

Source: Nasal swab

Collected: Oct 6, 2024

Comments:	

Susceptibility Information			Analysis Time: 12.23 hours	Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam	8	S	Meropenem	2*	I
Piperacillin/Tazobactam	8	S	Amikacin	<= 1	S
Cefotaxime	16	R	Gentamicin	<= 1	S
Ceftazidime	0.25	S	Ciprofloxacin	<= 0.06	S
Ceftazidime/Avibactam	0.5	S	Tigecycline	<= 0.5	S
Ceftolozane/Tazobactam	0.5	S	Colistin		
Cefepime	1	S	Trimethoprim/ Sulfamethoxazole	<= 20	S
Imipenem	0.5	S			

* = AES modified ** = User modified

AES Findings	
Confidence:	Consistent with correction

bioMérieux Customer:

Microbiology Chart Report

Printed October 5, 2024 10:15:27 PM CDT

Patient Name: N 6, .

Patient ID: 1

Location:

Physician:

Lab ID: 1

Isolate Number: 2

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae*

Source: Nasal Swab

Collected: Oct 4, 2024

Comments:	

Susceptibility Information			Analysis Time: 18.03 hours	Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam	4	S	Imipenem	<= 0.25	S
Piperacillin/Tazobactam	<= 4	S	Meropenem	<= 0.25	S
Cefotaxime	<= 0.25	S	Amikacin	<= 1	S
Ceftazidime	<= 0.12	S	Gentamicin	<= 1	S
Ceftazidime/Avibactam	<= 0.12	S	Ciprofloxacin	<= 0.06	S
Ceftolozane/Tazobactam	<= 0.25	S	Tigecycline	<= 0.5	S
Cefepime	<= 0.12	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

AES Findings	
Confidence:	Consistent

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:26:57 AM CDT

Patient Name: N2, .

Patient ID: 24

Location:

Physician:

Lab ID: 24

Isolate Number: 1

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae ssp pneumoniae*

Source: Nasal swab

Collected: Oct 6, 2024

Comments:	

Identification Information	Analysis Time: 3.93 hours	Status: Final
Selected Organism	99% Probability <i>Klebsiella pneumoniae ssp pneumoniae</i>	
ID Analysis Messages	Bionumber: 6607735753565150	

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	IHISa	+	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:23:48 AM CDT

Patient Name: M11, .

Patient ID: 21

Location:

Physician:

Lab ID: 21

Isolate Number: 2

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae ssp pneumoniae*

Source: Milk

Collected: Oct 6, 2024

Comments:	

Susceptibility Information		Analysis Time: 17.90 hours		Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam	4	S	Meropenem	<= 0.25	S
Piperacillin/Tazobactam	<= 4	S	Amikacin	2	S
Cefotaxime	<= 0.25	S	Gentamicin	<= 1	S
Ceftazidime	<= 0.12	S	Ciprofloxacin	<= 0.06	S
Ceftazidime/Avibactam	<= 0.12	S	Tigecycline	<= 0.5	S
Ceftolozane/Tazobactam	<= 0.25	S	Colistin		
Cefepime	<= 0.12	S	Trimethoprim/ Sulfamethoxazole	<= 20	S
Imipenem	<= 0.25	S			

AES Findings	
Confidence:	Consistent

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:22:24 AM CDT

Patient Name: M11, .

Patient ID: 21

Location:

Physician:

Lab ID: 21

Isolate Number: 1

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae ssp pneumoniae*

Source: Milk

Collected: Oct 6, 2024

Comments:	

Identification Information	Analysis Time: 7.90 hours	Status: Final
Selected Organism	85% Probability <i>Klebsiella pneumoniae ssp pneumoniae</i>	
ID Analysis Messages	Bionumber: 6607731753567553	

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	(-)	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	+	53	IHISa	+	56	CMT	-	57	BGUR	+
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	+	64	ILATa	+			

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:24:14 AM CDT

Patient Name: M10, .

Patient ID: 22

Location:

Physician:

Lab ID: 22

Isolate Number: 1

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae ssp pneumoniae*

Source: Milk

Collected: Oct 6, 2024

Comments:	

Identification Information	Analysis Time: 5.92 hours	Status: Final
Selected Organism	95% Probability <i>Klebsiella pneumoniae ssp pneumoniae</i>	
ID Analysis Messages	Bionumber: 6607715753565010	

Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	SKG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(-)	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed October 11, 2024 5:25:11 AM CDT

Patient Name: M10, .

Patient ID: 22

Location:

Physician:

Lab ID: 22

Isolate Number: 2

Organism Quantity:

Selected Organism : *Klebsiella pneumoniae* ssp *pneumoniae*

Source: Milk

Collected: Oct 6, 2024

Comments:	

Susceptibility Information	Analysis Time: 17.88 hours	Status: Final
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Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam	8	S	Meropenem	<= 0.25	S
Piperacillin/Tazobactam	<= 4	S	Amikacin	2	S
Cefotaxime	<= 0.25	S	Gentamicin	<= 1	S
Ceftazidime	<= 0.12	S	Ciprofloxacin	<= 0.06	S
Ceftazidime/Avibactam	2*	S	Tigecycline	<= 0.5	S
Ceftolozane/Tazobactam	<= 0.25	S	Colistin		
Cefepime	<= 0.12	S	Trimethoprim/ Sulfamethoxazole	<= 20	S
Imipenem	<= 0.25	S			

* = AES modified ** = User modified

AES Findings	
Confidence:	Consistent with correction

الخلاصة

أظهرت هذه الدراسة الحالية إمكانية الكشف عن بكتيريا كليبسيلا من إفرازات الأبقار المصابة. تم جمع ١٢٠ عينة ومسحة، مُقسّمة إلى (٤٠ عينة من إفرازات الأنف، و ٤٠ عينة من إفرازات المهبل، و ٤٠ عينة من عينات الحليب). تم زراعة هذه العينات على أوساط زراعية مختلفة، وتمّ تحديدها باستخدام الخصائص الزراعية والمظهر المجهرى. تم تحديد بكتيريا كليبسيلا الرئوية باستخدام نظام ViteK2 ، وتمّ تأكيد التشخيص النهائي لهذه البكتيريا باستخدام اختبار تفاعل البوليميراز المتسلسل (PCR) (16S rRNA). استخدم تفاعل البوليميراز المتسلسل للكشف عن بعض الجينات K1 و K2 (وهو أحد الجينات المسؤولة عن تكوين الكبسولات)، وكذلك جين blaSHv ، وهو أحد الجينات المرتبطة بمقاومة الأدوية المتعددة. أظهرت نتائج الفايتهك ان معدل عزل البكتيريا من ١٢٠ عينة ومسحة ٥٨% (٤٨,٣%) والتي تضمنت بكتيريا سلبية مختلفة. من نتائج عينات الحليب أظهرت ان ٢٠ (٥٠%) منها تحتوي على عزلات بكتيرية في حين ان ٢٠ (٥٠%) من العينات كانت سلبية لعزلات بكتيرية، من العزلات الايجابية من عينات الحليب تم تحديد عده عزلات بكتيرية سالبة لصبغة كرام مثل E.coli ٥ (25%)

Leclerciaadecarboxylata2(10%) ، Enterobacter cloaca complexe ٣ (١٥%)

(Acinetobacterspp. 2(10% ، (Klebsiellapneumoniae2(10%)

Pseudomonas aeruginosa 2 (10%) Stenotrophomonasmaltophilia ، بينما العزلات الاخرى

Providenciarettgeri, Pseudomonasstutze فسلجت كل منها5(١٠%) على التوالي.

اما من المسحات الانفية فأجمالي العينات ٤٠ مسحة لم يكن هناك نمو الا في ٢٠ عينة (50%) وشملت على

E.coli ١٣ (65%) , Burkholderiacepacia 1(5%) ،

Achromobacterdenitrificans1(5%) ، (Coronobactersakazakii 1(5%)،

Pseudomonasalcaligenes1(5%),Burkholderiagaldioli1(5%),Klebsiellapneumonia

(2(10%). في حين ان من بين ٤٠ عينة مهبلية لم يكن هناك نمو في ٢٢ مسحة بينما كان هناك نمو في ١٨

عينة بنسبة (45%) وهي كالتالي ، Escherichiahermannii 1(5,5%) ، E.coli 13(72,5%) ،

Achromobacterlowffii 1(5,5%) ، Achromobacterlowffii 1(5,5%)،

Aeromonashydrophila 1(5,5%) Achromobacterlowffii 1(5,5%)

(*Pseudomonasaerugiosa* 1(5,5%), *Enterobaxtercloacaecomplex* 1(5,5%
تم عزل الكلبسيلا الرئوية من عينات الحليب (٢٠/٢) اي بنسبة (١٠%) وبنفس هذه النسبة تم عزلها من
العينات الانفية ، ومن جهة اخرى لم تعزل من العينات المهبلية ١٨/٠. تم ايضا اختبار عزلات الكلبسيلا
الرئوية ضد ١٤ نوع من المضادات الحيوية باليات عمل مختلفة مثل، (Ampicilin/ sulbactam,
Piperacillin/Tazobactam, Cefotaxime, Ceftazidime, Ceftazidine/Avibactam,
Ceftazidine/Tazobactam, Cefepime, Imipenem, Meropenem, Amekacin, Gentamicin
sulfamethoxazole). , Ciprofloxacin, Tigecycline and Trimethoprim/
أظهرت النتائج هذه الدراسة ان جميع سلالات الكلبسيلا الرئوية حساسة لجميع هذه المضادات الحيوية
بمعدل ١٠٠%. تشير النتائج ايضا الى انه لم يتم الكشف عن جينات K1,K2 في جميع عينات الكلبسيلا
الرئوية وان جميع العزلات تحمل الجين blaSHV ،وان الكلبسيلا الرئوية ممكن اعتبارها مسببا رئيسيا
للامراض المختلفة التي تؤثر على الابقار في منطقة الشطرة في محافظة ذي قار .



جمهورية العراق
وزارة التعليم العالي والبحث العلمي/جامعة الشطرة
كلية الطب البيطري
فرع الاحياء المجهرية

الكشف عن انواع الكلبسيلا من مختلف افرازات المجترات في محافظة ذي قار

رسالة ماجستير

تقدم الى مجلس كلية الطب البيطري –جامعة الشطرة كجزء من متطلبات نيل شهادة الماجستير في الاحياء
المجهرية

مقدمة من قبل

ارجوان محسن عجمي

بكالوريوس طب وجراحة بيطري

(٢٠٠٣)

بأشراف

أ.د. حيدر خميس شنان

د.رباب جبار صخي