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Isolation and Identification of *Klebsiella Pneumoniae* from Respiratory Infected Sheep in Al-Anbar Province

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ABSTRACT

This study was aimed to isolate and diagnose *Klebsiella pneumoniae*, which causes respiratory infections in sheep, and to study the genes present in *Klebsiella pneumoniae* responsible for taking iron from the host, involved collection 100 nasal cotton swabs from respiratory infected sheep from several fields located in Al- Anbar province. Identifying and isolating *Klebsiella pneumoniae* by using routine diagnostic techniques (Gram stain, culture on plate and biochemical tests) followed by using the Vitek2 system. Out of the 100 samples collected, the Vitek2 system's results showed 7 (7%) of *Klebsiella pneumoniae* isolates and other bacterial isolates were obtained. And to confirm of *K. pneumonaie* isolates using PCR (16S rRNA gene); the results showed positive for all *Klebsiella pneumoniae* isolates 7(100%) and molecular detection was done for some virulence genes of iron acquisition genes (siderophores synthesis genes *entB* gene, *ybtS* gene and iron uptake system *Kfu* gene), siderophore the results showed *entB* gene positive for 7(100%) isolates, and *ybtS* gene positive for 4(57%) isolates, and the result of *kfu* gene (*Klebsiella* ferric iron uptake) showed positive for 4(57%) isolates. Antimicrobial susceptibility test for *K. pneumoniae*, which targeted 14 antibacterial drugs, the results showed that all 7 isolates (100%) were susceptible to both Imipenem and Meropenem. Moreover, the seven (100%) isolates shown resistance to Tetracycline, Pipracilline, and Cefixime. The study's conclusion is that respiratory tract infections were more common in one-year-old sheep than in older livestock. Additionally, *K. pneumoniae* showed greater susceptibility to Imipenem, Meropenem, Ciprofloxacin, and Trimethoprim than to other antibiotics.

Keywords: *Kfu gene, entB*, Molecular detection, PCR (16 SrRNA gene) , *ybtS*.

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INTRODUCTION

One of the major health issues affecting sheep of all ages worldwide is respiratory tract infections. Regardless of the cause, respiratory infections can strike animals at any age and account for 5.6% of all small ruminant diseases (Aziz & Lafta, 2022). Numerous factors have been linked to the accuracy of respiratory diseases in domestic animals, which have resulted in deaths without a competent diagnosis or course of treatment (Mahdi et al., 2019). As a member of the *Enterobacteriaceae* family, *Klebsiella pneumoniae* is gram negative, rod-shaped, facultative anaerobic, non-motile, and has a noticeable polysaccharide capsule. It is also a fermentative, catalase positive, oxidase negative lactose fermenter that converts nitrate to nitrite (Quinn et al., 2011). They can grow on nutrient agar and they can form pink mucoid colonies on MacConkey agar (Quinn et al., 2011). Due to its animal-borne nature, this bacterium is regarded as one of the most significant diseases in terms of public health (zoonoses) (Cantas & Suer, 2014). This bacterium may be detected in human intestinal tracts and nasopharynx, leading to hospital-acquired infections in the bloodstream, urinary tract, and respiratory tract. Although *K. pneumoniae* infection can occur practically anywhere in the body, respiratory tract infections have the highest occurrence. It is a significant nosocomial pathogen that most commonly causes pneumonia (Brisse et al.,

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2014). The common pathogen *Klebsiella pneumoniae* is responsible for a number of illnesses, including urinary tract, nosocomial pneumonia, and intra-abdominal infections; abscesses in the liver, lungs, and brain; pyogenic meningitis; septic arthritis; osteomyelitis; and endophthalmitis (Al–Samrraee, 2017). This bacterium typically expresses a smooth lipopolysaccharide (O antigen) and capsule polysaccharide (K antigen) on its surface, both contributing to the pathogenesis of diseases caused by this species (Remya et al., 2019). This study was aimed to isolate and diagnose *Klebsiella pneumoniae*, which causes respiratory infections in sheep, and to study the genes responsible for taking iron from the host, which are present in *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Specimens collection: For this investigation, a total of one hundred cotton swabs from the nasal cavities of one year to four-year-old local sheep were collected. One hundred swabs were taken from sheep exhibiting symptoms 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. The collected swabs were embedded in transport media (brain heart infusion broth) and transported within cool box not more than two hours to the laboratory of Microbiology Department, College of Veterinary Medicine, University of Baghdad.

Isolation and Identification of bacterial isolates: The all isolates was from infected sheep that were streaked onto MaCcnokey agar, eosin methylene blue (EMB) agar and blood agar, and it was then incubated aerobically at 37°C for 24 hours (Al-Mahemdy & Abdullah, 2020). Every sample was these cultures were examined for bacterial growth. Bacteria, pink and mucous-textured colonies were subcultured on MacConkey agar. These isolates, the colonies appear pink to purple on EMB agar and are non-hemolytic on blood agar media, exhibiting gamma hemolysis (γ-hemolysis) (Patilaya et al., 2019).

Maintenance of stock culture: The stock culture was maintained following the procedures of pure culture of the isolated organisms were stored in sterilized 80% glycerin and used as stock culture (Islam et al., 2014). Identification of the isolates: Identification of supposed isolates was doing according to the colony morphology, hypermucoviscosity test, and biochemical tests (MacFaddin, 2000). In addition, conformation by (VITEK2 system).

Culture characteristics: All isolates were recognized mainly based on the general culture characteristics (color, shape, texture, and size) of the colony on MacConky agar, Blood agar, and EMB agar following an overnight incubation at 37°C. Other features discovered were lactose fermentation, the colonies turn red or pink on MacConky agar, and nonfermenters do not change color (Quinn et al., 2011).

Hypermucoviscosity test (string test): The bacteria was cultured onto MacConkey agar and incubated for 24 hours at 37°C. When a colony was contacted by a loop and pulled vertically from the surface of an agar plate, a mucous phenotype was identified as the presence of a string-like growth. *Klebsiella* spp. developed a string less than 5 mm in length, except for *K. pneumoniae* colonies which give ≥ 5 mm displaying the hypermucoviscosity trait (MacFaddin, 2000).

VITEK 2 system for identification of isolates: The VITEK 2 system, which includes 20 antibiotic tests and 64 biochemical tests, is used to diagnose bacterial isolates. In this study, the *Klebsiella pneumoniae* isolates were identified using the VITEK 2 system (Espinar et al., 2011).

Molecular detection of *Klebsiella pneumoniae*: DNA extraction: By using Genomic DNA Extraction Kit, Genome Quick Bacteria (Dongsheng Biotech Co., Korea). This kit provides a straightforward and quick approach for obtaining high-quality genomic DNA from Gram-Positive and Gram-Negative Bacteria, which may then be utilized as the analysis target (Abbas & Musawi, 2016).

PCR detection of 16S rRNA gene: To finish this stage, add 3 µl of DNA sample, 1 µl of each primer (10 pmol/µl for forward and reverse), 12.5 µl of OneTaq (NEB®) mastermix, and 7.5 µl of free-nuclease water.

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The optimal PCR conditions for the gene were used to conduct the reaction, as showed in Table (1), and the primer sequence in Table (3).

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

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

PCR detection of *entB, ybtS* and *kfu* genes: PCR detection of these genes were carried out by adding 12.5 µl from OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1 µl 10 pmol/µl from each primer and 7.5 µl of free-nuclease water. The reaction done under the optimal PCR conditions for gene as shown in Table (2) and primer sequences of these genes in Table (3).

Table 2. PCR conditions for *entB, ybtS and kfu genes*

Cycle No.	Stage	Temperature	Time
1 Cycle	Initial Denaturation	94 ºC	5 min.
35 Cycle	Denaturation	94 ºC	30 sec.
	Annealing	47 ºC	45 sec.
	Extension	72 ºC	45 sec.
1 Cycle	Final Extension	72 ºC	7min.

Table 3. Primers were used to determine the target genes in *Klebsiella pneumoniae* isolates

primer		Primer sequence (5'-3')	Size bp
16S rRNA	F	CGGTCTGTCAAGTCGGATGT	193
	R	AGCGTCAGTCTTTGTCCAGG	
EntB	F	CATCATCCGCACCGAATCCAG	400
	R	GCACCTGATGGCGCTGAAATA	
Ybts	F	CCCTAAAAACGCTGCCCTGAA	242
	R	CGGTGTAGATTGCGCGATGAT	
Kfu	F	CTCGCCTACTATCCGTTTATT	487
	R	ACAGGACCAGTAAGGGAAC	

Disk diffusion susceptibility method: The disk diffusion susceptibility method was used in accordance with Bauer-kirby instructions (Bauer et al., 1966). This investigation determined *Klebsiella pneumoniae* susceptibility to fourteen distinct antibiotic agents. The susceptibility tests were interpreted using methods described in the Clinical and Laboratories Standards Institute CLSI (Clinical and Laboratory Standards Institute [CLSI], 2023). Every isolate underwent this test, and the average diameters were recorded. The antimicrobials used to test the sensitivity of *Klebsiella pneumoniae* isolates included: Amikacin, Amoxycillin/Clavulanic acid, Cefixime, Ceftriaxon, Chloromphinicol, Ciprofloxacin, Gentamycin, Imipenem, Meropenem, Nitrofuration, Pipracilline, Streptomycin, Tetracycline, and Trimethoprim.

Statistical analysis: SAS (Cary, 2012) was used to undertake the statistical analysis of the data. To evaluate the statistically significant variations between percentages, the chi-square test was used. A significant difference was defined as \le 0.05 (Cary, 2012).

RESULTS AND DISCUSSION

Bacterial isolation: In the present study, the 100 nasal swabs taken from deep nostrils of respiratory infected sheep, and the isolates were diagnosed by routine diagnosis techniques and confirm the results by Vitek2 system we obtained Klebsiella pneumoniae 7 (7%) isolates and other isolates included 16 (16%) Staphylococcus aureus, 12 (12%) Raoutltella 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 4).These results and the percentages of microorganisms isolated from infected sheep

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are partly consistent with Aziz and Lafta (2021) and agreed with Mebratu Asaye et al. (2015) 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 (2007) who confirm an aerobic bacteria were cultivated from the nasal cavity were apparently pathogenic.

Table 4. Number and percentage of bacterial species isolated from infected sheep with respiratory tract.

Identified bacterium	Number of isolates	Percentage%
Klebsiella pneumoniae	7	7%
Staphylococcus aureus	16	16%
Raoutltella planticola	12	12%
Pseudomonas fluorescens	2	2%
Pseudomonas aeruginosa	3	3%
Pseudomonas putida	2	2%
E. coli	19	19%
Butliauxella agrestis	5	5%
Pantoea spp.	3	3%
Cronobacter sakazakii group	1	1%
Aeromonas salmonicida	1	1%
Leclercia adecarboxylata	4	4%
Negative samples(no appear growth)	25	25%
Total isolates	100	100%
Chi square $\times^2$ value	____	53.2 *

* Significant at P≤0.05

dentification of *Klebsiella pneumoniae* isolates: **Culture characteristics:** Main identification of bacterial isolates were done after incubated aerobically on MacConkey agar at 37 °C for 24–48 hrs. On MacConkey agar, Klebsiella pneumoniae colonies were lactose-fermenting colonies and gave pink color, regular edge, round, mucoid texture with large size according to Quinn et al. (2011) (Figure 1).

Hypermucoviscosity test (string test): In the present study, all 7 isolates of *K. pneumoniae* were identified as hypermucoviscous by the string test. The positive string test result, signifying hypermucoviscosity phenotype as determined by the creation of a string according to MacFaddin (2000) and Ali and Sana'a (2019) (Figure 2).

Figure 1. Mucoid colonies of *K. pneumoniae* on MacConkey agar at 37C° for 24 hrs

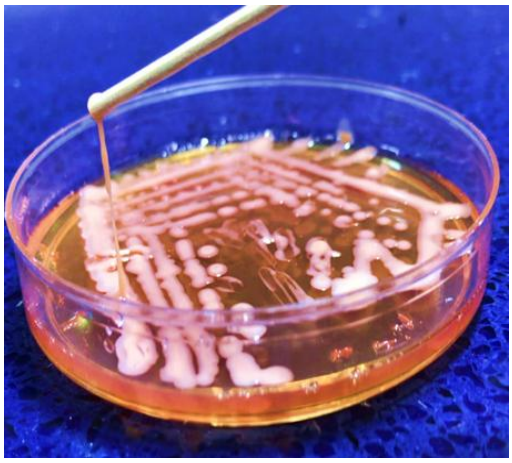

Figure 2. The Hypermucoviscosity phenotype (string test)

Biochemical tests: The biochemical tests were used for additional identification of bacterial isolates are mentioned in (Table 5) reveals routine diagnosis of isolates of *K. pneumoniae* according to Winn et al. (2006) and Abbas and Hussain (2021).

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Table 5. Biochemical tests results for *K. pneumoniae*:

Test	Result
Indole	-ve
Methyl Red (MR)	-ve
Voges-Proskauer (VP)	+ve
Citrate Utilization	+ve
H_2S production	-ve
Triple sugar iron	A/A +gas production
Urea hydrolysis	+ve
Lactose fermentation	+ve
KOH test	+ve
Catalase	+ve
Oxidase	-ve

+ve: positive, -ve: negative, A/A: acid/acid

Diagnosis using Vitek2 System: VITEK 2 system is made up of 20 antibiotic tests and 64 biochemical assays that are used to diagnose bacterial isolates. Isolates suspected to be *K. pneumoniae* were tested in Vitek2 system to identified *K. pneumoniae* subsp. *pneumoniae* according to Espinar et al. (2011) and Bdaiwi et al. (2019). 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 *Buttiauxella agrestis*, 3 *Pantoea* spp., 1 *Cronobacter sakazakii* group, 1 *Aeromonas salmonicida*, and 4 *Leclercia adecarboxylata* detected by this method, as shown in (Figure 3), (Figure 4).

bioMérieux Customer: Microbiology Chart Report Printed January 9, 2023 11:30:03PM CST

Patient Name: Ns-14 Patient ID:185
Location: Physician:
Lab ID:185 Isolate Number:1
Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: nasal swab Collected:

Comments:	

Identification information	Analysis Time: 4.08 hours	Status: Final
Selected Organism	99% Probability	Klebsiella pneumoniae ssp pneumoniae
	Bionumber:	6607734553564010
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	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	-			

Figure 3. A sample of *K. pneumoniae* subsp. *pneumoniae* identified by the Vitek2 system (biochemical tests).

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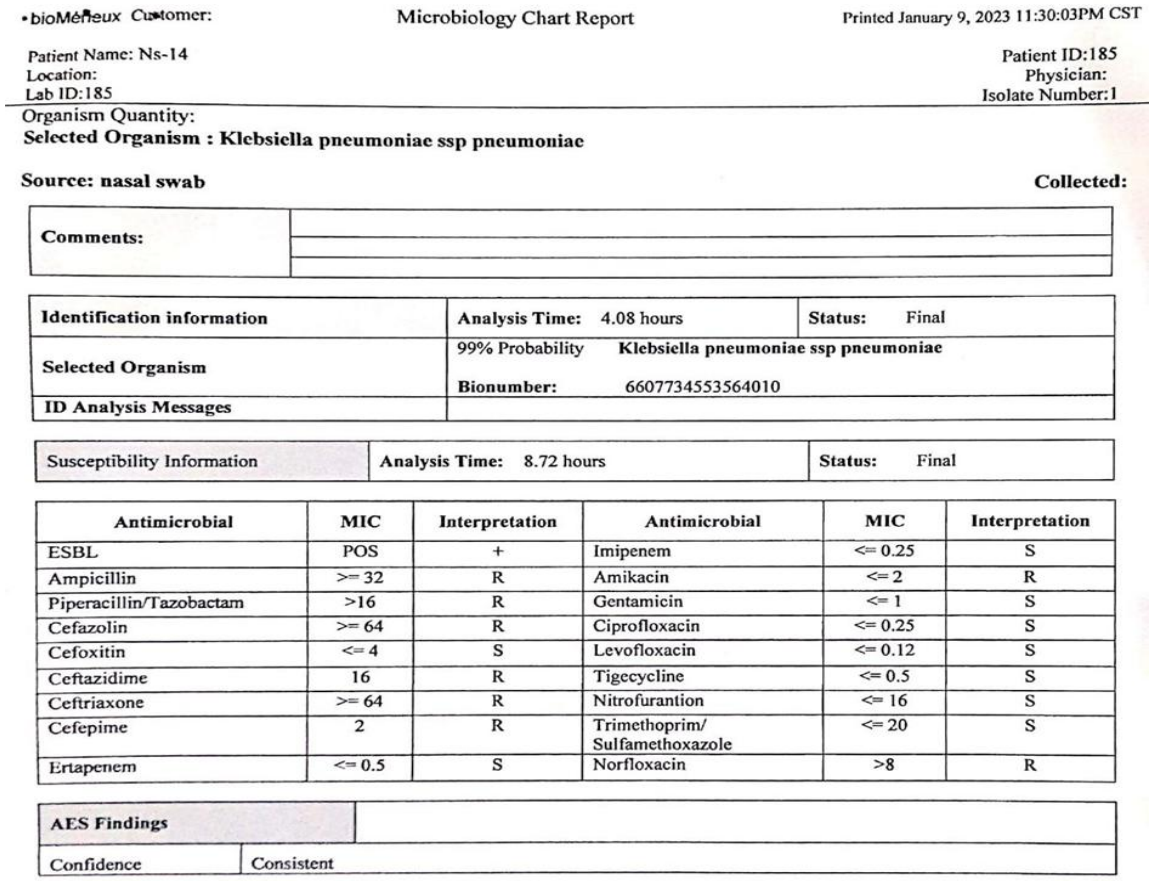

•bioMérieux Customer: Microbiology Chart Report Printed January 9, 2023 11:30:03PM CST

Patient Name: Ns-14 Patient ID:185
Location: Physician:
Lab ID:185 Isolate Number:1
Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: nasal swab **Collected:**

Comments:	

Identification information	**Analysis Time:** 4.08 hours	**Status:** Final
Selected Organism	99% Probability **Klebsiella pneumoniae ssp pneumoniae**	
	Bionumber: 6607734553564010	
ID Analysis Messages		

Susceptibility Information	**Analysis Time:** 8.72 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Imipenem	<= 0.25	S
Ampicillin	>= 32	R	Amikacin	<= 2	R
Piperacillin/Tazobactam	>16	R	Gentamicin	<= 1	S
Cefazolin	>= 64	R	Ciprofloxacin	<= 0.25	S
Cefoxitin	<= 4	S	Levofloxacin	<= 0.12	S
Ceftazidime	16	R	Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantion	<= 16	S
Cefepime	2	R	Trimethoprim/ Sulfamethoxazole	<= 20	S
Ertapenem	<= 0.5	S	Norfloxacin	>8	R

AES Findings	
Confidence	Consistent

Figure 4. A sample of *K. pneumoniae* subsp. *pneumoniae* identified by the Vitek2 system (antibiotic tests).

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 (Patel, 2001). 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 (Figure 5). 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 similar to those obtained by Turton et al. (2010), where all the isolates gave positive results and were identified as *K. pneumoniae*. Results of PCR amplification proved that all isolates were *K. pneumoniae*, and confirmed the previous results. All of the 7 isolates were submitted to molecular identification utilizing PCR amplification of the 16S rRNA using K 16S rRNA-F and K 16S rRNA-R primers, which are specific primers for the PCR amplification of the 16S rRNA of *K. pneumoniae* according to Mohammed (2023).

Amplification of Iron acquisition genes: Siderophores synthesis genes (*entB* and *ybtS*) Iron uptake system *kfu* gene (*Klebsiella* ferric iron uptake)

Amplification of entB gene: In the present study, the *entB* gene was positively detected in all isolates of *K. pneumoniae* at size 400 bp; as shown in (Figure 6); the *entB* gene is a siderophore-associated gene of this bacteria. *K. pneumoniae* mainly acquires iron via the secretion of siderophores, which have a higher affinity for iron than host transport proteins; iron is essential for bacterial survival (Liu et al., 2019). In the present study, the detection rate of the *entB* gene was 100%, which agrees with Liu et al. (2019) and El Fertas-Aissani et al. (2013) whose studies showed positive results for this gene.

Amplification of ybtS gene: The *ybtS* gene in this study was detected in most isolates (4 isolates) of *K. pneumoniae* at size 242 bp; as

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shown in (Figure 7). The discovery rate of the *ybtS* gene was 57.14%. These results for this gene are in partial agreement with those of Hsieh et al. (2008) and Bachman et al. (2011), who proved that yersiniabactin has been observed in only ~18% of classical but 90% of hypervirulent (HV) *K. pneumoniae* clinical isolates. However, this agrees with Bachman et al. (2011), who recorded that in conjunction with enterobactin, it is overrepresented in *K. pneumoniae* isolates from the respiratory tract, and in most cases, yersiniabactin is expressed during lung infection.

Amplification of kfu gene: The *kfu* gene is a chromosomal virulence gene for an iron uptake system. The results proved the presence of bands that indicate positive results for four isolates of *K. pneumoniae* at size 487 bp; as shown in (Figure 8). The detection rate of the *kfu* gene was 57.14%. The *kfu* gene is a supposed pathogenic gene, and it is associated with the virulent hypermucoviscosity phenotype and purulent tissue infections (Li et al., 2014). This is in partial agreement with Li et al. (2014), who reported that the *kfu* gene is significantly associated with the virulent hypermucoviscosity phenotype; in this study, the string test observe in all isolates of *K. pneumoniae* was positive.

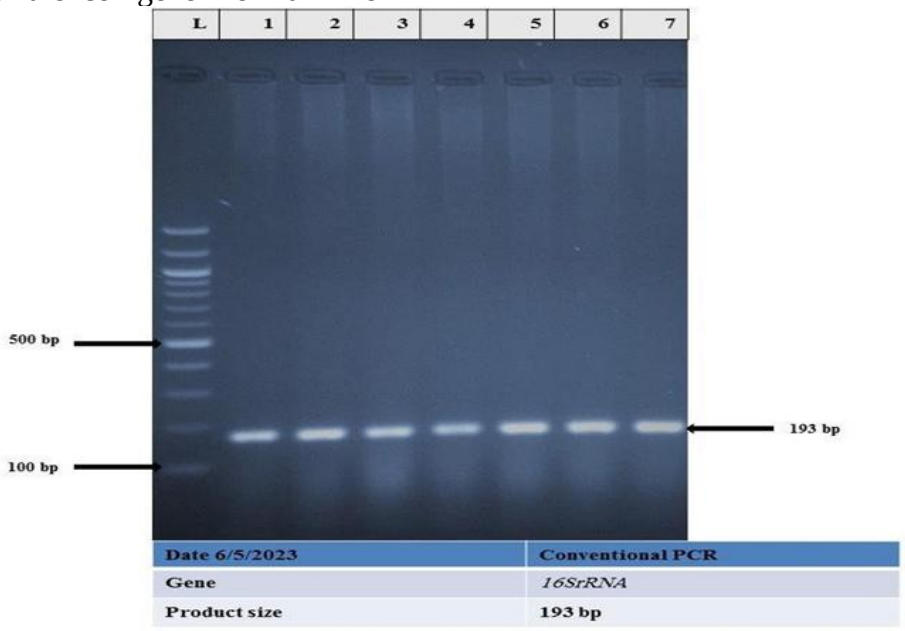

Figure 5. The electrophoresed agarose gel. The PCR products of *K. pneumoniae* targeting the *16S rRNA* gene. At size 193bp

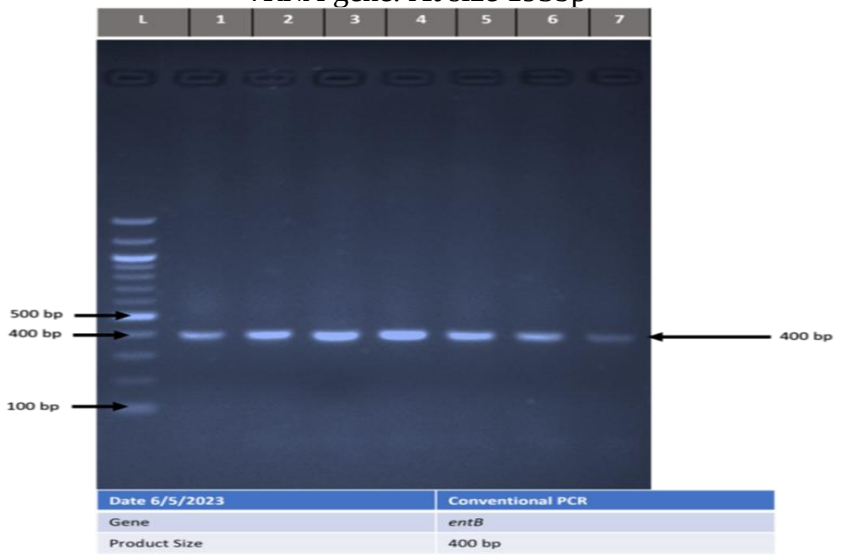

Figure 6. The electrophoresed agarose gel. The PCR detect present band of *entB* at size 400 bp

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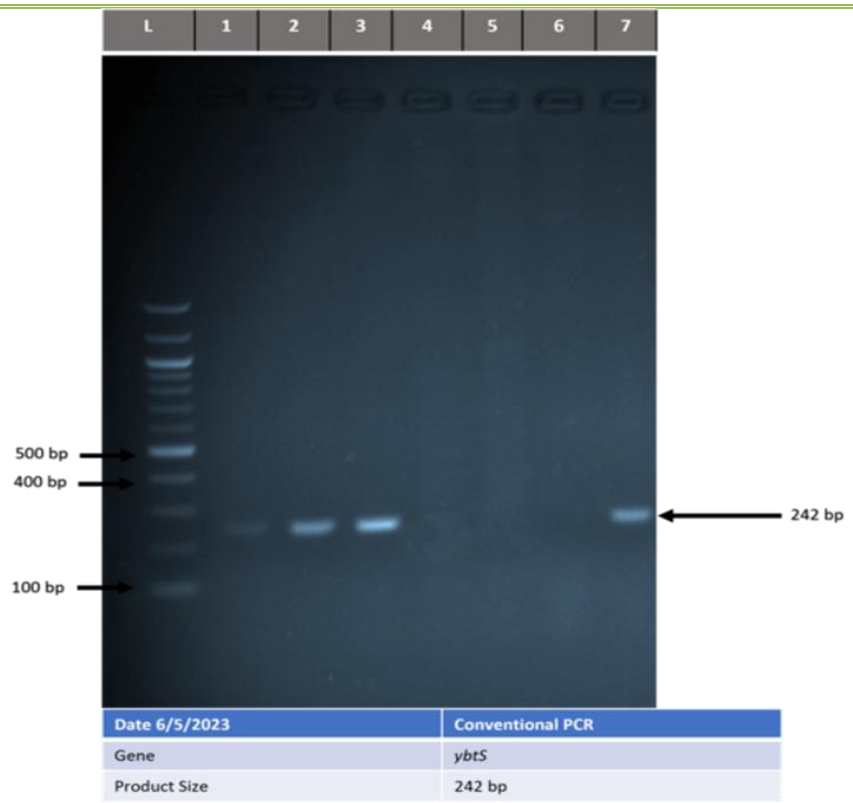

Figure 7. The electrophoresed agarose gel. The PCR detect present band of *ybtS gene* at size 242 bp

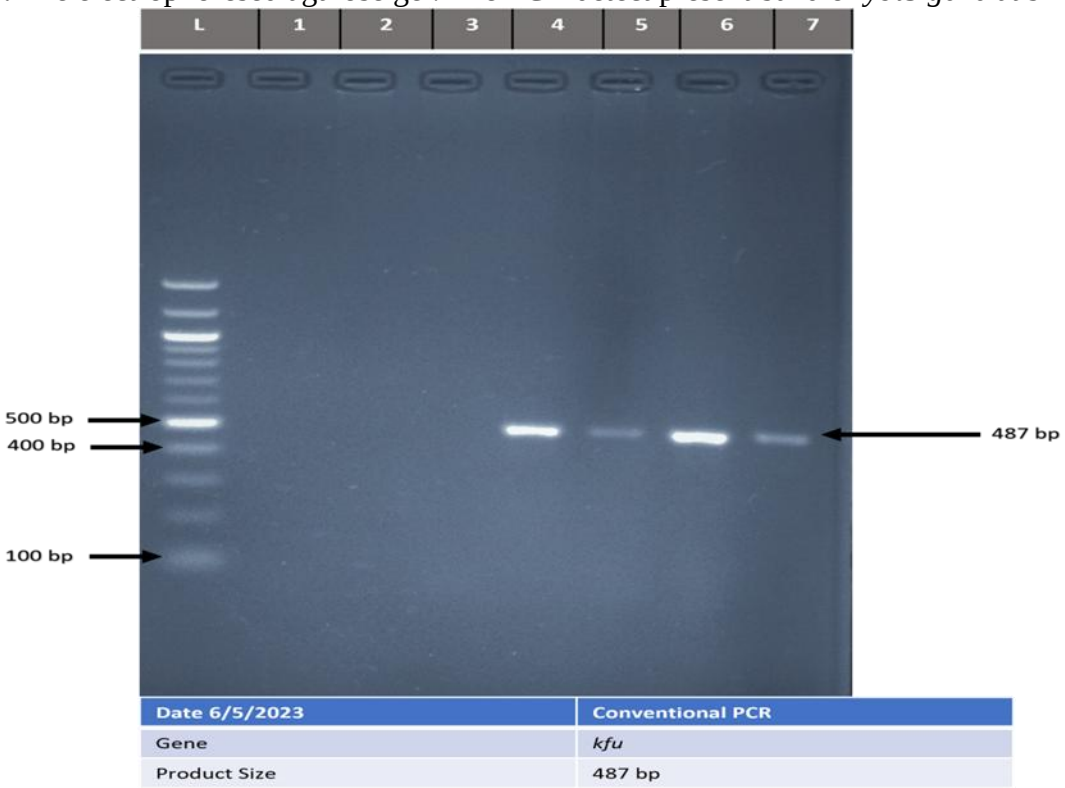

Figure 8. The electrophoresed agarose gel. The PCR detect present band of *kfu* gene at size 487 bp

Susceptibility of *K. pneumoniae*: Upon testing the seven isolates of *K. pneumoniae* to 14 antimicrobial agents, it looked that all of the 7 isolates (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 Chloramphenicol, Gentamycin and Streptomycin, While 3 (43%) of the organisms were sensitive to Amikacin and Nitrofurantoin, and 2 (29%) of the organisms were sensitive to

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Amoxycillin/Clavulanic acid. On the other hand, the 7 (100%) isolates showed resistance against Cefixime, Piperacillin and Tetracycline. In addition, 5 (71%) of isolates showed resistance against Amoxycillin/Clavulanic acid and Ceftriaxone, and 2 (29%) of isolates showed resistance against Nitrofurantoin, and 1 (14%) of isolates only was resistant to Amikacin, Chloramphenicol, 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, Chloramphenicol, Nitrofurantoin, Streptomycin and Trimethoprim, and 1 (14%) of isolates intermediate to Ciprofloxacin (Table 6). The results of this study are partially consistent with those of Aljanaby and Alhasani (2016) and Rahal et al. (2021), who found that *K. pneumoniae* isolates were more sensitive to Imipenem (10 µg) and Meropenem (10 µg). Furthermore, this study agrees with Aziz and Lafta (2021), who found that *K. pneumoniae* isolates had high resistance to Tetracycline (30 µg), which is also in agreement with the results of the study obtained by Rawy et al. (2020), who recorded high resistance to each of Cefixime (5 µg), Ceftriaxone (30 µg) and Piperacillin (100 µg).

Table 6. Susceptibility tests of *K. pneumoniae* to 14 antimicrobials

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

* Significant at P≤0.05, NS Non significant

CONCLUSION

Respiratory tract infections were more common in young sheep aged 1 year than older age. Each of *K. pneumoniae*, *E. coli*, *R. planticola*, and *S. aureus* were the most frequent species isolated from sheep. In addition, *K. pneumoniae* were sensitive for the Imipenem, Meropenem, Ciprofloxacin, and Trimethoprim**.**

CONFLICT OF INTEREST

There is no conflict of interest, according to the authors, when it comes to publicizing the current inquiry.

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عزل وتشخيص *Klebsiella pneumoniae* من الاغنام المصابة بالجهاز التنفسي في محافظة الانبار

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المستخلص

هدفت هذه الدراسة إلى عزل وتشخيص *Klebsiella pneumoniae* المسببة لالتهابات الجهاز التنفسي في الأغنام، وايضا" دراسة الجينات الموجودة في *Klebsiella pneumoniae* المسؤولة عن أخذ الحديد من المضيف، حيث تم جمع 100 مسحة قطنية من الأنف من الأغنام المصابة بالجهاز التنفسي من عدة حقول تقع في محافظة الانبار. وتم تشخيص وعزل *Klebsiella pneumoniae* باستخدام تقنيات التشخيص الروتيني (صبغة كرام والزرع على الطبق والاختبارات البايوكيميائية) ومن ثم استخدام نظام Vitek2. من بين 100 عينة تم جمعها، أظهرت نتائج نظام Vitek2 أنه تم الحصول على 7 (7٪) من عزلات *Klebsiella pneumoniae* وعزلات بكتيرية أخرى. ولتأكيد عزلات *K. pneumonaie* باستخدام PCR (جين الرنا الريباسي S16)؛ أظهرت النتائج إيجابية لجميع عزلات *Klebsiella pneumoniae*7(100٪)، وتم الكشف الجزيئي لبعض جينات الضراوة لجينات اكتساب الحديد (جينات تصنيع حاملات الحديد جين entB وجين ybtS وجين نظام امتصاص الحديد Kfu) وأظهرت النتائج ان جين entB موجبة لـ 7 عزلات (100٪) وكانت نتيجة جين ybtS موجبة لـ 4 عزلات (57٪) وكانت نتيجة جين kfu (امتصاص الحديديك *Klebsiella*) إيجابية لـ 4 عزلات (57٪). وان اختبار الحساسية للمضادات الميكروبية لـ *K. pneumoniae*، والذي استهدف 14 دواءً مضادًا للبكتيريا، أظهرت النتائج أن جميع العزلات السبعة (100٪) كانت حساسة لكل من الإيميبينيم والميروبينيم. علاوة على ذلك، أظهرت العزلات السبعة (100٪) مقاومة للتتراسيكلين والبيبراسيللين والسيفيكسيم. وخلصت الدراسة إلى أن التهابات الجهاز التنفسي كانت أكثر شيوعا في الأغنام البالغة من العمر سنة واحدة مقارنة بالماشية الأكبر سنا. بالإضافة إلى ذلك، أظهرت *K. pneumonaie* تحسسا" أكبر للمضادات الحيوية الأخرى مثل الإيميبينيم والميروبينيم والسيبروفلوكساسين والتريميثوبريم.

الكلمات المفتاحية : جين Kfu، entB ، الكشف الجزيئي،(PCR 16SrRNA)، ybtS .