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PREVALENCE OF BLA *TEM* AND BLA *KPC* RESISTANCE GENES IN *KLEBSIELLA PNEUMONIAE* ISOLATED FROM ANIMAL SOURCES

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ABSTRACT

Klebsiella pneumoniae has been recorded as one of the most infectious Enterobacteriaceae which cause huge economic losses in the poultry and animal industry. Moreover, it represents a critical reservoir for the spread of antimicrobial resistance in humans due to its globally increasing antimicrobial resistance prevalence and MDR. This study aimed to detect the prevalence of *K. pneumoniae* among chicken and neonatal calves, antimicrobial resistance profile and molecular detection of the most important resistance genes related to *K. pneumoniae* bla *TEM* and bla *KPC* and to highlight their zoonotic significance by sequencing. Twenty (17.4%) *K. pneumoniae* were isolated from chicken (14.3%) and neonatal calves (22.2%). All 20 *K. pneumoniae* isolates were positive for the 16s RNA species-specific gene and the sequencing result showed their zoonotic occurrence. Isolated *K. pneumoniae* showed the highest resistance against Ampicillin (90%), Sulfamethoxazole-Trimethoprim (75%), Amoxicillin-Clavulanic acid (70%) and Tetracycline and ceftriaxone (65%). Twelve and eleven isolates showed resistance to Ciprofloxacin and Gentamicin, respectively. Moderate resistance was against chloramphenicol and Imipenem recorded the highest percentage of sensitivity (85%). Moreover, MDR was detected in 55 % of isolates. The *bla-TEM* gene was detected in 8 out of 20 isolates (40%) and the *bla-KPC* gene was detected in 9 out of 20 isolates (45%). Strict rules for antimicrobial use in human beings and veterinary medicine to overcome the growing global antimicrobial resistance prevalence and prevent the spread of resistance among bacteria and hosts. Moreover, antimicrobial resistance monitoring in animals should be regularly assessed worldwide.

Key words: *K. pneumoniae*, antibiotic resistance, animal, *bla-TEM* and *bla-KPC*

INTRODUCTION

Klebsiella pneumoniae is a Gram-negative coccobacillus, non-motile and encapsulated, that classified within the Enterobacteriaceae family. *K. pneumoniae* has progressively converted to multidrug-resistant and is recently considered one of the most complicated Gram-negative bacteria in the healthcare setting (McElheny *et al.,* 2025). In recent decades, *K. pneumoniae* has risen to prominence as a major pathogen in both human and veterinary medicine, due to its capacity to cause a broad range of infections and its growing resistance to several antibacterials

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(Kot and Witeska, 2024). In humans, *K. pneumoniae* is a major cause of nosocomial infections, including septicemia, pneumonia and urinary tract infection. On the other hand, *K. pneumoniae* has been observed to be a community-based pathogen responsible for severe infections, such as liver abscess, pneumonia and endophthalmitis (Boonsarngsuk *et al.,* 2015 & McElheny *et al.,* 2025). In various animal species, *K. pneumoniae* serves as a cause of environmental mastitis in dairy cows, leading to economic losses due to reduced milk yield and quality (Hogan *et al.,* 1999), metritis and respiratory infections in horses and septicemia in birds (Alchalaby et al., 2024). Moreover, in pet animals, such as dogs and cats, it has been associated with respiratory tract infections, urinary tract (UT) and wounds (Radostits *et al.,* 2007). Livestock are principal sources of resistance and MDR *K. pneumonia*, which can be disseminated from the faeces of livestock to humans through farms, abattoirs and meat preparation (Doosti *et al.,* 2015; Kot and Witeska, 2024). Moreover, *K. pneumonia* was recorded as a significant foodborne pathogen through contamination of different food sources, such as milk, meat, fish and vegetables (Kakatkar *et al.,* 2017; Kot and Witeska, 2024).

Antimicrobial resistance is a potential global health risk. Multiple bacteria have acquired resistance due to the inappropriate use of antimicrobials and over-prescribing in humans, veterinary medicine (such as animal livestock, poultry industry, and aquaculture) and agriculture (Sivaraman *et al.*, 2021; Mahmoud *et al.,* 2022). Plasmids are the site of ESBL genes that can be easily transferred between bacteria. Various ESBL genes are present but the main types (*TEM, SHV and CTX* types) are the most widespread (Chong *et al.,* 2011). It has been recognized that the strains carrying ESBL genes tend to show a higher antimicrobial resistance not only to beta-lactam antibiotics but also to other antibiotics (Perez *et al.,* 2007 and Drawz *et al.,* 2014).

Enterobacteriaceae, mainly *E. coli* and *Klebsiella* species, have started to develop resistance to ESBLs (Montso *et al.,* 2019). The resistance mechanism mediated by plasmid is of increasing concern as it emphasizes dissemination of the resistance genes to other bacteria (Davies & Davies, 2010; Alzahrani *et al.,* 2022). Carbapenems are the last resort treatment for the infection caused by ESBL-bacteria. However, resistance to carbapenems has appeared and is increasing worldwide (Chander & Shrestha, 2013). One of the most important carbapenem resistance mechanisms is the production of carbapenemases enzymes (Shin *et al.,* 2012). Carbapenemases are divided into different classes, *KPC* is a Class A carbapenemase that is associated with *K. pneumoniae* (Patel & Bonomo, 2013). Awareness and concern about the supplementation of antibacterial in veterinary medicine are urgent to prevent the dissemination of antimicrobial resistance and maintain the powerful action of antibiotics (Alzahrani *et al.,* 2022). This study aimed to detect the prevalence of *K. pneumoniae* among chickens and neonatal calves, antimicrobial resistance profile and molecular detection of the most important resistance genes related to *K. pneumoniae* bla *TEM* and bla *KPC* and sequencing of the isolated *K. pneumoniae* to highlight their zoonotic significance.

MATERIALS AND METHODS

1. Samples

The study was conducted from September to October 2024 in Babylon city, Iraq. A total of 115 anal samples were obtained from animals (chicken, n = 70 and neonatal calves, n=45) that had suffered from gastrointestinal disturbances and diarrhea. All examined animals were not treated with antibiotics before sampling. Swabs were then kept in sterile 2 mL of 1%

peptone in a microfuge tube containing water (Oxoid, Basingstoke, UK). All samples were labeled and transported in the icebox to the Microbiology Laboratory in the College of Veterinary Medicine at Al-Qasim Green University for micro-biological examination.

2. Bacterial Isolation and Identification:
The peptone tubes with swabs were vortexed and 10 µL was cultured onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated at 37 ◦C for 24 h. Colonies were pink, mucoid and lactose fermenting which were selected and subcultured for purification. Pure isolates were identified based on colony morphology, Gram staining, oxidase test, citrate utilization, urease activity, and indole test (Madec *et al.,* 2017). Furthermore**,** all isolated strains were identified biochemically by the automated Vitek 2 compact system (BioMérieux, France).

3-Molecular confirmation of isolated bacteria by PCR and Sequencing:
1-DNA extraction:
According to (Ramadan et al., 2016), a bacterial suspension consisted of 3 to 5 colonies of each sample and one mL of sterile distilled water. Destruction of the resuspended bacterial cells was performed by heat for 15-20 min. at 100 °C and then centrifugation at 15,000 rpm for 15 minutes. The obtained DNA from supernatant was preserved at -20 °C until used for PCR. The DNA concentration was estimated using nanodrops.
2- 16S rRNA gene amplification and sequencing:
The 16S rRNA gene was amplified using the conditions described by Miller *et al.,* (2013). The primer was listed in Table (1). Agarose gel (1.5%) stained with ethidium bromide in 1x TBE buffer was used for running the PCR products. The bands were visualized under UV light, imaged by a gel documentation system and computer software was used to analyze the data.

4-Sequencing of the amplified 16S rRNA gene:
The PCR products were purified and sequenced. Sequences were subjected to analysis through the National Centre for Biological Information (NCBI) Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

5-Antimicrobial Susceptibility Testing:
Positive isolates were evaluated for their susceptibility to 9 different antimicrobial discs using Muller Hinton agar, including: Ampicillin (AMP), Amoxicillin-Clavulanic acid (AMC), Tetracycline (TET), Gentamicin (GEN), Ceftriaxone (CRO), Ciprofloxacin (CIP), Sulphamethoxazole +Trimethoprim (SXT), Chloramphenicol (CHL) and Imipenem (IMP). It was applied according to the guidelines given by the National Committee for Clinical Laboratory Standards (CLSI 2024).

6-Detection of Antimicrobial-Resistance Genes:
Antimicrobial-resistance genes associated; an ESBL gene (bla *TEM*) and metalobetalactamase gene (bla *KPC)* were determined by PCR. Primers are presented in **Table (1).**

Table 1: The primers used in molecular confirmation and detection of resistance genes.

Gene name		Primer sequences (5–3°)	Product size (bp)	Reference
16sRNA	F	AGAGTTTGATCCTGGCTCAG	1500	**Miller *et al.*, (2013)**
	R	GTTACCTTGTTACGACTT		
Bla(KPC)	F	CGTCTAGTTCTGCTGTCTTG	798	**Poirel *et al.*, 2011**
	R	CTTGTCATCCTTGTTAGGCG		
Bla(TEM)	F	GCTCACCCAGAAACGCTGGT	686	**Liang *et al.*, 2015**
	R	CCATCTGGCCC CAGTGCTGC		

7-Result analysis:

Data analysis was done using the Excel program. Data were presented as numbers and percentages.

RESULTS

1-Isolation and Identification of *K. pneumoniae*:

From 115 anal samples (Chicken, n=70 and Neonates calves, n=45), 20 (17.4%) *K. pneumoniae* strains were isolated. All isolates were identified biochemically. Ten *K. pneumoniae* strains were isolated from both chicken (14.3%) and neonatal calves (22.2%) **(Table 2).**

Table 2: Number and percentage of isolates of *K. pneumoniae* from anal samples (n=115).

Animal	No.of samples	No. of isolated *K. pneumoniae*	% of isolated *K. pneumoniae*
Chicken	70	10	14.3%
Neonatal calves	45	10	22.2%
Total	115	20	17.4%

2-Molecular confirmation of *K. pneumoniae* and sequencing:

All 20 *K. pneumoniae* isolates were positive for 16s RNA species-specific gene **(Figure 1)**. The result of 20 *K. pneumoniae* sequencing and blasting according to NCBI was presented in **Figure (2)**.

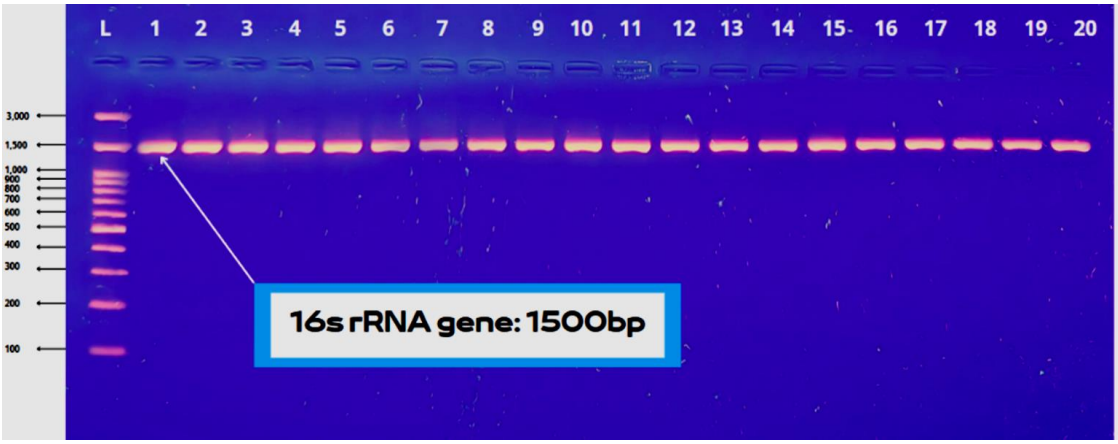

Figure (1): Agarose gel electrophoresis of amplified 16sRNA gene of *K. pneumoniae*: Lanes L: Marker lane 100-3000 bp DNA ladder; Lanes 1-20: positive amplification of 1500 bp of 16sRNA gene.

3-Antimicrobial resistance profile:

Antimicrobial resistance results of overall 20 isolated *K. pneumoniae* are collected in **Table (3) and Figure (3)** which exhibited the highest resistance against ampicillin (90%), Sulfamethoxazole-trimethoprim (75%), Amoxicillin-Clavulanic acid (70%). Thirteen (65%) isolates revealed resistance to tetracycline and ceftriaxone and 12 and 11 isolates were resistant to ciprofloxacin Gentamicin, respectively. However, 8 of the isolates conveyed resistance to chloramphenicol. In the present study, Imipenem recorded the highest percentage of sensitivity with only 3 (15%) isolates showing resistance and 17 (85%) isolates were sensitive. Interestingly, eleven (55%) isolates showed multidrug resistance.

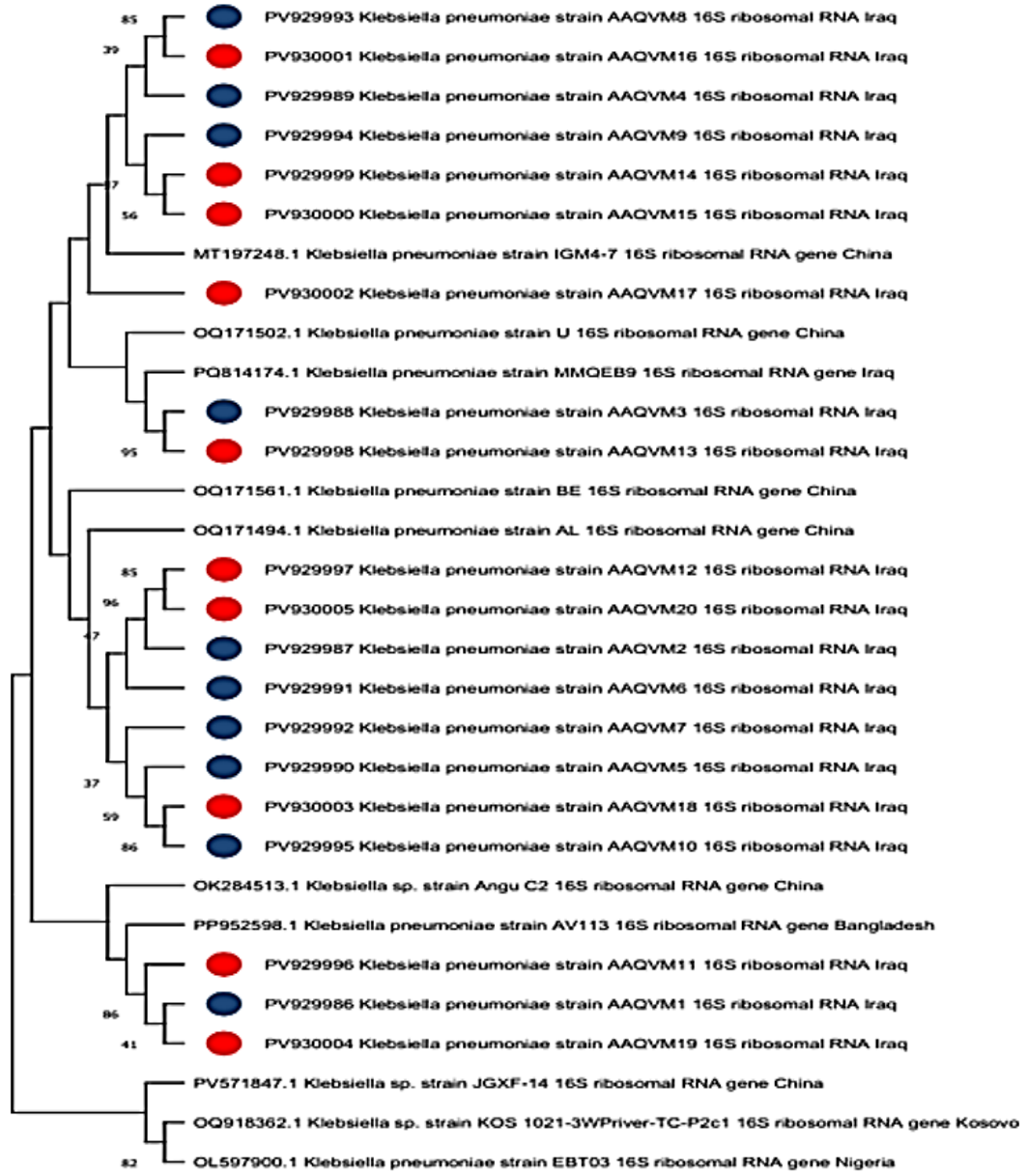

Figure (2): The phylogenetic tree of the *K. pneumoniae* isolates; the red circles refer to the ten *K. pneumoniae* isolated from chickens and the blue circles refer to the ten *K. pneumoniae* isolated from neonatal calves.

Table 2: Antimicrobial susceptibility profile of *K. pneumoniae* isolates (n=20).

Name of antibiotic	Resistance pattern			
	Sensitive		Resistance	
	No.	%	No.	%
Ampicillin	2	10%	18	90%
SXT	5	25%	15	75%
Amoxicillin-Clavulanic acid	6	30%	14	70%
Tetracycline	7	35%	13	65 %
Ceftriaxone	7	35%	13	65 %
Ciprofloxacin	8	30 %	12	60%
Gentamicin	9	45%	11	55 %
Chloramphenicol	12	60%	8	40%
Imipenem	17	85%	3	15%

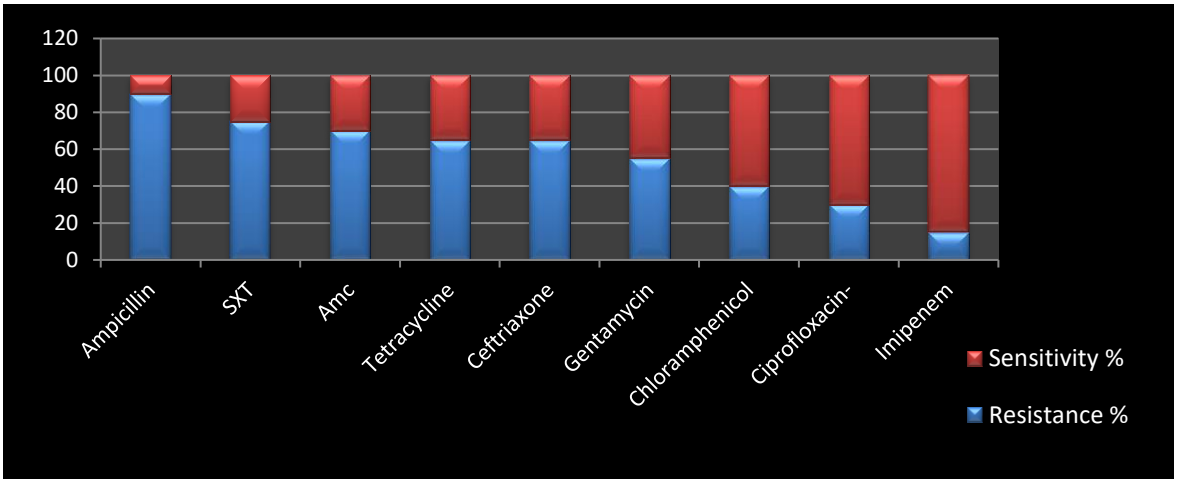

Figure 3: Antimicrobial susceptibility profile of *K. pneumoniae* isolates.

4-Detection of Antimicrobial-Resistance Genes:

PCR amplification results **(Tables 4 & 5) Figures (4-6)** showed that the *bla-TEM* gene was detected in 8 out of 20 isolates (40%) and the *bla-KPC* gene was detected in 9 out of 20 isolates (45%). It was observed that 3 isolates (No. 2, 6 and 20) harbored both genes. Moreover, only 3 isolates of the nine that had carried the bla *KPC* gene were resistant to imipenem. All the 8 isolates that harbored bla *TEM*, were resistant to ampicillin and AMC and 5 were resistant to ceftriaxone. Regarding the animal source, bla *TEM* was detected in 40% of both chicken and neonatal calves and bla *KPC* was detected in 40% and 50 % in chicken and neonatal calves, respectively.

Table 4: Prevalence of bla *TEM* and bla *KPC* in chicken and neonatal calves.

Genes	Chicken isolates (No.10)		Neonatal calves (No.10)	
	No.	%	No.	%
bla-TEM only	4	40%	4	40%
bla *KPC* only	4	40%	5	50%
bla-TEM+bla *KPC*	2	20%	1	10%

Table 5: Existence of *bla-TEM and* bla *KPC genes in* isolated *K. pneumoniae* (n=20)

Genes	Number of isolates	Percentage %
bla-TEM only	8	40%
bla *KPC* only	9	45%
bla-TEM + bla *KPC*	3	15%

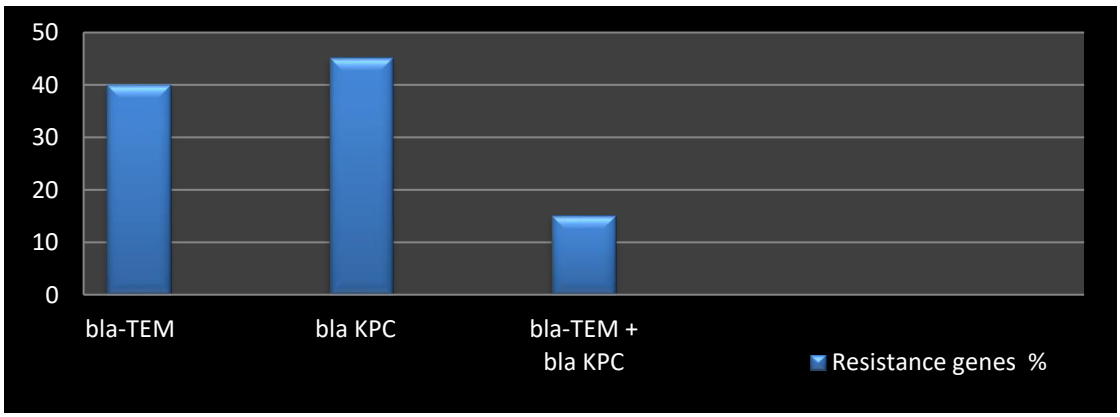

Figure (4): Percentage of *bla-TEM and* bla *KPC genes in* isolated *K. pneumoniae* (n=20).

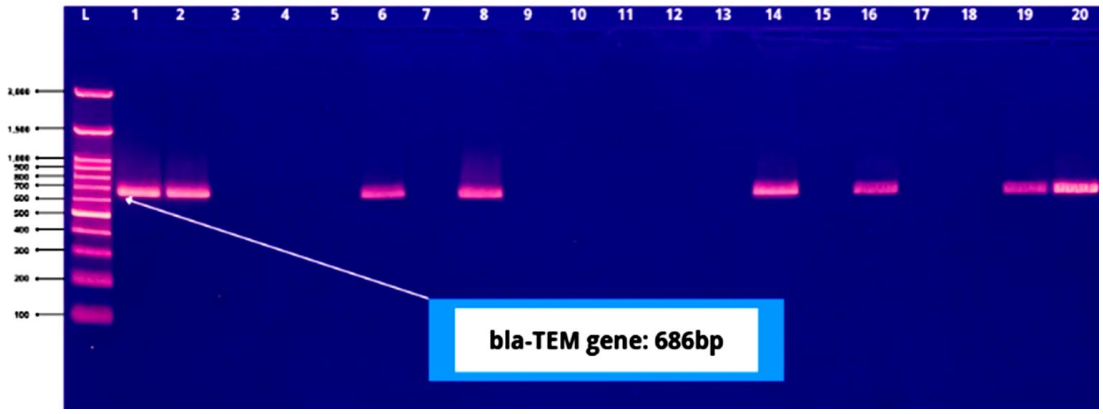

Figure (5): Agarose gel electrophoresis of amplified bla *TEM* gene of *K. pneumoniae* : Lanes L: Marker lane 100-3000 bp DNA ladder; Lanes (1,2,6,8,14,16,19 & 20): positive amplification of 686 bp of bla *TEM* gene.

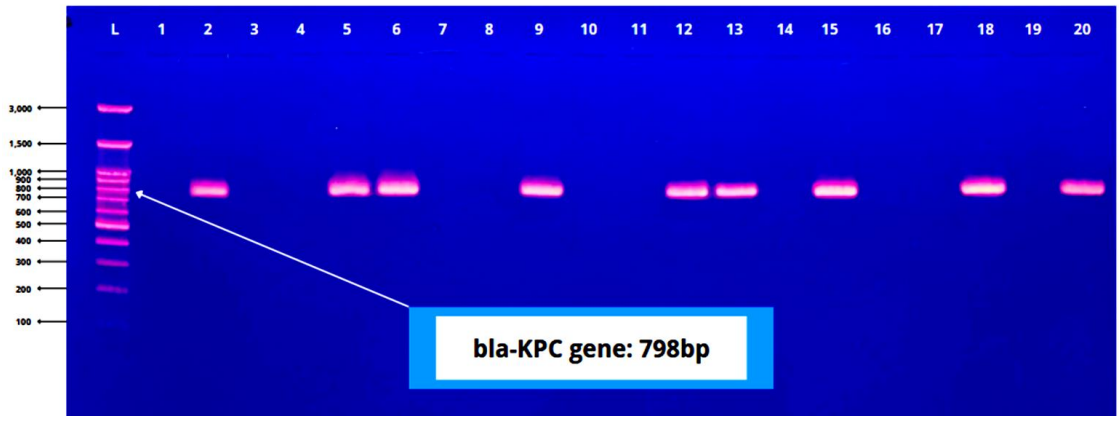

Figure (6): Agarose gel electrophoresis of amplified bla KPC gene of *K. pneumoniae* : Lanes L: Marker lane 100-3000 bp DNA ladder; Lanes (2, 5, 6, 9, 12, 13, 15, 18 & 20): positive amplification of 798 bp of bla KPC gene.

DISCUSSION

K. pneumoniae considered one of the dominant zoonotic and pathogenic bacteria which cause huge Economic losses in poultry and animal farms (Cheng *et al.,* 2020 & Kahin *et al. 2024*). Moreover, it represents a critical vehicle for trans-mission of antibiotic resistance to humans as it recorded a high antimicrobial resistance prevalence and MDR (Cheng *et al.,* 2018).

In the present study *20 (17.4%) K. pneumoniae* were detected from chicken (14.3%) and neonatal calves (22.2%), similar to previous findings by (Alaa, *et al.,* 2024). However, the current prevalence of *K. pneumoniae* in calves was higher than results detected in bovine by Cheng *et al.* (2018) (15%), Aslan *et al.* (2002) (20%) and Ramadan, (2023) (10.5%) and was lower than records of Wu *et al.* (2022) and Mario *et al.* (2023), who both detected a percentage of 27%. Regarding chicken, current findings were higher than those determined by Tantawy *et al.* (2018) (6.6%), Permatasari *et al.* (2020) (9%) and Li *et al.* (2022) (5%). The higher incidences of *K. pneumoniae* in chicken were reported by Franklin-Alming *et al.* (2021) (40%) and Kahin *et al.* (2024) (67.2%). Antimicrobial resistance and multidrug resistance (MDR) are considered a complicated public health concern that is affecting both humans and animals (Tigabie *et al.,* 2023). The global increase in *K. pneumoniae* MDR causes problems

in the treatment of diseases and makes it very difficult and complicated for *K. pneumoniae* to show resistance against cephalosporins, carbapenems, amino-glycosides (Ferreira *et al.,* 2019).

In the present study, isolated *K. pneumoniae* showed the highest resistance against ampicillin (90%), Sulfametho-xazole-trimethoprim (75%), Amoxicillin-Clavulanic acid (70%). Tetracycline and ceftriaxone (65%) and 12 and 11 isolates were resistant to cipro-floxacin and gentamicin, respectively. Moderate resistance was against chloram-phenicol. Imipenem recorded the highest percentage of sensitivity (85%). Moreover, MDR was detected in 55% of isolates. Nearly similar results were observed in other studies **(Yang *et al.,* 2019; Permatasari *et al.,* 2020).**

Safika *et al.* (2022) reported a high frequency of resistance in *K. pneumoniae* isolates from poultry and MDR *K. pneumoniae*. A percentage of 100% of MDR *K. pneumoniae* was recorded in Germany (Daehre *et al.,* 2018) and in Egypt (Elmonir *et al.,* 2021). These findings were conversely with Franklin-Alming *et al.* (2021), who noted a lower percentage of antimicrobial resistance among isolated *K. pneumoniae* from poultry.

The present results showed that the *bla-TEM* gene was detected in 8 out of 20 isolates (40%) and the *bla-KPC* gene was detected in 9 out of 20 isolates (45%). We observed that 3 isolates (No. 2, 6 and 20) harboured both genes. Regarding the animal source, bla *TEM* was detected in 40% of both chicken and neonatal calves and bla *KPC* was detected in 40% and 50% of chicken and neonatal calves, respectively.

Safika *et al.* (2022) showed that all *K. pneumoniae* isolates from poultry had bla*TEM* genes. Moreover, In Egypt, 37% *K. pneumoniae* isolates from chickens harboured bla*TEM* (Elmonir *et al.,* 2021) and 38% frequency of blaTEM was found by (El-Tawab *et al.,* 2022). Various studies reported the presence of blaTEM gene in different sources from cattle, such as percentage of 23% from cattle and raw beef (Montso *et al.,* 2019), 88% from milk (Enferad and Mahdavi 2021) and 93.4% from swabs (Cheng *et al.,* 2018).

K. pneumoniae resistance to carbapenems has recently been the fastest-growing antimicrobial resistance threat in China (Tian *et al.,* 2023) and in Europe (Cassini *et al.,* 2019). In Egypt, a study determined a high prevalence of *K. pneumoniae* from broiler chicken and 11 isolates of them harbouring bla *KPC* (Hamza *et al.,* 2016).

Coexistence of Bla*KPC* (51%) and bla *TEM* (30%) was noted in *K. pneumoniae* from cattle swabs (Yang *et al.,* 2019) and from pneumonic lung by percentage of 28% and31% for bla*TEM* and bla*KPC*, respectively (Qi *et al.,* 2023). The occurrence of MDR-ESBL and MBL *K. pneumoniae* in samples from animal sources is resulting in public health hazards and has adverse economic consequences (Kot and Witeska, 2024).

CONCLUSION

The presence of *K. pneumoniae* obtained from animal sources which exhibited a high percentage of resistance and MDR and harbored bla*TEM* and bla *KPC* genes, act as a reservoir for resistance genes and can disseminate them to other bacterial species and trigger the use of antimicrobials that are unused for their toxicity, such as colistin. Strict rules for antimicrobial use in human and veterinary medicine to overcome the growing global antimicrobial resistance prevalence and prevent the spread of resistance among bacteria and hosts. Moreover, anti-microbial resistance monitoring in animals should be regularly assessed worldwide.

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مدى انتشار جينات مقاومة bla TEM وbla KPC في بكتيريا *الكلبسيلا الرئوية* المعزولة من مصادر حيوانية

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تعتبر *الكلبسيلا الرئوية* واحدة من أكثر أنواع البكتيريا المعوية المسببة للأمراض والتي تتسبب في خسائر اقتصادية ضخمة في صناعة الدواجن والحيوانات. علاوة على ذلك، فهي تمثل مستودعًا خطيرًا لانتقال مقاومة المضادات الحيوية إلى البشر بسبب انتشار مقاومة المضادات الحيوية على مستوى العالم ومقاومة الأدوية المتعددة. هدفت هذه الدراسة إلى الكشف عن انتشار *الكلبسيلا الرئوية* بين الدجاج والعجول حديثي الولادة، ومقاومتها للمضادات الحيوية، والكشف الجزيئي عن أهم جينات المقاومة المرتبطة بـها bla TEM و bla KPC، وتسليط الضوء على أهميتها بالنسبة للانتقال للانسان من خلال التسلسل الجيني. من ١١٥ مسحة شرجية, تم عزل عشرين (١٧,٤٪) من *الكلبسيلا الرئوية* من الدجاج (١٤,٣٪) والعجول حديثي الولادة (٢٢,٢٪). كانت جميع عزلات *الكلبسيلا الرئوية* العشرين إيجابية بالنسبة للجين الخاص بنوع 16S RNA، وأظهرت نتائج التسلسل اشتراكها مع عينات خاصة بالانسان. أظهرت عزلات *الكلبسيلا الرئوية* أعلى مقاومة ضد الأمبيسلين (٩٠٪) والسولفاميثوكسازول-تريميثوبريم (٧٠٪) والأموكسيسيلين-حمض الكلافولانيك (٧٠٪). كانت المقاومة للتتراسيكلين والسيفترياكسون (٦٥٪) و اظهرت ١٢ و ١١ عينة مقاومة للسيبروفلوكساسين والجنتاميسين على التوالي. سجلت مقاومة معتدلة ضد الكلورامفينيكول، وسجل الإيميبينيم أعلى نسبة حساسية (٨٥٪). علاوة على ذلك، تم الكشف عن مقاومة متعددة للأدوية في ٥٥٪ من العينات. تم الكشف عن جين bla-TEM في ٨ من أصل ٢٠ عينة (٤٠٪) وتم الكشف عن جين bla-KPC في ٩ من أصل ٢٠ عينة (٤٥٪). تستوجب نتائج هذه الدراسة اتخاذ قواعد صارمة لاستخدام مضادات الميكروبات في الطب البشري والبيطري للتغلب على تزايد انتشار مقاومة مضادات الميكروبات على الصعيد العالمي ومنع انتشار المقاومة بين انواع البكتريا الاخري. علاوة على ذلك، ينبغي تقييم مراقبة مقاومة مضادات الميكروبات في الحيوانات بانتظام في جميع أنحاء العالم.