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## Detection of *Klebsiella pneumoniae* in Broiler Chickens at Ismailia City, Egypt

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### Abstract

*Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic bacterial pathogen belongs to the *Enterobacteriaceae* family. Although the bacteria are commonly present in the intestinal tract of animals, it's represent a major threat to the public health in other circumstances. The emergence of hypervirulent strains of *K. pneumoniae* may induce critical community-acquired and hospital-acquired infections. This study aimed to the detect *K. pneumoniae* in broiler chickens from Ismailia City, Egypt. A total of 481 samples (liver, spleen, heart, lung, trachea and cloacae) of broiler chickens were submitted for bacteriological examination. Furthermore, isolates were confirmed by detection of 16S rRNA gene using PCR and serologically tested. Overall *K. pneumoniae* isolates, based on the conventional culture and biochemical identification, were 65 out of 481 chicken's organ samples (13.5 %). The recovery rate was variable according to the organ of isolation, where a higher detection rate was reported from heart (30%) followed by lung (14.11%) and spleen (13.68%), while liver (10%), tracheal (9.76%) and cloacal swabs (8%) showed lower rates. Using PCR, all tested isolates were positive for 16S rRNA gene. The hypermucoviscous phenotype (HMV/hvKp) of *K. pneumoniae* isolates was detected using "string test". The occurrence of hvKp (85%) was higher than classic *K. pneumoniae* (cKp) (15%). In detection of capsular serotypes K1 and K2 among the examined *K. pneumoniae* isolates; K1 serotype was more prevalent among hvKP isolates (88,2%) while the K2 serotype was less prevalent (15%).

Findings in this study showed a high occurrence of *K. pneumoniae* in apparently healthy broiler chickens indicating that these chickens might be an important reservoir for human and animal infections and suggesting their potential threat to food safety.

**Keywords:** *K. pneumoniae*, Broiler chicken, PCR, Egypt.

## Introduction

*Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative encapsulated rod-shaped opportunistic facultative anaerobic bacterium that can produce fatal diseases in humans and animals, moreover, it can be transmitted from one person to another (Rønning et al., 2019). Although the *K. pneumoniae* bacteria are commonly known for colonizing the mammalian gut, they are also regarded as a serious public health hazard due to their capacity to cause severe infections in hospital settings and their association with antibiotic resistance (Wyres and Holt, 2018). Poultry is one of the most widespread food industries, which can successfully fill the gap of animal meat shortage worldwide. In chicks, *K. pneumoniae* is a serious infectious illness that causes significant economic losses. Also, it possesses food safety threats, as it may contaminate poultry meat and eggs, posing a serious risk to consumer health (Aly et al., 2014 and Navon-Venezia et al., 2017). The mucoviscosity character of *K. pneumoniae* isolates protects it from the interaction with anticapsule-specific antibodies to avoid

phagocytosis (Nelson et al., 2007). String test is a tool that is used to identify hypermucoviscous strains of *K. pneumoniae*, which appear as sticky colonies on plates of nutrient agar and form a long string when grasped with a loop (Yu et al., 2007). The hypermucoviscous strains of *K. pneumoniae* (hvKp) is more virulent than the classic ones (cKp), and can cause nosocomial and community-acquired infections in healthy individuals (Ye et al., 2016). Moreover, presence of K-capsular antigens in the bacteria protect it against opsonophagocytosis and serum-mediated death (Brisse et al., 2006). Although several studies had been conducted about *K. pneumoniae*, there is always a continuous need to track this pathogen. Therefore, the present study aimed to investigate to which extent *K. pneumoniae* is present in broiler chickens prepared for human consumption in Ismailia City, Egypt.

## Material and Methods

### Ethical statement

Sample collection and processing for this study was reviewed and approved by the Ethical Committee for Scientific Research, Faculty of

Veterinary Medicine, Suez Canal University, Ismailia, Egypt (No. 2019032).

### **Study area**

This study was carried out between March, 2022 and September, 2022 in Ismailia city, Egypt. Ismailia city is the capital of Ismailia Governorate and it is situated in north-eastern Egypt on the west bank of the Suez Canal about 120 km to the north east of Cairo. The city has a population of approximately 1.325000 inhabitants.

### **Sample collection**

Chicken samples (481) were taken from 100 broiler chickens from different chicken body sites; liver (100), spleen (95), heart (60), lung (85), trachea (41) and cloacae (100). These samples were collected from slaughtered broiler chickens prepared for human consumption from public markets distributed in Ismailia City, Egypt. Liver, spleen, heart and lung tissue samples were collected in labeled sterile plastic bags, while the tracheal and cloacal swabs were inserted directly into test tubes containing sterile buffered peptone water (Oxoid, UK) under aseptic conditions.

### **Isolation and identification of *K. pneumoniae***

Chicken samples from liver, spleen, heart and lung (340) were processed under aseptic conditions and inoculated into test tubes containing 10 mL sterile buffered peptone water. Tracheal and cloacal swabs (141) were directly

inserted into test tubes containing sterile buffered peptone water (Oxoid, UK). All inoculated samples were incubated for 24 h at 37°C. A loopful from each broth culture of chicken samples were sub-cultured by streaking onto EMB (Himedia, India) and MacConkey agar (Himedia, India) and incubated for 18-24h at 37°C. The colonies that morphologically resembling *K. pneumoniae* were carefully picked and repeatedly sub-cultured onto EMB and MacConkey agar until pure cultures with homogenous colonies were obtained. Characteristic colonies appear as pink to purple in color without green metallic sheen on EMB agar; and large, mucoid, and pink in color on MacConkey agar (*Masruroh et al., 2016*). Gram's stain was used to identify suspected *K. pneumoniae* isolates morphologically, while Citrate utilization, Urease, Indole, Methyl Red, Vogues-Proskaur, Catalase and TSI tests were used to identify the isolates biochemically according to (*Cruickshank et al., 1975 and ISO, 2013*).

### **Detection of 16S rRNA gene in *K. pneumoniae* isolates**

Selected twenty representative *K. pneumoniae* isolates, identified by the conventional cultural and biochemical tests, were examined for the 16S rRNA gene. DNA was extracted according to QIAamp DNA mini kit manufacturer instructions (QIAGEN, USA). Briefly protease (20 µl) was added to

200 µl broth containing 2-5 pure colonies with 200 µl buffer AL in a microcentrifuge tube. After incubation at 56°C for 10 min., the mixture was washed several times before the DNA was eluted from the QIAamp mini spin column using elution buffer. PCR amplification was carried out using 16S rRNA Oligonucleotide Primers; F: TGGAGCATGTGGTTTAATTCG A and R: TGCGGGACTTAACCCAACA (Metabion, Germany). The cycling conditions consisted of primary denaturation at 94°C/5 min. followed by secondary denaturation at 94°C/30 sec., annealing at 55°C/30 sec., extension at 72°C/30 sec. and final extension at 72°C/7 min. (Cunningham et al., 2013). *K. pneumoniae* reference strain supplied by the Animal Health Research Institute, Zagazig, was used as positive control in the PCR run. Finally, expected product size (159-bp) was photographed using a gel documentation system after being electrophoresed at room temperature using 1.5% agarose gel in 100 ml TBE buffer against a 100 bp DNA Ladder.

String test was used for detection of mucoviscosity in the previously selected twenty *K. pneumoniae* as performed by Shon et al. (2013). Using the loop, a loopful from each isolate was stretched on nutrient agar plate. Any generated viscous string more than 5 mm in length was considered positive and defined as

hypermucoviscous *K. pneumoniae* (hvKp). However, negative isolates were nominated as classic *K. pneumoniae* (cKp).

#### **Serological identification of *K. pneumoniae* isolates**

The previously selected twenty *K. pneumoniae* isolates were serologically identified in the Food Analysis Center at Faculty of Veterinary Medicine, Benha University using Quellung test "Neufeld reaction" (Edmondson and Cooke, 1979). Isolates were serologically identified for K1 and K2 antigens of *K. pneumoniae*. Specific sera were purchased from Statens Serum Institute, Copenhagen, Denmark. Accordingly, Quellung test was carried out according to the producer instructions.

#### **Results**

Bacteriological examination revealed that the recovery rate of *K. pneumoniae* was 13.5% (65 out of 481 examined chicken's organ samples). The isolation percentage in broilers was variable according to the organ of isolation, as illustrated in Table (1) and Fig. (1). The higher detection rate was reported from heart (30%) followed by lung (14.11%) and spleen (13.68%) and the lower rates were reported in liver (10%), trachea (9.76%) and cloacal content (8%). Twenty representative *K. pneumoniae* isolates (representing at least one isolate from each

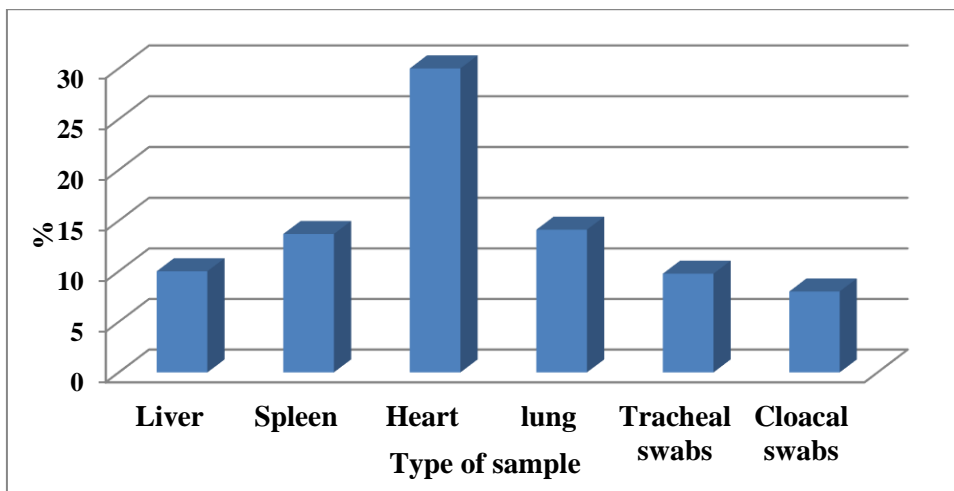
positive bird) were examined for the 16S rRNA gene using PCR and they were identified serologically. All tested isolates showed the 16S rRNA gene specific bands (Fig. 2). Regarding serotyping, the hypermucoviscous (HMV) phenotype was detected in chicken *K. pneumoniae* isolates using the “string test (Table 2). The hvKp

(17, 85%) was more prevalent than cKp (3; 15%) among the examined isolates. In detection of capsular serotypes K1 and K2 among the examined chicken *K. pneumoniae* isolates; K1 serotype was more prevalent among hvKP isolates (15/17; 88,2%) and generally K2 serotype was less prevalent (3/20; 15%).

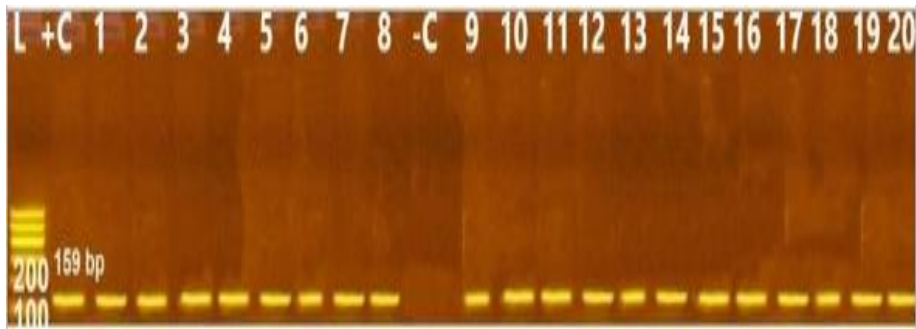
**Table (1):** Recovery rate of *K. pneumoniae* in broiler chicken based on

Total number of examined samples	No. of positive <i>K. pneumoniae</i> (%)	Positive No of <i>K. pneumoniae</i> / Total No. of each examined organ					
		Liver (n= 100) No. (%)	Spleen (n=95) No. (%)	Heart (n=60) No. (%)	Lung (n= 85) No. (%)	Tracheal swabs (n=41) No. (%)	Cloacal swabs (n=100) No. (%)
481	65 (13.5)	10 (10)	13 (13.68)	18 (30)	12 (14.11)	4 (9.76)	8 (8)

conventional cultural and biochemical identification



**Fig. (1):** Recovery rate of *K. pneumoniae* in broiler chicken organs based on conventional cultural and biochemical identification.



**Fig. (2):** Agarose gel electrophoresis of amplified DNA showing the specificity of the single reaction for the detection of the 16S rRNA gene (159-bp) in isolates from broiler chicken. Lanes: L, molecular marker (100-bp DNA ladder), +C, 16S rRNA positive control, -C, negative control, 1–20, 16S rRNA positive *K. pneumoniae* strains from chicken samples.

**Table (2):** Serotyping profile of *K. pneumoniae* isolates from broiler chicken samples.

Type of sample	No. of isolates	Sample Code	String test	Serodiagnosis
Liver	3	3	HVKP	K1
		4	HVKP	K1
		14	HVKP	K2
Spleen	4	2	HVKP	K1
		9	HVKP	K2
		12	HVKP	K1
Heart	6	19	HVKP	K1
		1	HVKP	K1
		10	HVKP	K1
		11	CKP	K1
		13	HVKP	K1
		15	CKP	K1
Lung	5	16	HVKP	K1
		5	HVKP	K1
		6	HVKP	K1
		7	CKP	K2
		8	HVKP	K1
Cloacal swabs	2	17	HVKP	K1
		18	HVKP	K1
		20	HVKP	K1

## Discussion

*Klebsiella pneumoniae* is an opportunistic pathogen of a major

threat to public health. It is present in the intestinal or the respiratory tract of animals and it causes

infections whenever the immune system of the infected bird is impaired. *Klebsiella* strains are now known in clinical microbiology laboratories, using automated devices based on classical biochemical testing. The biochemical assays can differentiate between *K. pneumoniae* and *K. oxytoca* (Hansen et al., 2004). The morphological examination showed that suspected colonies were observed as pink to purple in color without green metallic sheen on EMB agar; and large, mucoid, and pink in color on MacConkey agar (Dashe et al., 2013 and Masruroh et al., 2016). The biochemical identification proved that the isolated strains were methyl red-negative, indole-negative, citrate-positive, voges-Proskauer positive, urea hydrolysis positive, TSI produce yellow slant and butt with gas production but no H<sub>2</sub>S production, catalase-positive, so they were identified as *K. pneumoniae* (Barbara et al., 1994). In this study, *K. pneumoniae* was recovered from 65 out of 481 chicken's organ samples with an overall isolation rate of 13.5%. Nearly similar results had been previously stated by Yang et al. (2019) in China (15.6%) and El-Tawab et al. (2022) in Egypt (10.5%), however, lower detection rates had been documented in other studies by Hossain et al. (2013) in Bangladesh (6%), Hayati et al. (2019) in Indonesia (9.2%),

Bhardwaj et al. (2021) in India (7.80%), Elmonir et al. (2021) in Egypt (9%) and Li et al. (2022) in China (4.67%). The recovery of *K. pneumoniae* in broiler chickens was variable according to the organ of isolation; the higher detection rate was reported from heart (30%) followed by lung (14.11%) and spleen (13.6%), while the lower rates were reported from liver (10%), trachea (9.76%) and cloaca (8%). In this regard, Tantawy et al. (2018) reported that the isolation rate was higher in the lungs (60%) than in the liver (40%), with the intestine (10%) having the lowest isolation rate. In another study, *Klebsiella* spp. isolation rates from lungs, liver, spleen and heart were 46.67%, 20%, 20%, and 13.33 %, respectively (Younis et al., 2016). Also, Dashe et al. (2013) isolated *K. pneumoniae* from lung and livers of apparently healthy chickens at a rate of 8%. The presence of *K. pneumoniae* in the chickens' lung, heart, spleen and liver might imply concomitant extraintestinal infections (Younis et al., 2019).

A number of DNA-based approaches have been developed for detecting pathogenic *Klebsiella* spp., including PCR based on the 16S rRNA gene (Jonas et al., 2004). Because it is highly conserved segment within the species, the 16S rRNA gene is ideal candidate for bacterial identification (Gutell et al., 1994). In this study, all tested *K. pneumoniae* isolates, yielded

specific bands of 16S rRNA gene in PCR analysis. Similar findings have been previously reported by **Reham (2017)** and **Wong et al. (2018)**. **He et al. (2016)** in their study, on comparative evaluations of phenotypic techniques and sequencing of 16S rRNA genes for identification of *K. pneumoniae* clinical isolates, revealed that 16S rRNA gene might be beneficial for isolate identification up to the genus level.

The hvKp is more virulent than cKp, and can cause nosocomial and community-acquired infections in healthy people (**Ye et al., 2016**). In the present study, the hypermucoviscous (HMV) phenotype was detected in chicken *K. pneumoniae* isolates using the “string test. The hvKp (17, 85%) was more prevalent than cKp (3; 15%). Similar finding had been previously reported by **Ye et al. (2016)**. In detection of capsular antigens (K1 and K2), generally K2 serotype was rather uncommon in *K. pneumoniae* isolates (3/20; 15%) and all hvKP had K1 antigen except 2 isolates had K2. Usually, K1 and K2 appear to be associated with invasive strains  
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(**Jung et al., 2013**), and the capsule is associated with hypervirulence, with the majority of hvKp strains having type K1 or K2 (**Russo and Marr, 2019**). In this regard, epidemiologically, the importance of detection of hvKP appears in harboring a large virulence plasmid that harbors many virulence-encoding genes which encode functions related with adhesion, protection (capsules) or siderophore production (**Wyres et al., 2020**).

In conclusion, findings in the present study clearly demonstrated a high occurrence of hypervirulent *K. pneumoniae* in apparently healthy broiler chickens, which might be considered as an important zoonotic reservoir for *K. pneumoniae*. The K1 serotype was more prevalent among hvKP chicken isolates, representing a potential threat to food safety and public health.

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الملخص العربي**الكشف عن الكليبيسيلا الرئوية في دجاج التسمين بمدينة الاسماعيلية ، مصر**

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عبدالكريم محمود أبو عيشه

الكليبيسيلا الرئوية هي بكتيريا تنتمي إلى عائلة البكتيريا المعوية، سالبة الجرام، وتتواجد في القناة المعوية للإنسان والحيوان، وتعتبر ميكروب انتهازي مسئول عن العديد من الأمراض حيث انها قد تسبب في بعض الأحيان الإسهال والنفوق في الدجاج، وأجريت الدراسة الحالية لمعرفة مدى تواجد هذه البكتيريا في دجاج التسمين بمدينة الاسماعيلية، مصر، وتم تجميع 481 عينة (100 من الكبد، 95 من الطحال، 60 من القلب، 85 من الرئة، 41 من الزور، و100 من الزرق) من عدد 100 طائر من دجاج التسمين من اماكن مختلفة بمدينة الاسماعيلية، وتم فحص هذه العينات باستخدام الإختبارات البكتيريولوجية (المستنبتات البكتيرية والتصنيف البيوكيميائي)، اختبار تفاعل عديد البلمرة المتسلسل (PCR) للكشف عن جين 16S rRNA، بالإضافة الى التصنيف السيرولوجي للعزلات. نتج عن الزرع البكتيري والتصنيف البيوكيميائي عزل عدد 65 عذلة من الكليبيسيلا الرئوية من دجاج التسمين بنسبة عزل كلى 13.5%، حيث كانت نسبة عزل هذه البكتيريا: 14.11, 30%, 13.68%، 10%، 9.76%، 8% من القلب، الرئة، الطحال، الكبد، الزور و مسحة الزرق، علي التوالي. تم فحص عدد 20 عذلة ممثلة من الاعضاء المختلفة باستخدام اختبار تفاعل عديد البلمرة المتسلسل للكشف عن جين 16S rRNA، حيث أكدت النتائج ايجابية كل العينات للكليبيسيلا الرئوية. بالكشف عن الظاهرة المخاطية، تم تحديد النمط المصلي لهذه العزلات، و أظهرت النتائج وجود نوعي الكليبيسيلا الرئوية (HVKP) (17 / 20) و (CKP) (3 / 20) وأن العزلات تحتوي علي نوعي (K1, K2).

وتبين من هذه الدراسة ارتفاع نسبة تواجد الكليبيسيلا الرئوية في دجاج التسمين السليم ظاهرياً مما يشير إلى أن هذه الطيور قد تكون مستودعاً مهماً لإصابة الإنسان والحيوانات بهذه البكتيريا، كما تبين أن النمط المصلي K1 أكثر انتشاراً بين عزلات الدجاج hvKp، مما يشكل تهديداً محتملاً لسلامة الأغذية والصحة العامة.