



## Molecular Detection of *Klebsiella pneumoniae* Isolated from Respiratory Infected Sheep and Histopathological Study in Rabbits



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### Abstract

RESPIRATORY disease is common in rabbits, which is frequently difficult to detectable in live animals until it becomes serious and complicated. This study was aimed to describe the histopathological changes of the lung, liver and kidney of rabbits after induction of infection by *Klebsiella pneumoniae* which is isolated from respiratory system infected sheep that detection by using routine diagnostic methods followed identification by using the Vitek2 system and by molecular detection of *K. pneumoniae* isolates with PCR using (16S rRNA gene). This experimental study has six rabbits; two animals for control and four animals for infection. After induction of infection had used of euthanasia to six rabbits and opened the thoracic cavity in laboratory. The organs were filmed. The main pathological lesions had seen in lung, liver and kidney of rabbits which infected with *Klebsiella pneumoniae*. Histological and morphological description of respiratory tract of rabbits were achieved, The trachea supported by double cartilage. The rabbits are valuable models in respiratory research due to their respiratory hyper responsiveness to asthma resembles in humans. The histopathological changes of lung rabbit infected with *K. pneumoniae* showing mild thickness of alveoli emphysema (atelectasis). The histopathological changes of liver showed hydropic degeneration in the cytoplasm of hepatocytes with infiltration of inflammatory cell in the parenchyma of liver and congestion of blood vessels. The Histopathological changes of kidney reveals infiltration of inflammatory cell congestion and stenosis of renal lead to atrophy of renal tubules. The results of antimicrobial susceptibility showed that all 7 isolates (100%) of *K. pneumoniae* were susceptible to both imipenem and meropenem.

**Keywords:** *Klebsiella pneumoniae*, Respiratory disease, histopathological, Rabbits.

### Introduction

*Klebsiella pneumoniae* is rod-shaped, facultatively anaerobic, gram negative, non-motile, and possessing a visible polysaccharide capsule. Additionally, it ferments lactose and produces nitrite from nitrate. It is catalase positive and oxidase negative. On nutrient agar, they may proliferate, and on MacConkey agar, they can create colonies of mucoid colonies that are pink in color [1].

Breeding small ruminants, like sheep and goats, is a significant part of the economy, particularly in Iraq, where it is seen as a vital source of food goods, such as meat, milk, dairy products, and leather, as well as illnesses that afflict ruminants.[2].

Respiratory diseases usually affect livestock animals such as sheep, which have a major economic impact on sheep production [3], over concerns such as mortality, reduced weight-gains in well again

animals, reduction in carcass quality, drugs, and costs [4]. Several bacterial causes of pneumonia were studied experimentally and at fields in Iraq [5]. Sheep in Iraq can be infected with *Klebsiella pneumoniae* at an early age and most cases tend to be chronic and showed difficulty in treatment [5].

Pneumonia is common in domestic rabbits, and *Klebsiella pneumoniae* is one of the bacteria that can be involved in this condition. *Klebsiella pneumoniae* infection in rabbits can lead to pathological changes in the trachea and lung. The infection can result in symptoms such as anorexia, listlessness, dyspnea, fever, and respiratory signs like sneezing and coughing [6].

### Material and Methods

#### Specimens' collection

This study involved the collection of a total of 100 cotton swabs from the nasal cavity of local sheep

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whose ages ranged between 1 year to 4 years old. The 100 swabs were collected from sheep showing signs of cold and respiratory tract infections, nasal discharge, coughing and depression. These samples were collected from flocks reared in ten farms located in Al-Anbar province, during the period from October 2022 to February 2023. After collection, the swabs were imbedded into transport media and transported within cool box not more than two hours to the laboratory of Microbiology Department, College of Veterinary Medicine, University of Baghdad. Every sample was streaked onto MacConkey agar and blood agar, and it was then incubated aerobically at 37°C for 24 hours. Gram stain was utilized to determine the bacteria's reaction to stain, arrangement, and form, which allowed for their identification based on microscopical features [1,7].

#### *Isolation and Identification of Klebsiella pneumoniae*

##### *Klebsiella pneumoniae isolation*

Every isolate was obtained from infected sheep that were cultivated on Blood agar, Eosin Methylene Blue agar, and MacConkey agar. After an overnight incubation period at 37°C in an aerobic environment, the cultures were examined for the presence of bacteria. The pink-colored and mucous-textured colonies were subcultured onto MacConkey agar to verify lactose-fermenting (pink) from non-lactose-fermenting (colorless) bacteria. On EMB agar, *Klebsiella pneumoniae* colonies exhibit a pink to purple coloration, while on blood agar medium, the non-hemolytic colonies exhibit gamma hemolysis ( $\gamma$ -hemolysis) [8].

##### *Identification of the isolates*

Based on colony shape, the hypermucoviscosity test, staining response, and biochemical testing, probable isolates were identified [9].

##### *Colony morphology*

All isolates were identified primary according to general cultural characteristic (color, shape, texture and size) of colony onto MacConky agar, Blood agar and EMB agar after incubated overnight at 37°C. In addition, other characteristics were observed like lactose fermentation according to Quinn *et al.* [1].

##### *Hypermucoviscosity test (string test)*

The bacterial culture was inoculated onto MacConky agar or nutrient agar, and then incubated at 37°C for 24 hrs. The colony touched by a loop then lifted vertically from the surface of agar plate, mucous phenotype was defined as being present when a string-like growth was observed. *Klebsiella* spp. formed a string < 5 mm in length except *K. pneumoniae* colonies showed  $\geq$  5 mm demonstrating the hypermucoviscosity phenotype [9].

##### *VITEK 2 system for identification of Klebsiella pneumoniae isolates*

VITEK 2 system is used for diagnosis of bacterial isolates, it consists of 64 biochemical tests and 20 antibiotic tests. The VITEK 2 system was used in this study to confirm the identification of *Klebsiella pneumoniae* isolates [10].

##### *PCR detection of 16S rRNA gene*

This step was carried out by adding 12.5  $\mu$ l from OneTaq (NEB®) mastermix, 3  $\mu$ l of DNA sample, 1  $\mu$ l 10 pmol/ $\mu$ l from each primer (forward and reverse) and 7.5  $\mu$ l of free-nuclease water. The reaction done under the optimal PCR conditions for gene as shown in Table (1). And the primer sequence of 16S rRNA gene in Table (2).

##### *Disk diffusion susceptibility method*

Disk diffusion susceptibility method was performed as mentioned in Bauer-kirby *et al.*, [11]. Fourteen different antimicrobial agents were used in this study to determine the susceptibility of *Klebsiella pneumoniae*. Applying approaches illustrated in the clinical and laboratories standards institute CLSI, [12], the susceptibility tests were interpreted. This test was performed for each isolate and the average diameters were taken. The antimicrobials used to test the sensitivity of *Klebsiella pneumoniae* isolates included: Amikacin, Amoxicillin/ Clavulanic acid, Cefixime, Ceftriaxon, Chloromphenicol, Ciprofloxacin, Gentamycin, Imipenem, Meropenem, Nitrofurantoin, Pipracilline, Streptomycin, Tetracycline and Trimethoprim.

##### *Statistical analysis*

Statistical analysis of data was performed using SAS (Statistical Analysis System, 2012). Chi-square test was applied to assess the significant differences among percentages.  $P \leq 0.05$  was considered as a significant difference [13].

## **Result and Discussion**

### *Bacterial isolation*

In the present study, the 100 nasal swabs taken from deep nostrils of respiratory infected sheep, we obtained *Klebsiella pneumoniae* 7(7%) isolates and other isolates included 16 (16%) *Staphylococcus aureus*, 12 (12%) *Raoultella planticola*, 2 (2%) *Pseudomonas fluorescens*, 3 (3%) *Pseudomonas aeruginosa*, 2 (2%) *Pseudomonas putida*, 19 (19%) *E. Coli*, 5 (5%) *Butliauxella agrestis*, 3 (3%) *Pantoea* spp, 1 (1%) *Cronobacter sakazakii* group, 1 (1%) *Aeromonas salmonicida* and 4 (4%) *Leclercia adecarboxylata*; as show in (Table 3). These results and the percentages of microorganisms isolated from infected sheep are partly consistent with (Aziz and Lafta, [14] and agreed with Asaye *et al.*, [15] who demonstrated that Infectious agents are present in the upper respiratory tract of infected sheep and these

microorganisms might cause respiratory infections under stress conditions and is consistent with the study of Yimer and Asseged [16] who confirm an aerobic bacteria were cultivated from the nasal cavity were apparently pathogenic.

#### Diagnosis using Vitek2 System

VITEK 2 system is used for diagnosis of bacterial isolates, it consists of 64 biochemical tests and 20 antibiotic tests.

Isolates suspected to be *K. pneumoniae* were tested in Vitek2 system to identified *K. pneumoniae subsp pneumoniae* according to Espinar *et al.*, [10]; Bdaiwi *et al.*, [17]. In addition, using the same system, some isolates were diagnosed as 16 *Staphylococcus aureus*, 12 *Raoultella planticola*, 2 *Pseudomonas fluorescens*, 3 *Pseudomonas aeruginosa*, 2 *Pseudomonas putida*, 19 *E. Coli*, 5 *Butliauxella agrestis*, 3 *Pantoea spp.* 1 *Cronobacter sakazakii* group, 1 *Aeromonas salmonicida*, 4 *Leclercia adecarboxylata* detected by this method, as shown in (Fig. 1 & 2).

#### Molecular detection of *K. pneumoniae* using PCR amplification

##### Amplification of 16S rRNA gene

The 16S ribosomal RNA (rRNA) gene has served as an important tool for determining phylogenetic relationships between bacteria. The features of this molecular target that make it a useful phylogenetic tool also make it useful for bacterial detection and identification in the clinical laboratory [18]. The results of the PCR method identity of the *K. pneumoniae* in seven samples according to the amplified piece of the 16S rRNA gene at size 193 bp (Fig. 3).

Using the specific primers (forward and reverse), the PCR results revealed full presence of a band of *K. pneumoniae* for 7 samples.

These results are the similar obtained by Turton *et al.* [19]. All the isolates gave positive results, and identified as *K. pneumoniae*. Results of PCR amplification proved that all isolates were *K. pneumoniae*, and confirmed the previous results. All of the isolates 7 were submitted to molecular identification utilizing PCR amplification of the 16S rRNA using K 16SrRNA-F and K 16SrRNA-R primers, which are specific primers for the PCR amplification of the 16S rRNA of *K. pneumoniae* according to Mohammed [20].

##### Susceptibility of *K. pneumoniae*

Upon testing the seven isolates of *K. pneumoniae* to 14 antimicrobial agents, it looked that all of the isolates 7(100%) were sensitive to each of Imipenem, and Meropenem. While 5 (71%) of the organisms were sensitive to Ciprofloxacin and Trimethoprim. And 4 (57%) of this bacteria sensitive to

Chloromphenicol, Gentamycin and Streptomycin, While 3(43%) of the organisms were sensitive to Amikacin and Nitrofuration, and 2(29%) of the organisms were sensitive to Amoxicillin/Clavulanic acid, On the other hand, the 7 (100%) isolates showed resistance against Cefixime, Pipracilline and Tetracycline. In addition 5(71%) of isolates showed resistance against Amoxicillin/Clavulanic acid and Ceftriaxone, and 2(29%) of isolates showed resistance against Nitrofuration, and 1(14%) of isolates only was resistant to Amikacin, Chloromphenicol, Ciprofloxacin and Streptomycin. Finally, intermediate results; 3(43%) of isolates were reported intermediate to Amikacin and Gentamycin, and 2(29%) of this bacterium intermediate to Ceftriaxone, Chloromphenicol, Nitrofuration, Streptomycin and Trimethoprim, and 1(14%) of isolates intermediate to Ciprofloxacin. (Table 4 & Fig.4). The results of this study are partially consistent with those of Ahmed and Alaa [21] and Rahal [22], who found that *K. pneumoniae* isolates were more sensitive to Imipenem (10 µg) and Meropenem (10 µg). And Aziz and Lafta [14], reported presence of high resistance to Tetracycline (30 µg) and agree with AL, T. T. K. A. B. [23], who found that *K. pneumoniae* isolates were high resistance to Tetracycline (30 µg) and this in agreement the results of study obtained by Rawy *et al.*, [24] who recorded high resistance to each of Cefixime (5 µg), Ceftriaxone (30 µg) and Pipracilline (100 µg).

##### Histopathological result

In the current study the result showed that the respiratory system of rabbits has susceptible to get of pneumonia by any certain infection. The upper respiratory tract refers to the section that lies above the sternal angle (on the exterior of the thorax), upper the folds of the voice. The larynx was located partially between the part of the upper and lower airway of the respiratory system. The pharynx and lower part of the respiratory involved a part of larynx trachea as show in histology section normal mucosa, submucosa, and hyaline cartilage (Fig. 5A) bronchi and normal lung structure of alveolus in control group (Fig. 5B) and was divided into two bronchi which are extended inside the parenchyma of the lung and branched into small primary and secondary bronchioles. The trachea lined by respiratory epithelia (Fig. 5A).

##### The microscopic examination of the pathological lung

*Klebsiella pneumoniae* was isolation from Iraqi sheep according to Khalaf, *et al.*, [25]. The epidemiology of tick in transmission of Enterobacteriaceae bacteria in buffaloes in Marshes of the south of Iraq. Veterinary world. 2018 was agreed with the presented study, that which isolates the bacteria from ticks infects sheep living in Basrah

province. But there is highly incidence of infection in lungs of sheep living in Kerbala province. Infected lungs of slaughtered sheep showed interstitial pneumonia with suppurative bronchopneumonia and fibrinous pneumonia according to immune status of animals (Fig. 6A).

This study showed moderate pathological change of the lung and some thickness of wall and there little of inflammatory cells in section (Fig. 6 B). That indicated as emphysema is a common condition in rabbits, and it can be caused by various factors, including exposure to bacterial infection and tumors. Clinical signs of respiratory distress may or may not be present, depending on the severity of the condition. This study agreed with Kamaruzaman, *et al.*, [26] who state that the emphysema is a type of lung disease that causes shortness of breath and difficulty breathing. It is a form of chronic obstructive pulmonary disease (COPD) and is usually caused by long-term exposure to irritants that damage the lungs and fibroblasts proliferation widespread in alveolus such as exposure to bacterial infection and tumors or dusts from the environment pollution. Lung diseases are extremely prevalent worldwide, As for any other disease, clinical investigation and epidemiological studies are needed to advance knowledge and improve disease management Keir and Page [27].

#### *The microscopic examination of the pathological kidney*

The results showed that the kidney infiltration of inflammatory cell congestion of blood vessels, stenosis of renal tubules is inflammation of the tiny filters in the kidneys (glomeruli). The excess fluid and waste that glomeruli remove from the bloodstream exit the body as urine. Glomerulonephritis can come on suddenly (acute) or gradually (chronic). The current study agreed with the author Jennette, *et al.*, [28] who state the

glomerulonephritis is a type of kidney disease that can also affect rabbits infiltration of inflammatory cell congestion and stenosis of renal lead to atrophy of renal tubules (Fig.7). (GN) is inflammation of the glomeruli, which are structures in kidneys that are made up of tiny blood vessels, this can cause to kidneys stop working properly.

#### *The microscopic examination of pathological liver*

This study show that there was a hydropic degeneration and blood vessels congestion and there was inflammatory cells infiltrating liver (Fig. 8 A and B). The bacterial etiology associated *K. pneumoniae*, observed in cases with findings suggest varying inflammatory response of upper respiratory tract of rabbit to a variety of naturally occurring infecting bacterial agents The present study agreed with Toky, *et al.*, [29] which confirmed that the histopathological changes of liver showed hydropic degeneration in the cytoplasm of hepatocytes with infiltration of inflammatory cell in the parenchyma of liver and congestion of blood vessels.

#### **Conclusions**

*Klebsiella pneumoniae* affects both the respiratory and urinary systems and also causes hydrolytic degeneration of the cytoplasm of liver cells with infiltration of inflammatory cells in the liver parenchyma and vascular congestion in experimental infection of rabbits.

#### *Conflicts of interest*

The author confirms that there's no evidence of any conflict of interest in relation to the publishing of this article.

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**TABLE 1. PCR conditions for 16S rRNA gene**

Cycle No.	Stage	Temperature	Time
1cycle	Initial Denaturation	94 °C	5 min.
	Denaturation	94 °C	30 sec.
38 cycle	Annealing	57 °C	45 sec.
	Extension	72 °C	45 sec.
1cycle	Final Extension	72 °C	7min.

**TABLE 2. Primer sequences of 16SrRNA for detection *K. pneumoniae***

primer	Primer sequence (5'-3')	Size bp
16SrRNA	CGGTCTGTCAAGTCGGATGT	193
	AGCGTCAGTCTTTGTCCAGG	

TABLE 3. Number and percentage of bacterial species isolated from infected sheep with respiratory tract

Identified bacterium	Number of isolates	Percentage%
<i>Klebsiella pneumoniae</i>	7	7%
<i>Staphylococcus aureus</i>	16	16%
<i>Raoultella planticola</i>	12	12%
<i>E. Coli</i>	19	19%
<i>Butliauxella agrestis</i>	5	5%
<i>Pseudomonas fluorescens</i>	2	2%
<i>Pseudomonas aeruginosa</i>	3	3%
<i>Pseudomonas putida</i>	2	2%
<i>Pantoea spp.</i>	3	3%
<i>Cronobacter sakazakii group</i>	1	1%
<i>Aeromonas salmonicida</i>	1	1%
<i>Leclercia adecarboxylata</i>	4	4%
Negative samples	25	25%
Total isolates	100	100%
Chi square $\chi^2$ value		53.2 *

\* Significant at  $P \leq 0.05$ 

bioMérieux Customer: Microbiology Chart Report Printed January 12, 2023 12:35:03PM CST

Patient Name: N5-11 Patient ID: 195  
 Location: Physician:  
 Lab ID: 195 Isolate Number: 1

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

Source: nasal swab Collected:

Comments:

Identification information	Analysis Time: 5.03 hours	Status: Final
Selected Organism	94% Probability <i>Klebsiella pneumoniae ssp pneumoniae</i>	
ID Analysis Messages	Bionumber: 6607715553564350	

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	ILATr	+	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	ILATr	-			

Fig. 1. A sample of *K. pneumoniae* subsp. *pneumoniae* identified by the Vitek2 system (biochemical tests).

bioMérieux Customer:		Microbiology Chart Report		Printed January 12, 2023 12:35:03PM CST	
Patient Name: Ns-11		Location:		Patient ID: 195	
Lab ID: 195		Organism Quantity:		Physician:	
Selected Organism: <b>Klebsiella pneumoniae ssp pneumoniae</b>				Isolate Number: 1	
Source: nasal swab				Collected:	
Comments:					
Identification information		Analysis Time: 5.03 hours		Status: Final	
Selected Organism		94% Probability <b>Klebsiella pneumoniae ssp pneumoniae</b>			
ID Analysis Messages		Bionumber: 6607715553564350			
Susceptibility Information		Analysis Time: 10.65 hours		Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>16	R	Meropenem	<= 0.5	S
Amoxicillin/Clavulanic Acid	>16	R	Amikacin	>= 64	R
Piperacillin/Tazobactam	>= 128	R	Gentamicin	8	I
Cefotaxime	>= 64	R	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Norfloxacin	>= 16	R
Cefepime	>= 64	R	Fosfomycin	32	S
Imipenem	<= 0.25	S	Nitrofurantion	256	R
			Trimethoprim/Sulfamethoxazole	>= 320	R
AES Findings					
Confidence	Consistent				

Fig. 2. A sample of *K. pneumoniae* subsp. *pneumoniae* identified by the Vitek2 system (antibiotic tests)

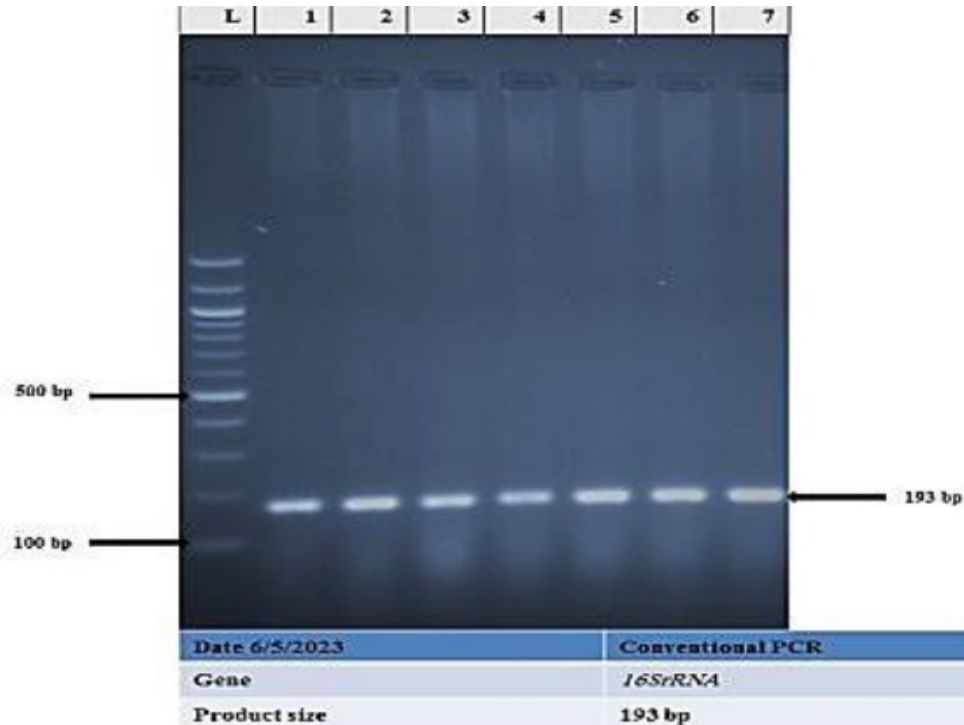


Fig. 3. Image of the electrophoresed agarose gel. The PCR products of *K.pneumoniae* targeting the *16S rRNA* gene. At size 193bp

**TABLE 4. Susceptibility of *K. pneumoniae* isolates tested against 14 antimicrobials**

Antibiotics	symbol	Con. Microgram/ disk	No. of resistance isolates	No. of Intermediate isolates	No. of sensitive isolates	Chi square $\chi^2$ value
Amikacin	AK	30 $\mu$ g	1(14.3%)	3(42.85%)	3(42.85%)	51.8*
Amoxicillin /Clavulanic acid	AMC	30 $\mu$ g	5(71.43%)	0	2(28.57%)	17.6*
Cefixime	CFM	5 $\mu$ g	7(100%)	0	0	94.1*
Ceftriaxone	CRO	30 $\mu$ g	5(71.43%)	2(28.57%)	0	17.6*
Chloromphenicol	C	30 $\mu$ g	1(14.3%)	2(28.57%)	4(57.14%)	28.6*
Ciprofloxacin	CIP	5 $\mu$ g	1(14.3%)	1(14.3%)	5(71.43%)	18.7*
Gentamycin	CN	10 $\mu$ g	0	3(42.85%)	4(57.14%)	31.7*
Imipenem	IPM	10 $\mu$ g	0	0	7(100%)	94.1*
Meropenem	MEM	10 $\mu$ g	0	0	7(100%)	94.1*
Nitrofuration	F	300 $\mu$ g	2(28.57%)	2(28.57%)	3(42.85%)	2.2 NS
Pipracilline	PRL	100 $\mu$ g	7(100%)	0	0	94.1*
Streptomycin	S	10 $\mu$ g	1(14.3%)	2(28.57%)	4(57.14%)	45.9*
Tetracycline	TE	30 $\mu$ g	7(100%)	0	0	94.1*
Trimethoprim	TMP	5 $\mu$ g	0	2(28.57%)	5(71.43%)	17.6*

\* Significant at  $P \leq 0.05$ , NS Non significant

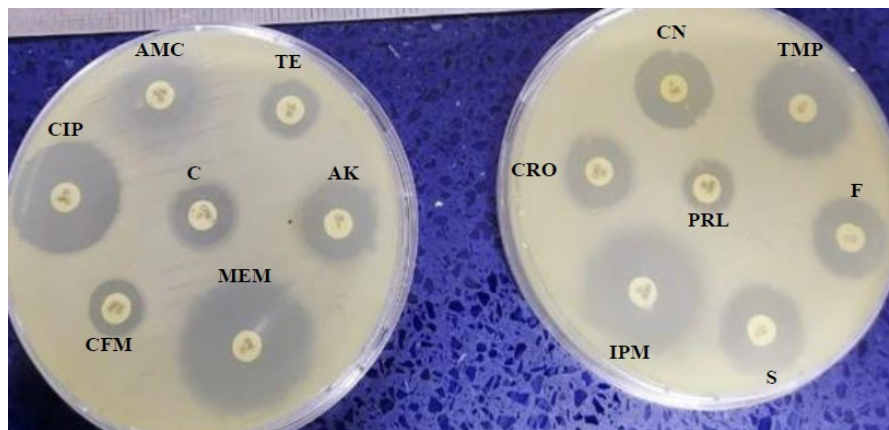


Fig. 4. Disk diffusion antibiotic susceptibility results of *K. pneumoniae* isolates tested against 14 antimicrobials

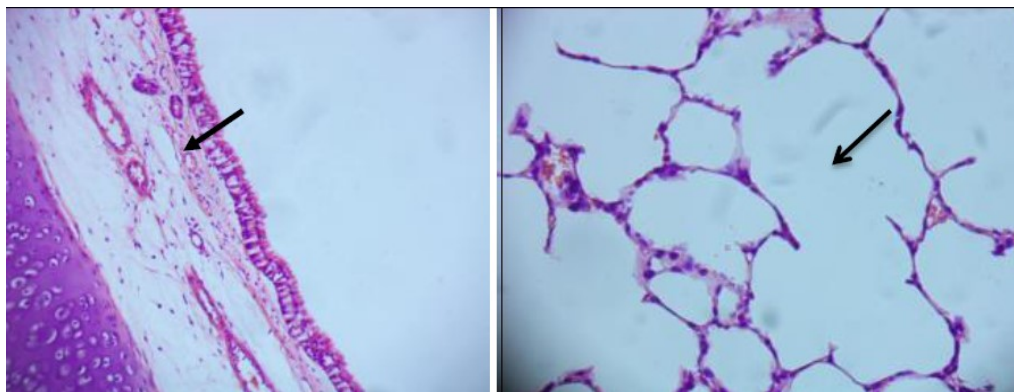


Fig. 5. Histological section in control group rabbit tracheal (A) showing normal histology structure of ciliated epithelia (black arrow) and lung (B) showing normal histology structure of alveolus sac H&E X200

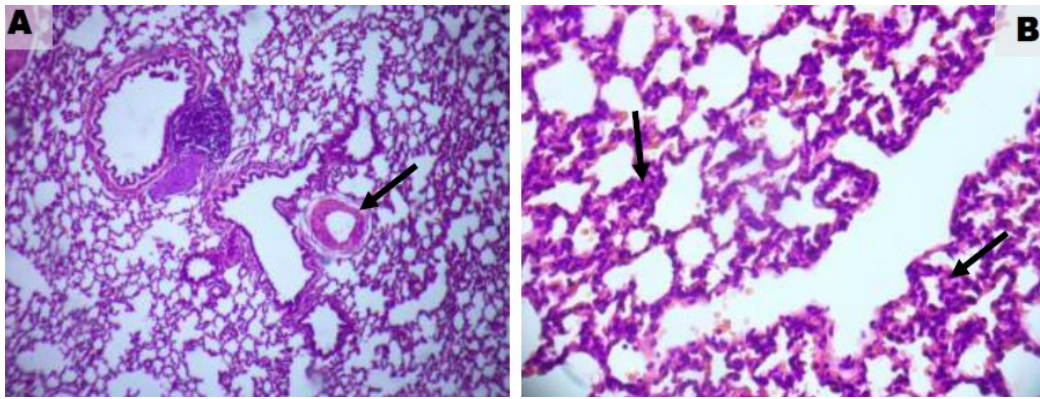


Fig. 6 Histopathological section of lung rabbit *K. pneumoniae* s infected showing increase thickness of inter alveolar septa and congestion of blood vessels (black arrow) H&E X100 (A) and mild thickness of alveoli (black arrow) H&E X200 (B).

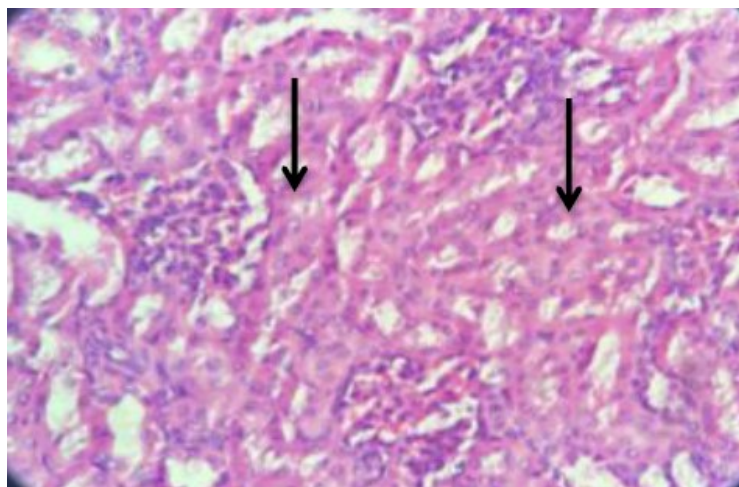


Fig. 7. Histopathological section of kidney showing infiltration of inflammatory cells, congestion and stenosis of renal tubules (black arrow) lead to atrophy of renal tubules H&E

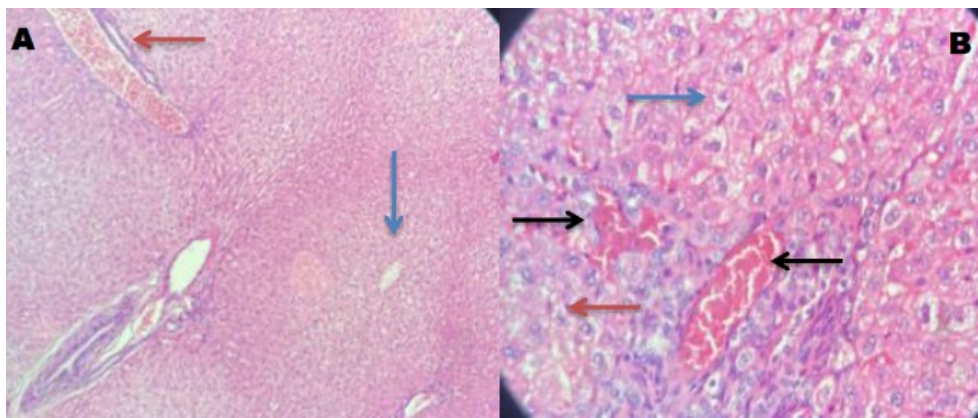


Fig. 8. Histopathological section of liver rabbit infected with *K. pneumoniae* showing infiltration of inflammatory cells around congested blood vessels and central vein and widespread areas of hydropic degeneration (blue arrow) blood vessel dilation (black arrow) and inflammatory cell infiltrations (red arrow) H&E (A), X40 and (B), X400



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### الكشف الجزيئي لبكتيريا الكلبسيلا الرئوية المعزولة من الأغنام المصابة بالجهاز التنفسي و دراسة نسجية مرضية في الأرانب

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أمراض الجهاز التنفسي شائعة في الأرانب، والتي غالباً ما يصعب اكتشافها في الحيوانات الحية حتى تصبح خطيرة ومعقدة. هدفت هذه الدراسة إلى وصف التغيرات النسيجية المرضية في الرئة والكبد والكلية في الأرانب بعد تحريض الإصابة ببكتيريا *Klebsiella pneumoniae* المعزولة من الأغنام المصابة بالجهاز التنفسي والتي تم الكشف عنها باستخدام طرق التشخيص الروتينية متنوعة بالتشخيص باستخدام نظام Vitek2 والجزيئي. الكشف عن عزلات *K. pneumoniae* بتقنية PCR باستخدام (جين S rRNA16). تحتوي هذه الدراسة التجريبية على ستة أرانب؛ حيوانان للتحكم وأربعة حيوانات للعدوى. وبعد تحريض العدوى تم استخدام القتل الرحيم لستة أرانب وفتح التجويف الصدري في المختبر. تم تصوير الأعضاء. وقد شوهدت الأفات المرضية الرئيسية في الرئة والكبد والكلية في الأرانب المصابة ببكتيريا *Klebsiella pneumoniae*. تم تحقيق الوصف النسيجي والمورفولوجي للجهاز التنفسي في الأرانب، والقصبة الهوائية مدعمة بالغضروف المزوج. تعتبر الأرانب نماذج قيمة في أبحاث الجهاز التنفسي بسبب استجابتها التنفسية المفرطة للربو التي تشبه البشر. التغيرات النسيجية المرضية في رئة الأرانب المصابة بـ *K. pneumoniae* تظهر سماكة خفيفة من انتفاخ الحويصلات الهوائية (الانخماص). أظهرت التغيرات النسيجية المرضية في الكبد انحطاطاً مائياً في سيتوبلازم خلايا الكبد مع ارتشاح الخلايا الالتهابية في حمة الكبد واحتقان الأوعية الدموية. التغيرات النسيجية المرضية للكلية تكشف عن ارتشاح احتقان الخلايا الالتهابية وتضيق الكلى مما يؤدي إلى ضمور الأنابيب الكلوية. أظهرت نتائج الحساسية للمضادات الميكروبية أن جميع العزلات السبعة (100%) من بكتيريا *K. pneumoniae* كانت حساسة لكل من الإيمبيينيم والميروبينيم.

**الكلمات المفتاحية:** الكلبسيلا الرئوية، أمراض الجهاز التنفسي، التشريح المرضي، الأرانب.