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Investigating Virulence and Multidrug Resistance Capability of *Klebsiella Pneumoniae* Isolates From Karish "Soft" Cheese and Chicken Carcasses Sold in Qena City, Egypt

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Abstract

Klebsiella pneumoniae significantly contributes to antimicrobial-resistant infections in humans, posing a considerable risk to both human and animal health worldwide, so this study was designed to identify the virulence and antimicrobial resistance profile of *K. pneumoniae* isolated from chicken carcasses and raw milk soft "Karish" cheese in Qena city, Egypt. One hundred food samples, 50 samples of each were examined for *K. pneumoniae* presence. A total of only seven isolates were confirmed to be *K. pneumoniae* through biochemical tests. PCR was performed for several virulence genes (*iut*A, *kfu* and *rmpA* genes) and antimicrobial resistance genes (*mcr-1*, bla_{SHV}, bla_{CTX-M} and bla_{CMY-2} genes). In total, 7% of food samples tested positive for *K. pneumoniae*, one isolate from chicken carcasses and six from Karish cheese samples. All isolates exhibited resistance to amoxicillin, ceftazidime and amoxicillin-clavulanic acid, while they were susceptible to imipenem, gentamicin, tobramycin, cefotaxime, aztreonam and meropenem. *K. pneumoniae* isolates from Karish cheese samples showed susceptibility to imipenem (100%), gentamicin and tobramycin (83.33%), while (100%) resistance was displayed against amoxicillin, ceftazidime and amoxicillin clavulanic acid. The most predominant virulence gene was *iut*A (100%), followed by *kfu* (85.7%). All *K. pneumoniae* isolates were found to carry bla_{SHV}. Five *mcr-1* and two bla_{CTX-M}-producing *K. pneumoniae* isolates were recovered, while bla_{CMY-2} was absent in all isolates. Our results raising serious concerns as *K. pneumoniae* poses significant public health risks because consumption of such products may transmit resistant strains to humans through the food chain, so stricter regulations are required to ensure food safety.

Keywords: Food safety; *K. pneumoniae*; virulence; antimicrobial resistance.

Introduction

Klebsiella pneumoniae, a member of *Enterobacterales*, is an important opportunistic bacterium, being a major cause of pneumonia, diarrhea, septicemia, urinary tract infections, and liver problems in humans [1]. Although it is a well-known hospital-acquired organism usually associated with nosocomial infections, *K. pneumoniae* has been considered an important pathogen of food-borne illness. It has been frequently recovered from various food types including meat and dairy products [2, 3, 4]. Many virulence genes are involved in the virulence process of *K. pneumoniae*; these genes play important roles in the pathogenesis of *K.*

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pneumoniae, Polymerase chain reactions were used to determine the different types and to detect potential virulence genes in all isolates [5]. Severity of infections caused by *K. pneumoniae* will depend on many factors, including the virulence profile of isolates, *rpmA* gene, which is associated with the hypervirulence of *K. pneumoniae* [6] as it may play a role in the survival of *K. pneumoniae* in low temperatures or may be linked to factors that increase the ability of *K. pneumoniae* to attach to the tank surface (e.g., biofilms). Aerobactin and its transporter *IutA* are present in hypervirulent *K. pneumoniae* strains [7]. The gene *kfu* encodes an iron transportation system and, therefore, is also involved in the acquisition of iron by *K. pneumoniae* [8]. The consideration of *kfu* gene as pathogenic may be due to its association with virulent tissue infections, capsule biosynthesis and purulent tissue infections characterized by hypermucoviscosity [8]. It is suggested that it plays a vital role in iron extraction from host cells, highlighting its significance in pathogenicity [8].

On the other side, the encounter of antimicrobial-resistant *K. pneumoniae* strains in the food chain is an alarming public health concern. Carbapenem-resistant and third-generation cephalosporin-resistant *Enterobacterales* (including *K. pneumoniae*) have been classified by the WHO as critical priority pathogens [9]. Irresponsible behavior and the overuse of antibiotics for treating infections have led to the emergence of multidrug-resistant (MDR) pathogenic bacterial strains. Furthermore, the addition of antimicrobial substances and growth promoters in chicken feed complicates the issue further [10, 11]. The emergence of MDR and pathogenic strains of *K. pneumoniae* were identified across a range of food products, encompassing meat, seafood, milk, and dairy products [12]. Consequently, their possibility to pose significant public health risks due to their resistance profiles and virulence factors is increasing [12]. The increasing emergence and spread of antimicrobial-resistant *K. pneumoniae* isolated from different food sources, including poultry and dairy products, is a worrying problem, as it severely endangers food safety and public health [5]. One of the most clinically significant mechanisms of resistance for gram-negative pathogens, including *K. pneumoniae*, is β-lactamase production, including Extended-spectrum β-lactamases (ESBLs) [13, 14]. ESBLs, particularly $bla_{\text{CTX-M-like}}$ genes, are responsible for conferring resistance to broad-spectrum cephalosporins and aztreonam in *K. pneumoniae* [15]. Humans can acquire infections with ESBL-producing *K. pneumoniae* through the ingestion of contaminated food or water [16]. The ESBL genes, specifically bla_{SHV} and $bla_{CTX\text{-}M}$, have been identified worldwide and are carried on plasmids, which facilitate horizontal gene transfer within strains of *K. pneumoniae* and across different genera of bacteria [17]. Along with the ongoing dissemination of multidrug-resistant hypervirulent *K. pneumoniae*, the emergence of colistin-resistant multidrug-resistant *K. pneumoniae* of human and animal origin has become more challenging to treat [18]. The extensive use of colistin in veterinary medicine, either as a growth promoter or prophylactic and therapeutic agent, has been a major reason for the emergence and spread of colistin-resistant bacteria in food-producing animals [13]. The plasmid-borne transferable colistin resistance gene "*mcr-1*" was first discovered in a Chinese pig in 2015 by Liu et al. [19] . Since then, there has been concern about plasmid-mediated colistin resistance. In Egypt, limited information is available about the prevalence rate of foodborne *K. pneumoniae* of food origin and their phenotypic and genotypic characteristics. Therefore, we aimed here to shed a light on the occurrence of multi-drug-resistant *K. pneumoniae* isolated from chicken carcasses and Karish cheese from different local markets in Qena city, Egypt, highlighting their virulence characteristics and antimicrobial resistance profile.

Material and Methods

Sampling

A total of one hundred food samples were collected, consisting of 50 samples of raw milk "Karish" cheese and 50 samples of chicken carcasses. These samples were obtained from Qena city in Egypt during the period of January and June 2024. Each sample was clearly labeled and transported in cold, aseptic containers for bacteriological examination.

Bacteriological analysis

Each sample (25 g) was separately homogenized and enriched in buffer peptone water (225 mL) for 24 h at 37°C with shaking for microbial propagation. MacConkey agar medium (MCA: Oxoid, UK) was employed to enumerate the enriched broth inoculum following a 24-hour incubation at 37°C. Colonies exhibiting lactose fermentation were identified by their rose-pink color; dome shape and mucoid appearance were subsequently isolated on MCA. These selected colonies were then streaked onto Eosin Methylene Blue agar medium (EMB: Oxoid, UK) and incubated overnight at 37°C. From the subsequent growth, pink mucoid colonies that lacked a metallic sheen were selected for further investigation. The biochemical characterization of the isolates was performed using established protocols [20], incorporating a series of diagnostic tests: catalase and oxidase activity, IMViC assays (indole, methyl red, Voges-Proskauer, and citrate utilization), triple sugar iron agar reaction, urease activity, lysine decarboxylase test, lactose fermentation, H_2S production, motility assessment, and capsule staining.

Antimicrobial susceptibility testing (AST)

K. pneumoniae isolates were tested for antimicrobial susceptibility against 9 antimicrobial agents. The antimicrobial susceptibility testing was executed employing the disk diffusion method on Mueller-Hinton agar plates, adhering to the recommendations set forth by the Clinical and Laboratory Standards Institute [21]. The following antibiotic disks (Himedia) were used: imipenem (IMP; 10µg), meropenem (MRP; 10 µg), gentamicin (CN; 30 µg), tobramycin (TOB; 10 µg), amoxicillin (AMX; 30 µg), amoxicillin/clavulanic acid (AMC; 20/10 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), and aztreonam (AT; 30 µg). Following 24 hours of incubation at 37°C, measurements of the inhibition zones were taken and interpreted. The multiple antibiotic resistances (MAR) index was determined by dividing the total number of resistances to antimicrobials for each isolate by the total number of tested antimicrobials [22].

PCR based confirmation of K. pneumoniae strains

All suspected *K. pneumoniae* isolates obtained were subjected to PCR targeting the *K. pneumoniae 16S-23S ITS* (internal transcribed spacer) gene, which is categorized as a *K. pneumoniae*-specific gene (Tables 1, 2).

Molecular detection of K. pneumoniae virulence and antimicrobial resistance genes

PCR assays were applied for the recognition of three virulence genes of *K. pneumoniae* strains (*iutA*, *kfu*, and *rmpA* genes). The detection of four antimicrobial-resistant genes (*mcr-1*, bla_{SHV}, $bla_{CTX-M,}$ and bla_{CMY-2} genes) were also done. The amplification reaction was performed using specific primers and conditions (Tables 1 and 2). PCRs were performed in 12.5 µL of 2X PCR master mixes (Takara, Code No. RR310A), 1 µL of 20 pmol of each primer (Metabion, Germany), and 5 µL of template DNA were used in the reaction mixture, and the reaction was completed to 25 µL with nuclease-free water. Agarose gel electrophoresis was used to visualize approximately 5 µL of each PCR product, using 1.2% agarose prepared in TBE. A 100-bp DNA ladder (Qiagen, USA) was used to determine the fragment sizes. The gel was visualized by a UV gel documentation device (Cleaver Scientific Ltd., ultraviolet gel documentation system, Rugby, Warwickshire, UK).

Results

Prevalence of K. pneumoniae in contaminated food samples

The presence of *K. pneumoniae* in the examined food samples was initially detected by using cultural and biochemical-based methods. *K. pneumoniae* strains were identified in 7 out of 100 samples, resulting in a prevalence rate of 7%. Specifically, the strains were found in 12% (6 out of 50) of raw milk cheese samples and in 2% (1 out of 50) of chicken carcasses.

By applying the PCR targeting the *16S-23S ITS* gene (a *K. pneumoniae* specific gene marker), all seven suspected isolates were successfully confirmed as *K. pneumoniae* isolates (Kp1, Kp2, Kp3, Kp4, Kp5 and Kp6 from raw milk cheese and Kp7 from chicken carcass) (Figure 1), therefore validating the biochemical identification.

Antimicrobial susceptibility of K. pneumoniae

In this study, various antimicrobial resistance patterns were observed among *K. pneumoniae* isolated from different sources. The single isolate (Kp7) obtained from chicken carcass showed susceptibility to imipenem, gentamicin, tobramycin, cefotaxime, aztreonam, and meropenem, while exhibiting resistance to amoxicillin, ceftazidime, and amoxicillin-clavulanic acid.

Cheese-derived *K. pneumoniae* isolates showed a high susceptibility level to imipenem (100%), gentamicin and tobramycin (83.33%), and meropenem (66.66%). All cheese-derived isolates (100 %) exhibited a high level of resistance to amoxicillin, ceftazidime, and amoxicillin clavulanic acid, followed by cefotaxime (83.33%), as shown in Table 3. Moreover, 50% of the isolates showed resistance against aztreonam, while the others were found to be susceptible.

Nine different antimicrobial classes were tested as shown in Table 4. Among cheese-derived isolates, 16.66% (1 out of 6) showed resistance to three antimicrobial agents, 33.33% (2 out of 6) showed resistance to four agents, 16.66% (1 out of 6) showed resistance to five agents, 16.66% (1 out of 6) showed resistance to six agents and 16.66% (1 out of 6) showed resistance to eight agents. The chicken-derived isolate showed resistance against three antimicrobial agents. The most prevalent resistance pattern involved resistance to four antimicrobial agents, particularly amoxicillin, ceftazidime, amoxicillin-clavulanic acid, and cefotaxime, representing the most common resistance profile observed among the tested isolates.

Molecular identification of K. pneumoniae virulence and antimicrobial resistance genetic profile

The PCR results showed distinct virulence and antimicrobial resistance profiles among the *K. pneumoniae* isolates, as detailed in table 5 and illustrated in figures 2-3. The most prevalent genes identified were *iutA* and bla_{SHV}, both present in 100% of the isolates, followed by *kfu* (85.7%) and *rmpA* and *mcr-1* (71.43%). In addition, bla_{CTX-M} was found in 28.6%, while bla_{CMY-2} was not detected at all, as shown in table 5.

All isolates harbored various combinations of resistance (*mcr-1*, bla_{SHV}, bla_{CTX-M}, and bla_{CMY-2}) and

virulence determinants (*iutA*, *kfu*, and *rmpA*), highlighting their dual threat potential. Molecular analysis revealed that one strain harbored three genes (*iutA*, *mcr-1*, and bla_{SHV}), and two strains carried four genes either (*iutA*, *kfu*, *mcr-1*, and bla_{SHV}) or (*iutA*, *kfu*, *rmpA*, and bla_{SHV}). Moreover, three strains carried five genes, with two strains having *iutA*, *kfu*, *rmpA*, *mcr-1*, and bla_{SHV}, and one strain had *iutA*, *kfu*, *rmpA*, $bla_{CTX-M,}$ and bla_{SHV}.

Only one strain carried all six tested genes (*iutA*, *kfu*, *rmpA*, *mcr-1*, bla_{CTX-M}, and bla_{SHV}). Among the 6 strains isolated from raw milk cheese, the genes (*iutA*, *kfu*, and bla_{SHV}) were identified in all of them (100%), followed by the *rmpA* gene (88.33%), the *mcr-1* gene (66.66%), then the bla_{CTX-M} gene (33.33%), while the bla_{CMY-2} gene was not detected in any of them. The single chicken-derived strain carried three genes, including *iutA*, *mcr-1*, and bla_{SHV}, while the other three tested ones (*kfu*, *rmpA*, and bla_{CMY-2}) were not absent.

Discussion

Klebsiella spp. originates not only from fecal contamination but also from various environmental sources [23]. Ensuring food safety and protecting public health require controlling the growth of contaminating microflora. Key factors ensuring food safety and influencing its shelf life and quality include the microbiological quality of raw materials, strict hygiene during manufacturing, and appropriate storage conditions [24]. In this study, *K. pneumoniae* strains were isolated from various food types that are commonly consumed (Karish cheese, and chicken meat). Overall, the raw milk Karish cheese has a higher prevalence (12%) of *K. pneumoniae* than chicken carcasses (2%), likely due to bacterial contamination from the udder and teats. In another study, *K. pneumoniae isolates* were identified in 6.4% (16 out of 234) of examnined milk and dairy product samples in Libya [25]. Additionally, *K. pneumoniae* was detected in Maasora cheese (19%), Ricotta cheese (7.6%), and imported soft cheese (16.6%), highlighting variability in contamination levels across different cheese types.

Klebsiella contamination with varying prevalence rates in food samples has been previously reported from China. For instance, *Klebsiella spp.* was identified in 96% of raw chicken samples [12], while in retail chicken meat, it was detecetd in 22.2% of samples (lower contamination rate) [26]. A higher rate of *Klebsiella* contamination (40% of samples) was found in raw milk samples, than observed in our study [27]. Gelbíčová et al. [28] detected *Klebsiella* spp. in 43% of tested samples, including swabs from cheese processing environments, personnel, and raw materials.

It is well known that *Klebsiella* spp., particularly *K. pneumoniae*, poses a significant global public health concern, as it is a leading cause of both hospital- and community-acquired infections due to its virulence factors and antibiotic resistance traits [29, 30].

Regarding antibiotic resistance profile, *Klebsiella spp*. exhibit intrinsic resistance to penicillin and certain cephalosporins due to the production of chromosomally encoded β-lactamases [31]. Recently, Extended spectrum beta-lactamase (ESBL)-producing *Klebsiella* spp. have increased dramatically among clinical isolates, posing a major threat to both human and animal health [32, 33].

According to Gelbíčová et al., none of the *Klebsiella* isolates detected was found to be resistant to cefotaxime, gentamicin, aztreonam, amoxicillin/clavulanic acid, or meropenem [28]. Contrastingly, another study revealed the presence of ESBL- producing *K. pneumoniae* in 26.3% of retail cheese samples [34], with 70% gentamicin-resistant strains. Azwai et al. reported *K. pneumoniae* isolates that were susceptible to levofloxacin, ciprofloxacin, amikacin, gentamicin, ertapenem, and chloramphenicol [24]. Resistance rates to cefotaxime and ceftazidime were 55% and 67.5%, respectively [27], which are notably lower than those recorded in our study.

A study conducted in Egypt reported that *K. pneumoniae* strains exhibited full susceptiblity to gentamicin (100%) [35], closely similar to our findings (83.33% susceptiblity). While 96.25% of chicken-meat derived *Klebsiella* isolates showed multidrug resistance (MDR) to cefotaxime, ceftriaxone, and aztreonam [26]. In another report, the same resistance pattern was observed in only 3.8% of the isolates [12]. In another study, the resistance of *K. pneumoniae* strains isolated from cow milk to gentamycin was 46% [36]. Such discrepancies in antibiotic resistance patterns can be contributed to geographical variations, treatment regimens, and antibiotic usage measures.

In addition to their resistance profiles, *K. pneumoniae* can be classified into classical (cKP) and hypervirulent (hv-KP) phenotypes [37]. Those hv-KP strains were first described in Asia from severe infections, including liver abscesses and meningitis. Such a hypervirulent phenotype is usually associated with increased capsule production and mucoviscosity, mediated by the rpmA gene, contributing to the hypervirulent phenotype [6].

Aerobactin and its transporter *IutA* are major virulence factors, responsible for increased siderophore production in hvKP compared to cKP [7, 38]. The gene *kfu* encodes an iron transportation system and, therefore, is involved in iron acquisition by *K. pneumoniae*. In a previous study, the *rpmA* gene was found to be absent in all *K. pneumoniae* tested [28]. The results reported by Cheng et al. [39] demonstrated that the *kfu* gene was frequently

detected in >50% of isolates, while aerobactin was detected in 39% and *rmpA* in 23%.

The CTX-M and SHV families are the most prevalent and clinically significant ESBL enzymes, with CTX-M enzymes emerging as the dominant type globally [40]. In the present study, SHV enzymes were the predominant type, being detected in 100% of the tested isolates. Other studies reported prevalence of CTX-M and SHV genes in *K. pneumoniae* isolates and found 62.5% and 42.5%, respectively [27], higher for CTX-M but lower for SHV compared to our findings. The SHV genes (97.1%) were previously found to be markedly more frequent than CTX-M genes in raw milk isolates (57.1%) [41]. In another study, the distribution of bla_{CTX-M} among ESBL-producing isolates was 30.8%, while no isolates were positive for bla_{SHV} and bla_{CMY} [26]. The ESBL producing strains of *Klebsiella spp.* were also frequently described from chicken meat sources [12]. The significant variations in the CTX-M and SHV gene frequencies across the different studies may be likely due to regional variations, isolation sources and sample types, and methodology.

Generally, the prolonged and excessive use of antibiotics, either to treat livestock diseases or as growth promoters, can significantly contribute to the emergence and spread of antibiotic-resistant bacteria. Consequently, the potential introduction of these resistant bacteria into the food chain warrants serious concern and attention [13]. Moreover, the surveillance and analysis of *Klebsiella spp.* originating from diverse food sources are crucial for understanding their role in human colonization and infection. Furthermore, assessing their virulence factors and patterns of multi-drug resistance is essential for developing effective strategies to combat these pathogens.

Conclusion

The emergence of resistance genes among different bacterial species, including commensal bacteria in humans and animals could be a critical factor of possible health pandemics that need to be mitigated. Thus, the limitation of the spread of antibiotic resistance genes in clinical and foodborne isolates are crucial. As a result, the robust surveillance and assessment of MDR bacteria, with a special focus on β-lactam resistance, in various food sources are essential for anticipating and mitigating potential public health risks associated with MDR infections. Moreover, enhancing knowledge of best hygiene practices is crucial, particularly at small-scale local production levels throughout the phases of production, handling and distribution.

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Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study was ethically approved by Research Ethics Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt (Code No: 06/2024/0282).

TABLE 1. Oligonucleotide primers sequences of *K. pneumoniae*.

Gene	Sequence	Amplified product	Reference
K. pneumoniae 16S-23S ITS	ATTTGAAGAGGTTGCAAACGAT TTCACTCTGAAGTTTTCTTGTGTTC	130 bp	[42]
rmpA	ACTGGGCTACCTCTGCTTCA CTTGCATGAGCCATCTTTCA	535 bp	[43]
iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300 bp	[44]
kfu	GAAGTGACGCTGTTTCTGGC TTTCGTGTGGCCAGTGACTC	797 bp	[35]
mcr-1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	308 bp	[45]
bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392 bp	[46]
bla_{CMY-2}	TGGCCAGAACTGACAGGCAAA TTTCTCCTGAACGTGGCTGGC	462 bp	[47]
bla_{CTX-M}	ATGTGCAGYACCAGTAARGTKATG GC TGGGTRAARTARGTSACCAGAAYCAGC GG	593 bp	[48]

TABLE 2. Cycling conditions of the primers during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
K. pneumoniae 16S-23S ITS	94˚C/5 min.	94˚C/30 sec.	55˚C/30 sec.	72˚C/30 sec.	35	72˚C/10 min.
rmpA	94˚C/5 min.	94˚C/30 sec.	50˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.
iutA	94˚C/5 min.	94˚C/30 sec.	63˚C/30 sec.	72˚C/30 sec.	35	72˚C/10 min.
kfu	94˚C/5 min.	94˚C/30 sec.	55˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.
mcr-1	94˚C/5 min.	94˚C/30 sec.	60˚C/40 sec.	72˚C/40 sec.	35	72˚C/10 min.
bla_{SHV}	94˚C/5 min.	94˚C/30 sec.	54˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.
bla_{CMY-2}	94˚C/5 min.	94˚C/30 sec.	55˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.
bla_{CTX-M}	94˚C/5 min.	94˚C/30 sec.	54˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.

TABLE 3. Antimicrobial resistance profile of *K. pneumoniae* isolates.

Antibiotics	Antimicrobial classes	Resistant		Sensitive	
		No.	%	No.	%
Amoxicillin	Aminopenicillin	7	100	0	0.00
Amoxicillin/clavulanic acid	Aminopenicillin	7	100	0	0.00
Ceftazidime	3rd generation cephalosporin	7	100	0	0.00
Cefotaxime	3rd generation cephalosporin	5	71.4	2	28.6
Aztreonam	Monobactam	3	42.9	4	57.1
Imipenem	Carbapenem	0	0.00	7	100
Meropenem	Carbapenem	2	28.6	5	71.4
Gentamicin	Aminoglycosides	1	14.3	6	85.7
Tobramycin	Aminoglycosides	1	14.3	6	85.7

TABLE 4. Antibiogram-resistant patterns and MAR index of *K. pneumoniae.*

*Antibiotypes	Resistance pattern	MAR index	Isolates	
			No.	%
AB-1	AMX, AMC, CAZ, CTX	0.44	2	28.6
AB-2	MRP, AMX, AMC, CAZ, CTX, AT	0.66	1	14.3
AB-3	MRP, GEN, TOB, AMX, AMC, CAZ, CTX, AT	0.88	1	14.3
AB-4	AMX, AMC, CAZ, CTX, AT	0.55	1	14.3
AB-5	AMX, AMC, CAZ	0.33	2	28.6

*Different antibiotypes (AB) from AB-1 to AB-5.

TABLE 5. Pattern of virulence and antimicrobial resistance genes of *K. pneumoniae* isolates.

Sample source	Code	Virulence genes			Antimicrobial resistance genes			
		kfu	*rmpA*	*iutA*	*mcr-1*	bla_{CMY-2}	bla_{CTX-M}	bla_{SHV}
Raw milk Karish	Kp1	+	+	+	+	-	-	-
cheese	Kp2	+	+	+	+	-	+	-
	Kp3	+	+	+	+	-	-	+
	Kp4	+	+	+	+	-	+	+
	Kp5	+	+	+	+	-	-	+
	Kp6	+	-	+	+	-	-	+
Chicken carcass	Kp7	+	-	-	+	-	-	+
Total no.		7	5	6	7	0	2	5
%		100	71.43	85.71	100	0	28.57	71.43

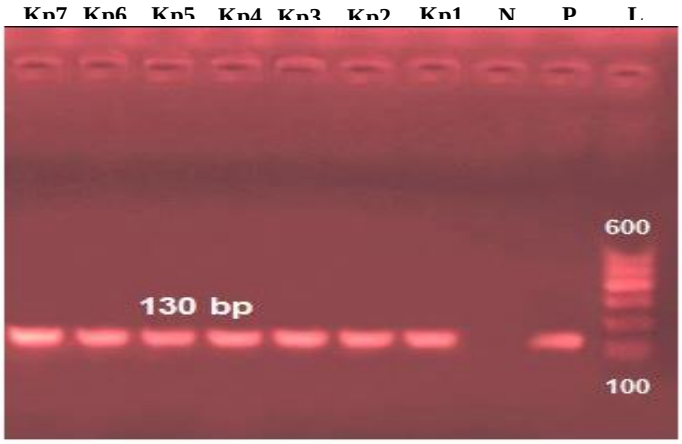
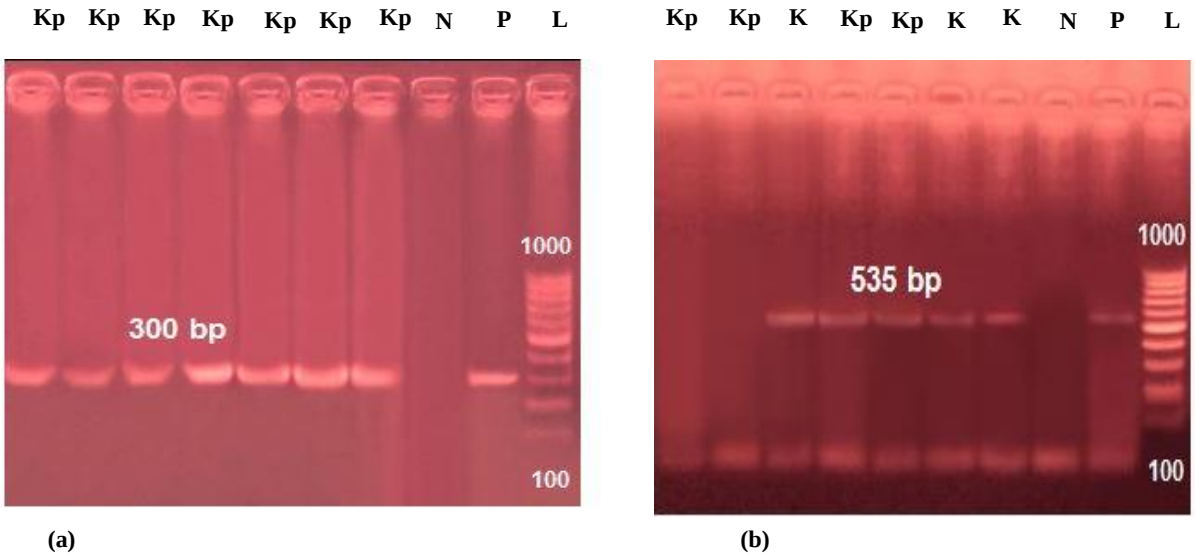
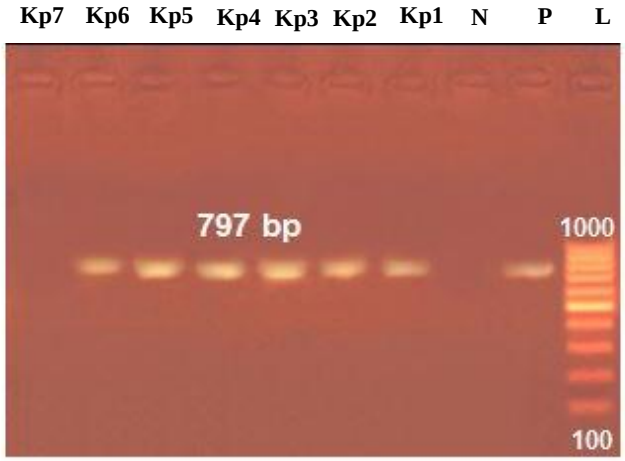


Fig. 1. PCR analysis of *16S-23S ITS* gene of *K. pneumonia* (marker). L, DNA ladder (100bp); P, positive Control; N, negative Control.



(a) **(b)**



(c)

Fig. 2. PCR analysis of *K. pneumoniae* different virulence genes: (a) *iutA* gene; (b) *rmpA* gene; (c) *kfu* gene. L, DNA ladder (100bp); P, positive Control; N, negative Control.

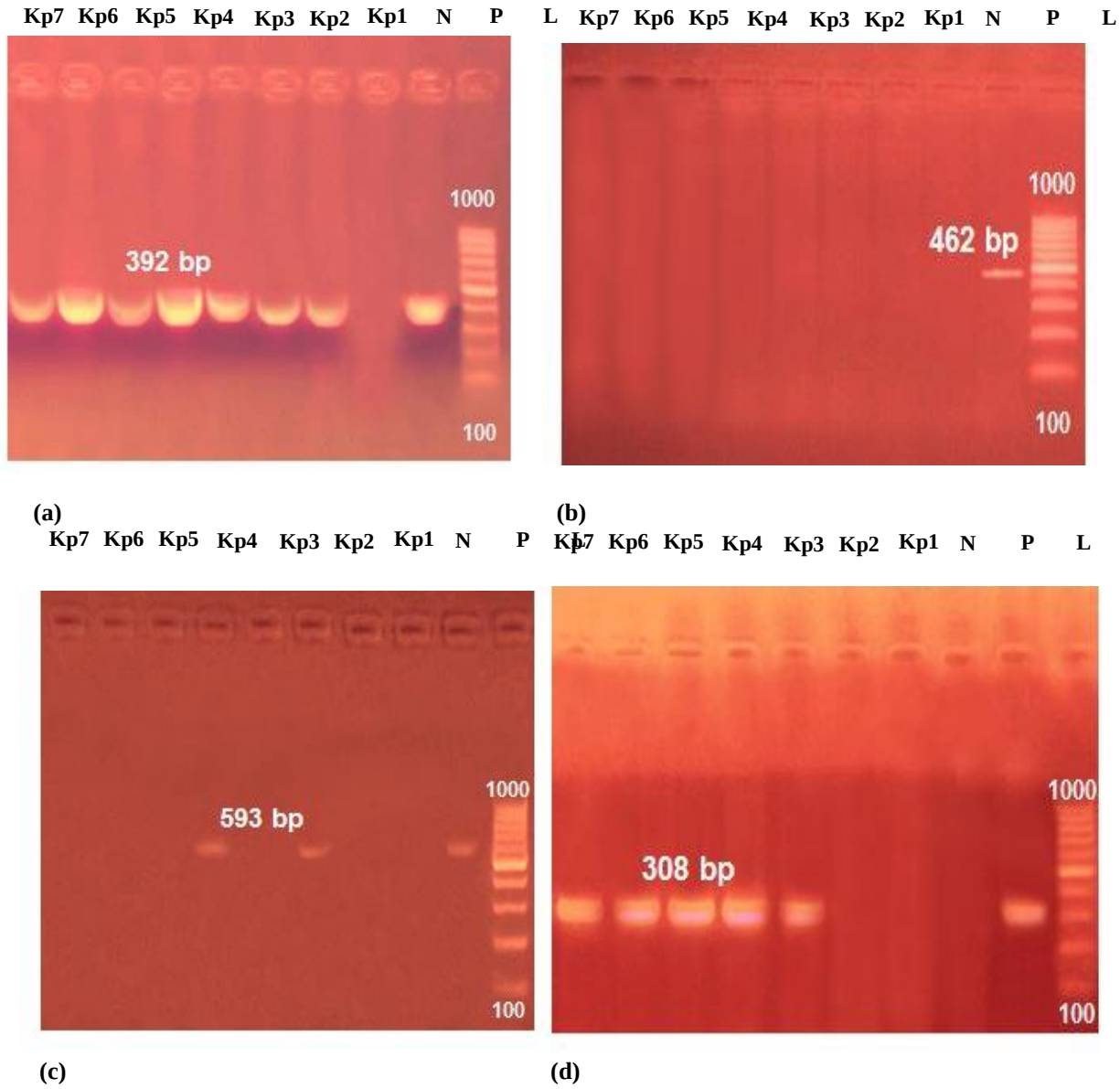


Fig. 3. PCR analysis of *K. pneumoniae* antimicrobial resistant genes: (a) bla_{SHV} gene; (b) bla_{CMY-2} gene; (c) bla_{CTX-M} gene; (d) *mcr-1* gene. L, DNA ladder (100bp); P, positive Control; N, negative Control.

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دراسة القدرة الإمراضية ومقاومة المضادات الميكروبية لعزلات الكليبسيلا *الرئوية* المعزولة من جبن "قريش" وذبائح الدجاج المباعة في مدينة قنا، مصر

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الملخص

تُعد *الكليبسيلا الرئوية* من المسببات الرئيسية للعدوى المقاومة للمضادات الميكروبية في البشر، مما يشكل خطرًا كبيرًا على الصحة البشرية والحيوانية عالميًا. لذلك صُممت هذه الدراسة لتحديد الصفات الإمراضية ومقاومة المضادات الميكروبية لعزلات *الكليبسيلا الرئوية* المعزولة من ذبائح الدجاج وجبن القريش المصنوع من اللبن الخام في مصر. تم فحص مائة عينة غذائية (٥٠ عينة من كل نوع) للكشف عن وجود *الكليبسيلا الرئوية* .تم تأكيد سبع عزلات فقط على أنها *الكليبسيلا الرئوية* باستخدام الاختبارات الكيميائية الحيوية. أُجري تفاعل البوليميراز المتسلسل (PCR) للكشف عن جينات الإمراضية (iutA, kfu, rmpA) وجينات مقاومة المضادات الميكروبية (*mcr-1*, bla_{SHV}, bla_{CTX-M}, bla_{CMY-2}). كانت نسبة العينات الإيجابية ٧%، حيث عُزلت عزلة واحدة من ذبائح الدجاج وست عزلات من جبن القريش. أظهرت جميع العزلات مقاومة للأموكسيسيلين، السيفتازيديم، والأموكسيسيلين-كلافولانات، بينما كانت حساسة للإيميبينيم، الجنتاميسين، التوبراميسين، السيفوتاكسيم، الأزترونام، والميروبينيم. أظهرت عزلات جبن القريش حساسية بنسبة ١٠٠% للإيميبينيم، و٨٣,٣٣% للجنتاميسين والتوبراميسين، بينما كانت مقاومتها ١٠٠% للأموكسيسيلين، السيفتازيديم، والأموكسيسيلين- كلافولانات. كان الجين *iutA*الأكثر انتشارًا (١٠٠%)، يليه (85.7%) *kfu* . حملت جميع العزلات جين bla_{SHV}، بينما عُثر على خمس عزلات تحمل (mcr-1) وعزلتين تحملان bla_{CTX-M}، ولم يُكتشف (bla_{CMY-2}) في أي عزلة. تثير نتائجنا مخاوف جادة نظرًا لأن *الكليبسيلا الرئوية* يشكل خطرًا على الصحة العامة، حيث قد يؤدي استهلاك هذه المنتجات إلى نقل سلالات مقاومة إلى البشر عبر السلسلة الغذائية، مما يستدعي تشديد اللوائح لضمان سلامة الغذاء.

الكلمات الدالة: سلامة الغذاء، *الكليبسيلا الرئوية*، الإمراضية، مقاومة المضادات الميكروبية.