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Research Article

Bacteriological Identification and Molecular Detection of *Klebsiella pneumoniae* from Pneumonic Camels in Al-Muthanna Province

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Abstract | Camels are one of the important animals in Al-Muthanna province; they exhibit meat, milk and hides for human consumption. Camels are characterized by resistance to hard condition, but still susceptible to many diseases such as bacterial infections. *Klebsiella pneumoniae* is one of the bacterial diseases that infect camels and induce pneumonia. In this study, one hundred camels were examined and samples were collected from lungs of *pneumonic* camels slaughtered in Al-Muthanna abattoir. Swabs were taken from lungs of slaughter camels, and cultured on blood and MacConkey agars. The bacterial growth was subjected into gram stain and subculture to *Klebsiella pneumoniae* selective media. Subsequently isolated bacteria were subjected to molecular detection using conventional PCR and sequencing. The finding showed that *Klebsiella pneumoniae* were 31 out of 74(41.89%) from total 100 camels that slaughter in the abattoir. This study indicates that *Klebsiella pneumoniae* reported higher infection rate as it is the predominant bacteria isolated from pneumonic camels which slaughter in Al-Muthanna abattoir.

Keywords | Camels, *Klebsiella pneumoniae*, pneumonia, PCR, Al-Muthanna province, Bacterial growth

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INTRODUCTION

The one-humped camels scientifically known as Camelus dromedaries, is a species within the Camillus genus that is commonly found in desert environments, particularly in the Arabian Peninsula. This type of camels possesses distinctive anatomical characteristics, including a single hump and elongated legs, which enable it to navigate and survive in the harsh desert conditions (Al-Tarazi, 2001). Camels (*Camelus dromedarius*) significantly contribute to the economy and social life of extensive areas in barren and semi-barren regions across various parts of the world (Abdul-Rahaman et al., 2018). Compared to other livestock species, the camel is a remarkably hardy and resilient animal, capable of enduring extreme temperatures, scarce water resources, and limited vegetation. As such, it has served as a valuable source of transportation, food, and other products for many cultures throughout history (Ibrahim, 2022). Moreover, due to its exceptional immune system, the camel is less susceptible to diseases and parasites that commonly afflict other livestock species in the same regions. Hence, it has become a crucial asset for the livelihoods of many people who inhabit arid and semi-arid regions around the world (Aldabbagh, 2022). Pneumonia is a frequently encountered respiratory disease in camels and other domestic animals, as it can cause damage to the lungs and make it difficult for the animal to breathe. It is a major cause of morbidity and mortality, leading to significant economic losses in animal husbandry. The colonization and multiplication of bacteria and virus

exhibits the accumulation of pus, fluid and development of the inflammatory process (Al-Khafaji, 2013). *Klebsiella pneumoniae* was firstly isolated at 1980 in Taiwan, when researchers had detected the bacterium from the patient's liver (Hashim, 2021). *K. pneumoniae* is a gram-negative bacterium within enterobacteriaceae family, rod in shaped, capsulated, non-motile, and also lactose-fermenting. It is an opportunistic pathogenic bacterium, facultative anaerobic, and a gamma hemolysis bacterium. *K. pneumoniae* is opportunistic pathogen multiplied in the lungs and intestine of humans and animals and in the environment (Das et al., 2019). *Klebsiella pneumoniae* is accountable for approximately 3 to 10% of cases. It is a significant pathogen that leads to various illnesses, such as pneumonia and bloodstream infections, primarily affecting newborns and patients in intensive care units (Hassan and Ibrahim, 2023). The capacity of *K. pneumoniae* to promote immune evasion is partly attributed to the production of a thick capsule that envelops the bacterial cell, protecting it from phagocytosis and enhancing its ability to adhere to host cells and tissue (Al-Samarraee, 2017). According to current evidence, the formation of bacterial biofilms within a host, particularly in the case of *E. coli* and *Klebsiella pneumoniae*, seems to primarily occur intracellularly. Clearly defining biofilms poses a challenge because their structure and composition vary significantly under diverse environmental circumstances. These microbial biofilms represent complex ecosystems that consist of microorganisms adhering to surfaces and enclosed within an organic polymer matrix originating from microbes themselves (Nadhom, 2018). In addition to pneumonia *K.pneumoniae* can be colonies the digestive system and exhibits diarrhea and enteritis in animals (Al-Darraji and Yousif, 2012). Over the recent years, the emergence of multi-drug resistance (MDR) in *K. pneumonia* and the widespread occurrence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have grown into a substantial global public health concern (Li et al., 2022). Identifying pneumonia in camels necessitates a comprehensive approach that takes into account the animals past clinical history, thorough clinical examination, and clinical indicators. The tell-tale signs of this condition include coughing, nasal discharge, labored breathing, and general lethargy, with congestion and consolidation of the lungs being a hallmark feature. It is typically necessary to conduct a bacteriological analysis (Farah et al., 2007). The bacteria can be purified from the samples through cultivation in culture media, MacConkey agar related with isolation of gram negative bacteria (Al-Hamadany, 2013). In addition to the enrichment methods, the PCR assay is one of the most important techniques used in definitive diagnosis of *K. pneumoniae* species. The sequences of 16SrRNA gene of *K. pneumoniae* are recently installed, sequence tends to be more genetically diverse and species specifically differ than 16SrDNA and 23S rDNA. PCR is the conventional technique for identification of *K. pneumoniae* in clinical sample (Salman and Al-Mathkhury, 2016). This study designed to isolate and identified the *K. pneumoniae* from camels suffered from respiratory signs. Additionally, to isolate and identified the *K. pneumoniae* and molecular detection using conventional PCR and sequencing.

MATERIALS AND METHODS

Animals

One hundred camels slaughtered at Al-Muthanna abattoirs were appeared to been suffered from pneumonia. The age, sex and breed were recorded for each animal. The physical examination was done for each animal accompanied with recording of case history and clinical signs.

Post mortem examination

Post-mortem inspection was performed to establish any relevant signs related with pneumonia followed by sample collection.

Samples collection

One hundred swab samples were collected from the 100 camel's lungs at slaughter house.

Bacterial culture

Lungs swab were cultured on MacConkey and nutrient agars and incubated at 37˚C for 24 hr. The suspected colonies on the primary culture were sub cultured on blood agar to determine the ability to hemolysis the blood. Additionally, it was culture on CHROM agar the selective media for *K. pneumoniae*.

Microscopic examination

A bacterial smear of the *K. pneumoniae* culture was stained by gram stain for microscopic examination.

Molecular detection

All isolates of *K. Pneumoniae* which diagnosed by culturing and microscopic examination were submitted to detection by conventional PCR. Universal primer of *K. pneumoniae* 16SrRNA gene, according to gene bank code (CP118936.1) was used as reported in Table 1.

Table 1: The primers used for detection of *K. Pneumoniae*; F (forward) and R (reverse).

Primer	Klebsiella pneumoniae sequence (5`→3`)	Amplicon size	Gene bank code
16rsRNA	F TGCCTGATGGAGGGGGATAA	625bp	CP118936.1
	R AGCGTCAGTCTTTGTCCAGG		

Statestical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors in our study parameters.

The PCR thermal cycles programs used for the detection of *K. pneumoniae* are listed in Table 2.

Table 2: PCR thermal cycles program for detection of *K. pneumoniae* in camels.

No	Stages	Temperature	Time	
1	Initial denaturation	94°C	7 mins	1
	Denaturation	94°C	40 s	35 cycles
	Annealing	55°C	30 s	
2	Extension	72°C	1 min	1
	Final Extension	72°C	7 mins	

RESULTS AND DISCUSSION

Physical examination

The most important clinical signs of pneumonia in camels that were brought to slaughterhouse were suffered from fever, mucopurulent nasal discharge, increase pulse rate, rapid shallow respiration, some cases were showed coughing, anorexia, and eyes lacrimation and some animals appeared emaciated. The clinical signs that reported in this study were relatively convenient with Hussain et al. (2018).

Post mortem examination

Post mortem inspection exhibited red consolidation that were prominently firm, resembling liver tissue (hepatized) with blunted end and displaying a lobular pattern. The post mortem finding were relatively convenient with Hussain et al. (2018), (Figure 1).

Percentage of infection

The study's findings revealed that 74% of the gram-negative bacteria were isolated from swab samples taken from the lungs of pneumonic camels at the Al-Muthanna abattoir. Among this percentage, 64.86% were single bacterial isolates, while 35.13% were samples that yielded mixed bacterial isolates (Table 3).

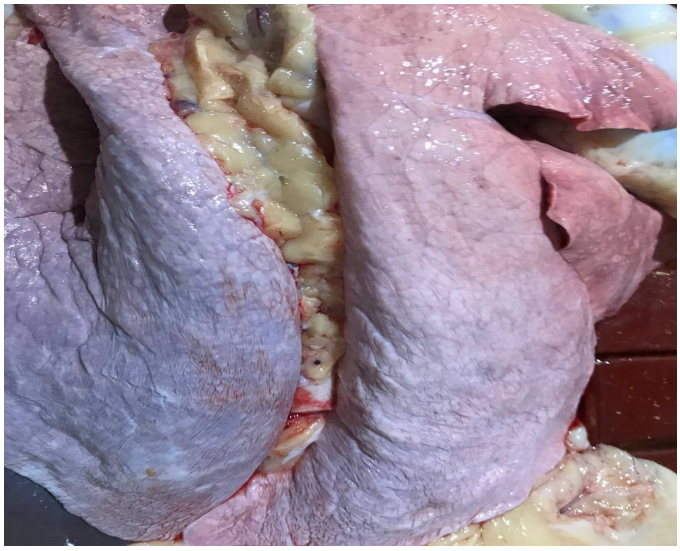

Figure 1: A post-mortem examination of the left lung in the camels unveiled a pneumonic lesion characterized by a firm, blunt end, along with petechial hemorrhaging and red consolidation.

Pneumonia in camels is a significant respiratory infection that can lead to serious health issues if left untreated. The identification of the causative agents and appropriate treatment are crucial in the management of the disease (Ismail, 2017).

The study presents intriguing findings on the prevalence of bacteriologically positive camels' lungs swap samples. According to the data in Table 2, out of the 100 camels examined, 74 (74%), were found to be positive for gram negative bacteria. Interestingly, the results of the study are relatively consistent with earlier research by Moawad et al. (2011) who reported the incidence of 76 gram negative bacterial isolates from 103 of pneumonic camels which were examined. However, other studies have shown different rates of gram negative bacterial isolates in camel lungs. Moustafa (2004) found lower incidences of 64 (25.1%) from total 255 of pneumonic camels which were examined. A sample carefully collected from the lungs of pneumonic camels, the bacterial isolates were meticulously analyzed to reveal a disturbing trend: 74 (74%) of the bacterial isolates were identified as gram-negative, this percent were classified as 31 (41.89%) of *Klebsiella pneumoniae*, 26 (35.13%) of *E. coli* and 17 (22.97%) was *pseudomonas aeruginosa* isolates, (Table 4).

Table 3: Gram negative bacterial isolates showing the samples give single or mixed bacterial isolates and other infection.

Health status of camels	No. of examined camels	No. of gram negative bacterial isolates	%	No. of samples gives single bacterial isolates	%	No. of samples gives mixed bacterial isolates	%	Other infection	%
Pneumonic camels	100	74	74	48	64.86	26	35.13	26	26
Total	100	74	74	48	64.86	26	35.13	26	26

Table 4: Isolation of gram negative bacteria from lungs of camels infected with pneumonia.

Type of gram negative bacterial isolates	No. of bacterial isolates	Percent %
Klebsiella pneumoniae	31	41.89%
Escherichia coli	26	35.1%
Pseudomonas aeruginosa	17	22.97%
Total	74	100%

The higher percent of prevalence in gram negative bacterial isolates was *Klebsiella pneumoniae* 41.89%, while lower percent recorded by Wareth et al. (2014) who reported 26.71% from one hundred lungs of slaughter camels which were examined. On the other hand, the results of the study are higher than Gebru et al. (2018) who reported 8(8.7%) of *klebsiella pneumoniae*. In addition, Ahmed et al. (2017) documented 9 (11.25%) of *klebsiella pneumoniae*.

These divergent results suggest that the prevalence of bacterial infections in camel's lungs may vary depending on multiple factors, including the study design, sample size, and geographic location, management practice, and immune status Moawad et al. (2011).

Identification of bacteria

Culture characteristics

All samples that were collected from lungs of camels infected with pneumonia are cultured on MacConkey, Nutrient and blood agars, then the gram negative bacteria were isolated and subculture into selective media such as CHROMagar orientation for bacterial characterization.

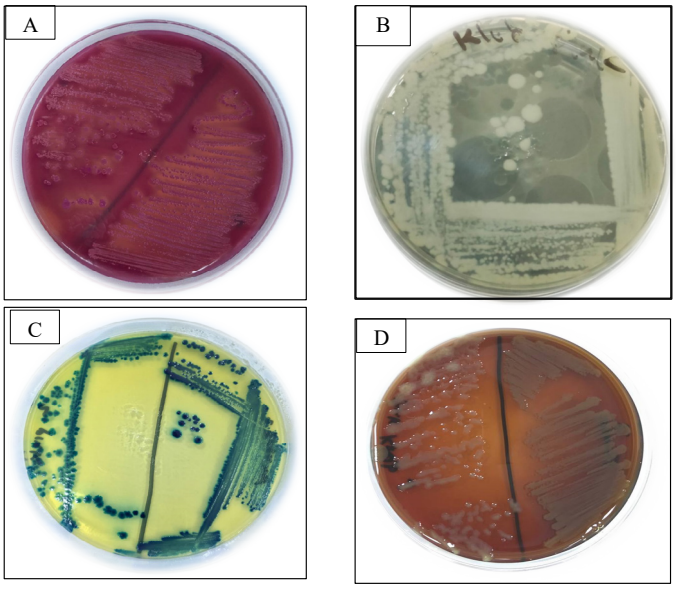

Figure 2: *K. pneumoniae* on MacConkey, nutrient, blood and CHROMagar orientation agars. (A) *K. pneumoniae* on MacConkey agar. (B) *K. pneumoniae* on nutrient agar. (C) *K. pneumoniae* on CHROMagar orientation. (D) *K. pneumoniae* is a non-hemolytic on blood agar.

Klebsiella pneumoniae colonies on MacConkey agar appeared smooth, convex, and round in shape and exhibit a moist, mucoid texture. The size of the colonies can vary from small to large, and their growth is characterized by an expanding and contracting motion. Meanwhile, the growth of *K. pneumoniae* on nutrient agar typically appears as large, smooth, mucoid, and gray-white or cream-colored colonies. *K. pneumoniae* is a non-hemolytic bacterium; they exhibited gamma hemolysis on blood agar. On the other hand, the Isolation of *K. pneumoniae* on CHROMagar orientation produced Steel blue colonies (Figure 2).

Microscopic characteristics

The microscopic examination exhibits morphological characterization of the bacterium such as color and colony shape. *K. pneumoniae* revealed rod in shape; also, the gram negative bacteria produced red color for gram staining (Figure 3).

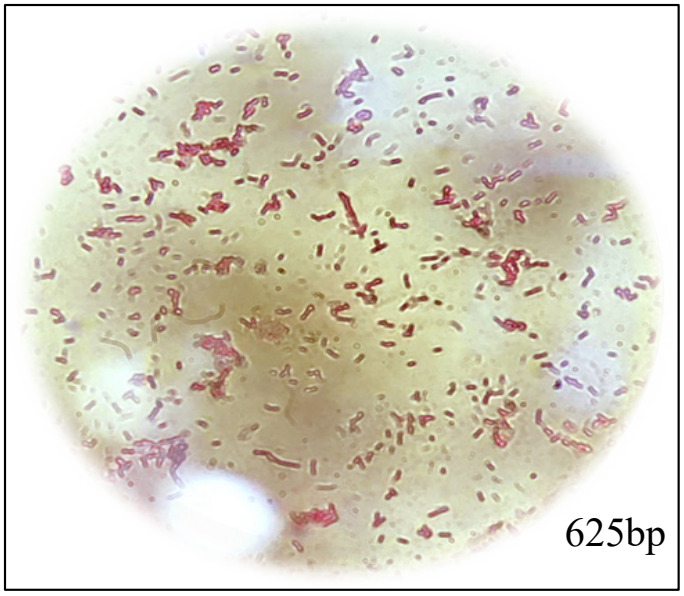

Figure 3: Gram stain of *K. pneumoniae*.

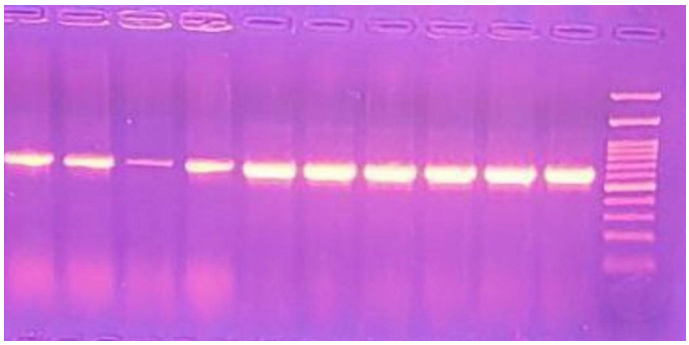

Figure 4: PCR Gel electrophoresis positive results of *K. pneumoniae* with red stained for amplification size 625bp of 16SrRNA *K. pneumoniae*.

Polymerase chain reaction of *K. pneumoniae*

The Polymerase Chain Reaction (PCR) technique has gained significant prominence in the field of microbiology

as a confirmatory technique for detecting bacteria. Its high sensitivity and specificity make it a standard and widely recognized diagnostic technique. As such, it is considered the definitive technique for confirming the presence of the pathological agents in clinical samples (Badawi and Yousif, 2020). In this study *K. pneumoniae* was the more prominent bacteria isolated from camels lungs. All *K. pneumoniae* isolates are submitted to detection using conventional PCR with specific primer and amplification size 625bp. The PCR results are demonstrated in Figure 4.

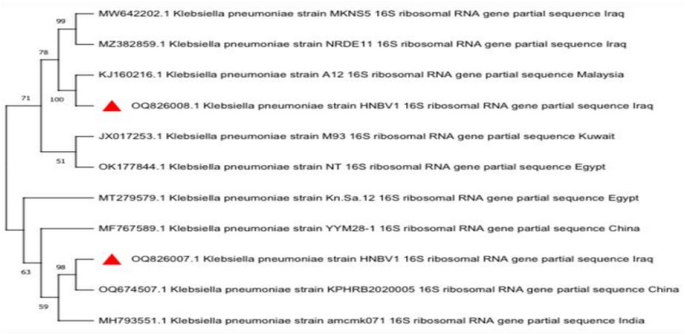

Figure 5: Phylogenetic tree of *K. pneumonia* in comparison with local and international isolates.

The molecular diagnosis of *K. pneumoniae* is done by using conventional PCR, a DNA samples were extracted and subjected to a conventional PCR assay targeting a specific gene, referred to as (16rsRNA) associated with *K. pneumoniae*. The PCR reaction produced an amplicon with a molecular weight of 625 base pairs. The detection of K. *pneumoniae* by conventional PCR relatively disagreement with Mohammed et al. (2020) who reported the detection of *K. pneumoniae* with amplification size 770bp with different primer. On the other hand, the detection of *K. pneumoniae* from diseased camels has also been reported in a previous study by Liu et al. (2008).

Sequencing of *K. pneumoniae* isolates

The study was conducted on 32 positive isolates of the *K. pneumoniae*, 5 isolates were submitted to sequencing. The isolates were sent to the National Center for Biotechnology Information (NCBI). The study results of the sequencing discussed in Figure 5.

Mong this study, the Phylogenetic tree of *K. pneumoniae* isolates was showed identical percent with numerous isolates. The higher percent of identification was reported with (OQ826008.1 *K. pneumoniae* strain HNBV1 16S ribosomal RNA gene partial sequence Iraq) and (KJ160216.1 *Klebsiella pneumoniae* strain A12 16S ribosomal RNA gene partial sequence Malaysia) followed by (MW642202.1 *Klebsiella pneumoniae* strain MKNS5 16S ribosomal RNA gene partial sequence Iraq), (MW642202.1 *Klebsiella pneumoniae* strain MKNS5 16S ribosomal RNA gene partial sequence Iraq) and consecutively as shown in Figure 5. The genetic sequencing can be effected by several evolutionary processes like natural selection, geographical origins and environmental factors such as temperature, humidity. So, the Bacterial isolates that are adapted to specific host organisms or environments may have acquired genetic traits that are not present in other isolates (Drancourt et al., 2000).

CONCLUSIONS AND RECOMMENDATIONS

This study indicates that *K. pneumoniae* reported higher infection rate as it is the predominate bacteria isolated from pneumonic camels which slaughter in Al-Muthanna abattoir.

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NOVELTY STSTEMENT

The novelty of the study is focus on the importance of using molecular technology and genetic sequencing in identifying the pathogen that exhibits morbidity and mortality in camels at Al-Muthanna abattoirs, Because of the inefficiency of health control in the Iraqi abattoirs and the lack the use of modern technology in the epidemiological survey, treatment and prevention of pathogen to transmission for humans and other animals.

AUTHOR'S CONTRIBUTION

WHM: Submitted the collection of samples and isolation of *K. pneumonia*. NMHA-M: Performed the molecular examination and statistical analysis of obtained results.

Conflict of interest

The authors have declared no conflict of interests.

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