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Prevalence of Hyper Virulence and Multidrug Resistance of *Klebsiella pneumoniae* Isolates from UTI Patients

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Abstract

Urinary tract infections (UTIs) are the most common bacterial infections and are often caused by *Klebsiella pneumoniae*. During the period from first of November 2022 to the February 2023,a total of 300 specimens were collected in Baqubah Teaching Hospital and Albatool Hospital to investigate the prevalence of bacterial isolates among patients.The detection results of some virulence factors isolates of *K. pneumoniae* showed negative result to hemolysin production were tested by using blood agar with ratio (100%). Capsule production was also investigated using nigrosin stain followed by direct microscopic examination and it was found that 28 (93.3%) of *K. pneumoniae* isolates were surrounded by a capsule as well as (100%) biofilm formers. 86.6%, 77% and 70% of the isolates were siderophors, protease and coagulase were giving positive results respectively. Biofilm production was tested using micro-titer plate (MTP) method. The results showed 16 (53%) of *K. pneumoniae* were biofilm-former isolates . The resistance to antibiotics was done and the results illustrated that the resistance toward ticarcillin-clavulante, cefotaxime, ceftriaxone and ciprofloxacin was 100%. While the resistance toward tetracycline and trimethoprime /sulfamethazole was 62% and streptomycin and norfloxacine was 51.7% and 48.2% respectively, additionally, the resistance towards gentamicine was 44.8%, while impenem and meropenem were 37.9% and 6.8% respectively.

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Resistance patterns for the isolates *K. pneumoniae* was also calculated and 16(53%) isolates were MDR which includes isolates that have resisted three groups of antibiotics and the 14(47%) was XDR which resisted four or five groups of different antibiotics. The minimum inhibitory concentration (MIC) was determined for a total of 30 isolates of *K. pneumoniae*. Ceftazidime and cefepime were used as antimicrobial agents, and the measurements were conducted on Muller-Hinton agar. Serial dilutions were generated, and the resulting MIC values were found to be 512 and 1024µg/mL, respectively. The production of extended-spectrum beta-lactamases (ESBLs) was assessed through the utilization of the double disc synergy test. Out of the total isolates of *K. pneumoniae*, 18 (60%) were identified as ESBL producers, while the remaining 12 (40%) were found to be non-producers. Molecular detection was carried out to detect some virulence genes in *K. pneumoniae*. Polymerase chain reaction was used to detect the adhesion gene *fimH, r*esults showed that (75%) of *K. pneumoniae* isolates had *fimH, rmp*A gene which is responsible for capsule formation and it was found in (66%) of *K. pneumoniae*, according to *bla*- TEM gene encoding ESBL, (71.2%) *K. pneumoniae* isolates had *bla*-TEM gene.

Keywords: *K. pneumoniae*, *FimH*, rmpA, *bla* TEMgenes, Biofilm formation, extended spectrum Beta-Iactamase (ESBL) production, siderophore production.

Introduction

Klebsiella pneumonia is a type of bacterium that is capable of surviving in both aerobic and anaerobic environments. It is classified as a Gram-negative bacterium and possesses a protective outer layer known as a capsule. Additionally, *K. pneumoniae* has the ability to ferment lactose. Due to its potential to cause severe infections and life-threatening conditions, this opportunistic pathogen continues to be a significant issue in the field of public health. *K. pneumoniae* is frequently identified as a causative agent in nosocomial infections, encompassing conditions such as bacteremia, UTIs, pneumonia, pyogenic liver abscesses, burn-related infections, and wound infections. Presently, *K. pneumoniae* exhibits a notable level of resistance to a wide range of pharmaceutical agents, including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides [1, 2] . The emergence of resistance has led to a global

issue with the selection of appropriate antibiotic therapy for illnesses acquired in healthcare settings [3]. The beta-lactam group encompasses a range of antibiotics that are widely administered on a global scale. This category includes penicillins, cephalosporins, monobactams, and carbapenems [4]. The primary signs of b-lactam antibiotic resistance are the formation of b-lactamase enzymes through the presence of b-lactam-insensitive cell wall transpeptidases, or the active ejection of b-lactam molecules from gram negative bacteria [5]. According to[6], carbapenems are considered the preferred beta-lactam antibiotics for the management of infections caused by bacteria that produce extended-spectrum beta-lactamase (ESBL), including *K. pneumoniae*. According to.[7], these antibiotics are also regarded as the final option for addressing severe healthcare-associated infections that pose a threat to life. Regrettably, there has been a notable rise in bacterial resistance to carbapenems, a phenomenon that has been extensively documented[8, 9]. This resistance has been further compounded by the emergence of various mechanisms, including the production of beta-lactamases, efflux pumps, and genetic mutations that modify the expression and/or functionality of porins and penicillin-binding proteins (PBPs) [10, 11]. The pathogenicity of *K. pneumoniae* is attributed to several virulence factors, namely the capsule, lipopolysaccharide (LPS), fimbriae (specifically types 1 and 3), and siderophors. *K. pneumoniae* strains have the ability to produce capsules belonging to various serotypes, ranging from K1 to K78. Notably, serotypes K1 and K2 have been found to exhibit heightened pathogenicity, as reported by [12]. This study was conducted to investigate the distribution of virulence genes and drug resistance among *K. pneumoniae* isolates from patients with UTIs in Baqubah city.

Materials and Methods

Patients and Specimens Collection

A case control study was conducted in Baqubah and Albatool Teaching Hospitals, during the period extended from the first of November 2022 to the end February 2023. A total of 300 urine specimens were collected from patients clinically. The ethical approval was given by the Medical Research at Baqubah Health Directorate ethical committee prior to the collection of the samples. The researchers ensured the preservation of patient privacy by maintaining

participant confidentiality and refraining from obtaining or utilizing identifying private data in the study. The identification of the bacteria as *K. pneumonia* was established through the examination of many factors, including the morphology observed in Gram's staining, the characteristics of the colonies grown on MacConkey agar (Oxoid, UK) and Hi chromo agar, and the biochemical properties as described by Macfideen [13]. The laboratory employed a set of standard biochemical tests, including oxidase, catalase, coagulase, sugar fermentation, motility, indol production, methyl red, Voges-Proskauer reaction, urease production, citrate utilization, and capsule detection. Additionally, the identification was confirmed using the VITEK compact system2.

Biofilm Formation: Sterile 96-well flat-bottomed tubes filled with 200 L of bacterial culture were inoculated with Tryptic soy broth (Hi Media India), the plate was incubated for 24 hours at 37°C. Afterwards, each well was drained, rinsed with 250 µl of sterile physiological saline, and then dried. Next 0.2 µl of 2% crystal violet solution was added to the plates and incubated for 5 minutes. Water was used to wash away any remaining stain in the plate. After that, the plates were dried by air, and the dye that had been bound to the adhering cells solubilized with 160 µl of 33% (v/v) glacial acetic acid. ELISA reader, and OD of each well measured at 650 nm [14].

Phenotypic detection of Hemolysis test

The activated isolates were cultured by streaking them onto the surface of blood agar plates. Subsequently, the plates were incubated at a temperature of 37°C for a duration of 24 hours. Following incubation, the types of hemolysis were seen and meticulously documented.

Detection of Siderophors

The detection of siderophors synthesis in bacteria was conducted using a universal Chrome Azurol S (CAS) agar medium, which was prepared according to the method outlined by [15]. The Malt Extract agar medium containing Chromo Azurol S (CAS), also known as blue agar, was injected with bacteria that were 24 hours old. The plate was then incubated at a temperature of 30 degrees Celsius for a duration of 72 hours. According to.[16], the presence of a yellow to

light orange halo surrounding the colony or a shift in the medium's color from blue to orange signifies the formation of siderophore.

Antimicrobial Susceptibility Testing. The examination and assessment of antimicrobial susceptibility of isolates were conducted in accordance with the standards set by the Clinical and Laboratory Standards Institute (CLSI 2020) using the disc diffusion method [11]. In this study, antimicrobial discs (Mast Group, Merseyside, UK) containing various antibiotics, namely tetracycline (TE, 5 µg), meropenem (MEM, 10 µg), imipenem (IMP, 10 µg), trimethoprim/sulfamethoxazole (STX, 5 µg), streptomycin (S, 10 µg), gentamicin (GEN, 5 µg), norfloxacin (NOR, 10 µg), ciprofloxacin (CIP, 5 µg), ticarcillin clavnic (TTC, 10 µg), cefepime (FEP, 30 µg), ceftriaxone (CRO, 30 µg), and cefotaxime (CTX, 30 µg), were employed. The MIC was determined for a total of 30 isolates of *K. pneumoniae*.

Detection of Extended spectrum β-lactamases production

The double disc synergy test (DDST) was utilized to detect the prevalence of ESBL synthesis in *K. pneumoniae* isolates as previously reported [17].

Detection of some virulence Genes by PCR.

The DNA extraction kit manufactured by Roche in Germany was utilized to extract genomic DNA from individual bacterial colonies cultured on LB culture plates, following the instructions provided by the manufacturer. PCR analysis was conducted to identify virulence genes (fimH and rmpA) using the specific primers in Table 1. PCR was performed using a reaction solution with a total volume of 25 µl. The solution consisted of 12.5 µl of 2× Master Mix (SinaClon, Iran, CAT), 1 µl of 10 pmol of each primer, 2 µl (20 ng) of DNA template, and 8.5 µl of sterile distilled water. The amplification reactions were conducted using a thermal cycler (Eppendorf, MasterCycler Gradient, Germany). A denaturation process was performed at 94°C for 5 minutes during the initial stage. Subsequently, 36 denaturation cycles were conducted at 94°C for 45 seconds. Following this, the subsequent step involved annealing at 54-65°C for 45 seconds. Subsequently, an extension step was performed at 72°C for 45 seconds, followed by a final one at 72°C for 5 minutes. To identify genes associated with ESBLs, a

targeted primer was employed to examine the presence of ESBL-encoding genes, specifically *bla*TEM, as outlined in Table 1. The agarose solution was cooled to approximately 50°C, following which 20µl of ethidium bromide was added, mixed, and agitated. The resulting mixture was poured into a gel tray, and combs were subsequently inserted. Once the gel had solidified, the comb was extracted. During the electrophoresis process, the gel was positioned horizontally within an electrophoresis apparatus filled with TBE buffer and ethidium bromide. The electrophoresis experiment was conducted using a voltage of 100 volts for 35 minutes [18].

Table 1: Primer sequencing of some virulence genes of *K. pneumoniae* isolates

Genes	Primer	bp	References
rmpA	ACTGGGCTACCTCTGCTTCA CTTGCATGAGCCATCTTTCA	535	[19]
fimH-1	TGCTGCTGGGCTGGTCGATG GGGAGGGTGACGGTGACATC	550	[20]
blaTEM	TGCGGTATTATCCCGTGTTG TCGTCGTTTGGTATGGCTTC	296	[21]

Result and Discussion

Isolation of *K. pneumoniae*

The result of the present study, a total of 30 *K. pneumoniae* were isolated from a total of 300 midstream urine samples collected from UTIs patients admitted to Al Batool and Teaching Hospital in Baqubah city center of Diyala province during the period From 1 st of November 2022 to the 28th February 2023 . In total samples, 220 (73.3%) showed positive growth, whereas the remaining 80 (26.6%) showed negative growth, as shown in Figure 1 A. Following the completion of morphological, microscopic, and biochemical testing as well as the application of the Vitek 2 System to confirm the diagnosis, 30(13.6%) *K. pneumoniae* isolates were obtained out of 220 positive growths while 190 (86.4%) isolates were from other bacterial species as shown in Figure 1B

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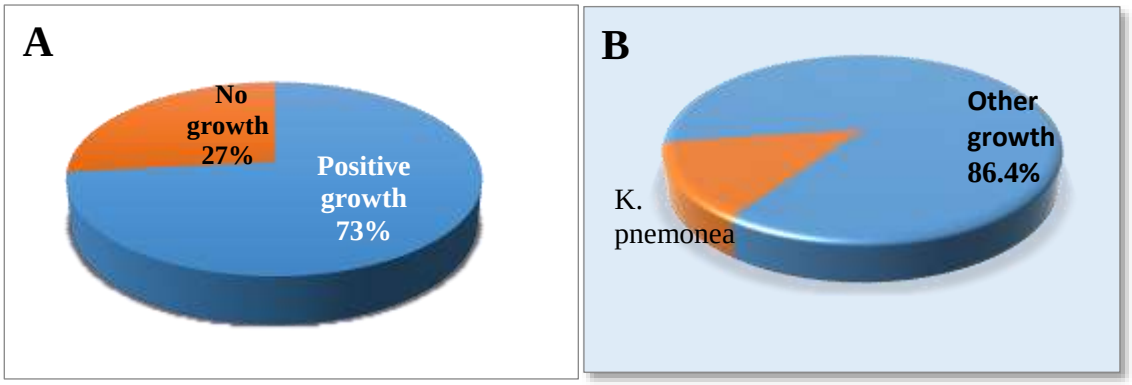

Figure 1: Percentage of growth across all samples: A. samples of positive and negative growths; B. The proportion of *K. pneumoniae* from the 220 positive growths.

Microscopic examination of *K. pneumoniae* showed a negative gram reaction, having rods appearance singly, in pairs or short chains, as mentioned in Levinsoni *et al*.,[22]. Morphological characteristic; the colonies of *K. pneumoniae* isolates appeared on blood agar, transparent, shiny and unable to hemolysis blood agar, while on MacConkey agar, a pink and mucoid due to lactose fermenter. *K. pneumoniae* grew as pink to purple colonies, dark-centered, mucoid colonies indicating lactose fermentation and acid production, as shown in Figure 2. The identification of the isolates was conducted using biochemical assays. As presented in Table 2, the results indicated that the isolates had positive reactions for catalase, citrate utilization, urease, and Voges-Proskauer. While it was negative for Indole ring production, oxidase, and methyl red, Although, , it could not liquefy gelatin, it could ferment lactose sugar. This bacteria rod is gram-negative and non-motile, as summarized in Table 2.

A **B** **C**

Figure 2: *K. pneumoniae* on A: Hichromo agar, B MacConkey agar, C: blood agar

Table 2: The Biochemical Characterization of *K. pneumoniae*

Biochemical test	Reaction
Gram stain	-
Motility test	-
Oxidase test	-
Catalase test	+
Indole production test	-
Methyl red test	-
Voges proskauer test	+
Citrate utilization test	+
H_2S production test	-
Urea hydrolysis (urease test)	+
Gelatin liquefaction test	-
Triple sugar iron	A/A
Lactose fermentation test	+

Detection of virulence factors of *K. pneumoniae*

Sheep blood agar was used to test isolates' ability to lyse red blood cells; produce hemolysin. The results as illustrated in Table 2, that all of the 30 (100%) of *K. pneumoniae* isolates were not able to hemolysis blood (ɣ-hemoysis). Hemolysin is an exotoxin (cytolysis toxin) which cause lysis of blood cells and therefore facilitate the dissemination of bacteria [23]. It was observed that 28 (93.3%) of *K. pneumoniae* isolates had capsules indicated by a clear halo surrounding the dyed cells while 2 (6.6%) did not clear any halos and were considered as negative false result to produce a capsule because the capsule is a key virulence factor that renders hyper virulence *K. pneumoniae* (hvKp) resistant to the complement system and to phagocytosis [24]. The primary factor influencing the pathogenicity of *Klebsiella* is the presence of capsular polysaccharides[25]. The existence of about 77 distinct capsular serotypes. *K. pneumoniae* strains have been documented and classified as serotypes K1 and K2 identified as the most pathogenic in murine models [26]. The majority of *K. pneumonia* strains possess K markers, with K1 and K2 variants exhibiting a notably higher resistance to phagocytosis compared to non-K1/K2 isolates [27, 28]. The findings indicated that all isolates of *K. pneumoniae* had a 100% rate of urease production. Urease, an enzyme involved in ureolysis, plays a significant role as a virulence factor in numerous bacterial species, enabling the establishment of successful infections. The ammonium produced through the process of ureolysis serves as both a nitrogen source for bacteria and as a means of neutralising acidity, so

safeguarding the bacteria against potential harm caused by acid stress [29]. The synthesis of urease is considered a significant virulence factor in bacterial pathogenicity due to the alkaline pH elevation caused by the ammonia generated by this enzyme. This pH alteration holds crucial medicinal consequences. Urease is a virulence determinant present in pathogenic bacteria responsible for inducing gastric ulcers, urinary stone development, pyelonephritis, and various other human health complications. [30]. The findings of our study indicate that a significant proportion, specifically 77%, of the *K. pneumoniae* isolates examined exhibited the ability to manufacture protease enzymes. In a separate investigation conducted by [31], it was observed that a significant proportion of *K. pneumoniae* strains, specifically 90%, were found to exhibit protease production. Protease enzymes are a class of proteolytic enzymes that catalyze the hydrolysis of peptide bonds inside proteins, forming shorter polypeptides and amino acids. Proteases, known by various names such as peptidase, proteinase, or proteolytic enzymes, can catalyse the hydrolysis of peptide bonds inside proteins [32]. The results showed that 70% of the isolates under study produced gelatinase enzyme and this result in agreement with [31], which was found 86% of *K. pneumoniae* producing for this enzyme. Gelatinases are specific proteases that hydrolyze gelatin through a two way process, first to polypeptides, then subsequently to amino acids especially of the alpha kind [33]. The results of the present study showed that 86.6% of *K. pneumoniae* isolates have the ability to produce Siderophors and this result was compatible with [31], which was detected 73.3%. Pathogenic bacteria require iron for their replication. *Klebsiella pneumoniae* produce siderophors to gain iron from host iron-chelating proteins or the terrain for survival and reduplication during mammalian infection. Following this, distinct outer membrane receptors acknowledge the iron siderophors complexes that facilitate the transportation of the matching material to the periplasm. In the periplasm, the siderophores bind with periplasmic proteins, enabling their transportation to the inner membrane [34, 35]. Prior research has demonstrated that hypervirulent *K. pneumoniae* (hvKP) has a notable 6- to 10-fold augmentation in siderophore synthesis compared to classical *K. pneumoniae* (cKP) [36, 37].

Extended-Spectrum β-Lactamase (ESBL) production

The isolates underwent testing for ESBL enzyme production by utilizing the double disc synergy test (DDST). The study indicated that 18 isolates (60%) were identified as ESBL producers. In contrast, the remaining 12 isolates (40%) did not exhibit the production of this particular enzyme, as illustrated in Table 3. A prominent mechanism of antibiotic resistance in *K. pneumoniae* involves the production of ESBLs through plasmid-mediated means. These enzymes render β-lactam antibiotics, such as Cephalosporin's and monobactams, ineffective by catalyzing the hydrolysis of their β-lactam ring [38]. The current findings are inconsistent with a study conducted in Iran by [20], where they reported an 84.6% prevalence of ESBL production. However, our results align with the findings of.[39], who observed that 40.8% of their isolates exhibited ESBL production. Furthermore, it is worth noting that most clinical strains of *K.pneumoniae* exhibit a diverse range of beta-lactamase enzymes, including ESBLs, AmpC beta-lactamases, and carbapenemases. These enzymes play a significant role in the emergence and dissemination of drug-resistant strains globally [40].

Table 3: Virulence factors of *K. pneumoniae*

Isolates	ESBL	Hemolysin	Capsule	Motility	Urease	Gelatinase	Siderophore	Coagulase	Protase
K1	+	-	+	-	+	-	+	-	+
K2	+	-	+	-	+	+	+	-	+
K3	+	-	+	-	+	-	-	-	+
K4	+	-	+	-	+	-	+	-	+
K5	+	-	-	-	+	+	+	-	+
K6	+	-	+	-	+	+	+	-	+
K7	+	-	+	-	+	+	+	-	+
K8	+	-	+	-	+	-	+	-	+
K9	-	-	+	-	+	+	+	-	-
K10	+	-	+	-	+	-	+	-	+
K11	-	-	+	-	+	+	+	-	+
K12	-	-	+	-	+	-	+	-	-
K13	+	-	+	-	+	+	+	-	+
K14	-	-	+	-	+	-	+	-	+
K15	-	-	-	-	+	+	+	-	+
K16	+	-	+	-	+	+	+	-	+
K17	+	-	+	-	+	+	+	-	+
K18	-	-	+	-	+	+	+	-	+

K19	+	-	+	-	+	-	+	-	+
K20	+	-	+	-	+	+	+	-	-
K21	+	-	+	-	+	+	+	-	+
K22	-	-	+	-	+	+	+	-	-
K23	+	-	+	-	+	+	+	-	+
K24	-	-	+	-	+	-	+	-	-
K25	+	-	+	-	+	+	+	-	+
K26	-	-	+	-	+	+	-	-	+
K27	-	-	+	-	+	+	+	-	+
K28	-	-	+	-	+	+	-	-	-
K29	-	-	+	-	+	+	-	-	+
K30	+	-	+	-	+	+	+	-	-
%	**60 %**	**0%**	**93.3%**	**0%**	**100%**	**70%**	**86.6%**	**0%**	**77%**

K= *K. pneumoniae*, - = negative, + = positive

K. pneumoniae demonstrates a variety of mechanisms to effectively acclimatize to its specific ecological environment and safeguard itself from the host's immune system. Bacterial virulence factors are implicated in the etiology of UTIs. Type 1 and 3 fimbriae, the iron uptake system involving siderophors, lipopolysaccharide (LPS), and the polysaccharide shell are among the significant virulence elements that contribute to the enhanced survival of bacteria and the development of disease[12, 41].

Detection of Biofilm formation

The result as showed in Table3, thirty isolates of *K. pneumoniae* had varied in their capacity to form biofilms as follow; 16/30 (53.33%) of the isolates were strong biofilm formation and 14/30(46.66%) moderate biofilm formation. The variation in formation of biofilm depended on several factors that influencing the capacity like the physicochemical characteristic of *K. pneumoniae*, physical interaction between the constituents, the type of surface where the biofilm attaches, temperature and pH [42]. The current result was consistent with what was reached by [43], as the percentage of biofilm formation 97.3%, and does not agree with what *K. pneumoniae* reported by [44].as the percentage of isolates producing biofilm was 52%. The bacteria that grow in the biofilm are tolerant to many antibiotics and for different environmental conditions, phagocytosis, opsonization, and resistance to delectableness. also resistance to selective osmosis pressures [44]. *K. pneumoniae* production of biofilm in urinary catheter devices is vigorous microorganisms biofilm-producing are responsible for infections and

diseases and increase their resistance to antibiotics which are difficult to treat due to the difficulty and restriction of antibiotic penetration of the biofilm [45].

Table 4: Absorbency and biofilm level for *K. pneumoniae* isolates

No. of sample	Absorbency at 630 nm	Biofilm level compared to (ODc=0.058)(2*ODc=0.116)	No.of sample	Absorbency at 630 nm	Biofilm level compared to (ODc=0.058)(2*ODc=0.116)
K.1	0.313	Strong	K.16	0.504	Strong
K.2	0.240	Strong	K.17	0.253	Strong
K.3	0.216	Moderate	K.18	0.183	Moderate
K.4	0.292	Strong	K.19	0.429	Strong
K.5	0.373	Strong	K.20	0.155	Moderate
K.6	0.286	Strong	K.21	0.245	Strong
K.7	0.225	Moderate	K.22	0.247	Moderate
K.8	0.235	Moderate	K.23	0.276	Strong
K.9	0.383	Strong	K.24	0.210	Moderate
K.10	0.208	Moderate	K.25	0.257	Strong
K.11	0.212	Moderate	K.26	0.222	Moderate
K.12	0.248	Strong	K.27	0.161	Moderate
K.13	0.258	Strong	K.28	0.157	Moderate
K.14	0.254	Moderate	K.29	0.303	Strong
K.15	0.223	Moderate	K.30	0.360	strong
			Control	0.121	
p.value = 0.0001 Strong= 16/30 (53.33%) ; Moderate 14/30(46.66%)					

Antimicrobial susceptibility test for *K. pneumoniae*

Antimicrobial susceptibility test of all isolates to locally commonly used in hospitals, antibiotics was determined by disk diffusion method. Our findings showed that the isolates appeared different susceptibility towards 12 antimicrobial agent. As shown in Table 5 and Figure 3.

Table 5: Antimicrobial sensitivity for (30) isolates *K. pneumonia*

Antibiotics classes	Antibiotics Type	Sensitive %	Resistant %
B-lactam	Ticarcillin- clavulante	(0%)	(100%)
Cephalosporins	Cefepime	(16%)	(86%)
	Cefotaxime	0(0%)	(100%)
	Ceftriaxone	0(0%)	(100%)
Carbapeneams	Imipenem	(64%)	(37.9%)
	Meropenem	(93.2%)	(6.8%)
Aminoglycoside	Gentamicin	(57.2%)	(44.8 %)
	Streptomycin	(48.3%)	(51.7%)
Tetracycline	Tetracycline	(38%)	(62%)
Fluoroquinolones	Norfloxcin	(51.8%)	(48.2%)

	Ciprofloxacin	(0%)	(100 %)
Sulfa drug	Trimethoprim – sulfamethoxazole	(38%)	(62%)
***P value*= 0.1074**			

At significance P ≤0.05

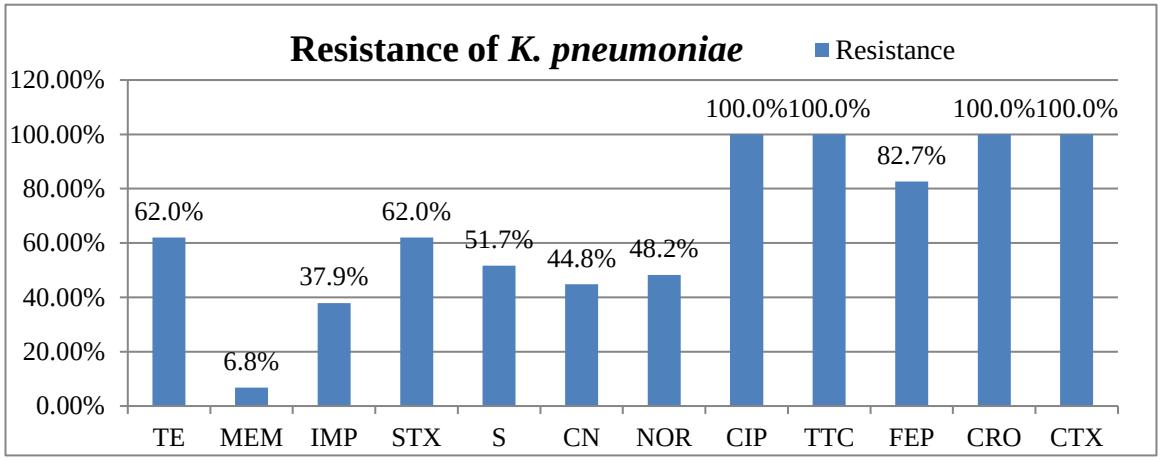

Figure 3: Antibiotic resistance rate of *K. pneumonia* against 12 antibiotics. TE : Tetracycline, MEM : Meropenem IMP : Impenem, STX : Trimethoprim/ sulfamethozole, S : Streptomycin, CN: Gentamycin, NOR :Norfloxacin, CIP : Ciprofloxacin, TTC :Ticarcillin clavnlanic, FEP: Cefepime, CRO: Ceftriaxone, CTX: Cefotaxime.

According to the results *K. Pneumoniae* isolates have the highest resistant rate (100% resistant) toward Ticarcillin- clavulante, Cefotaxime, Ceftriaxone, and Ciprofloxacin, this result agree with [48], when recorded 100% of the isolates were resistance toward these antibiotic. *K. pneumoniae* shows 62% resistance against Trimethoprim – sulfamethoxazole and this result almost agree with the result of [46], which the resistant rate was 53% and disagree with [47], when they recorded the rate of resistance was 72%. The resistance toward Tetracycline was 62% and this agree with the results of [48], which was 58.1% and with [46], that was 50.6%. Tetracycline and other antibiotics have been commonly employed in the treatment of ailments; nevertheless, the recurrent utilization of these compounds has regrettably led to the emergence of resistance strains. The reduced susceptibility of tetracycline in *K. Pneumoniae* is attributed to chromosomal abnormalities occurring in the outer membrane of the bacteria. These alterations result in a decreased ability of tetracyclines to penetrate the bacterial cell [49], acquisition of mobile genetic elements carrying tetracycline-specific resistance genes especially

tetB gene that resides on highly mobile genetic elements or *tetA* that located on plasmids and mutations within the ribosomal binding site leading to increased expression of intrinsic resistance mechanisms [50]. The resistance toward Norfloxacin was 48.2% this agree with [46], result that was 50.6% and disagree with [51], were the resistant rate was 31%. *K. pneumoniae* has a number of mechanisms for resisting Quinolone antibiotics, including Ciprofloxacin and Norfloxacin. These mechanisms involve the modulation of the target site that interacts with the antibiotics, achieved through the induction of chromosomal genetic mutations in the genes responsible for DNA gyrase or topoisomerase. Consequently, this leads to the production of distinct enzymes that confer resistance to these antibiotics. Additionally, efflux mechanisms are also implicated in the resistance of K. pneumoniae to Quinolones. The bacterial outer membrane is equipped with efflux pumps that extrude antibiotics from the cell, impeding their permeability [52]. The resistance rate against Cefepime was 82.7% which disagree with the results of [53], who observed 54.95% of their isolates was resist to this antibiotics. Resistance to cefepime in *K. pneumoniae* mainly mediated by over expression of Extended-spectrum beta-lactamases include *bla*OXA which have great activity against cefepime, plasmid-mediated AmpC beta-lactamases and outer membrane protein loss [54]. The most effective antibiotic against this bacteria was Meropenem (93.2% sensitive) and this result agree with [46], recorded 100% of the bacteria were sensitive towards Meropenem, while the resistance toward Imipenem, showed 37.9%, this nearly agree with [53], result which was 43%, but it was disagreed with the result obtained by [55], in Iran who found that only(20%) of isolates were resistance to imipenem [56], in Nigeria showed the resistance to imipenem was (10%) [57], demonstrated (5.26%) resistance to imipenem by *K. pneumoniae* in a surveillance study in hospitals in Baghdad. *K. pneumoniae's* resistance to imipenem may be caused by subtherapeutic concentrations of the antibiotic and improper antibiotic therapy duration. Production of KPC type carbapenemases, which also mediate resistance to extended-spectrum cephalosporins in addition to carbapenems, is strongly related with resistance to imipenem [58].The present study showed that the level of *K. pneumoniae* resistance to aminoglycosides used was 44.8% for Gentamicin, this result was similar to[53], which was 49% and for Streptomycin the resistance rate was 53%, this disagree with result of [59] that was 75%.

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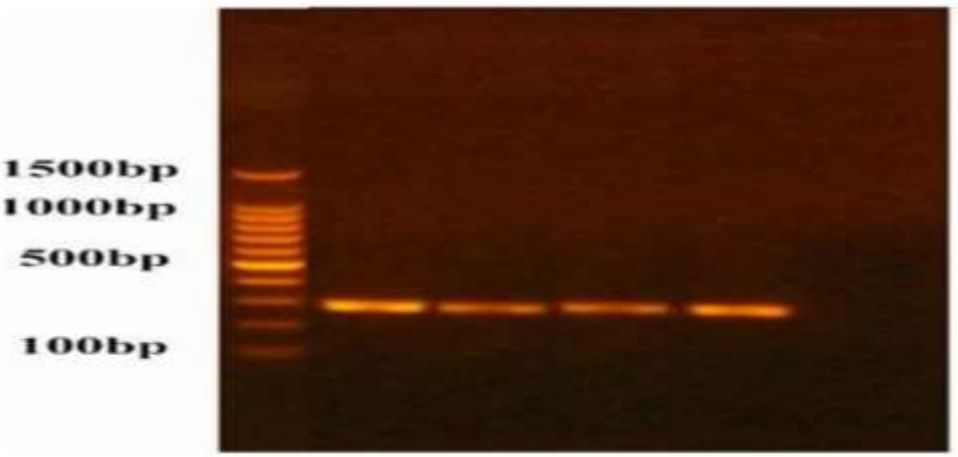
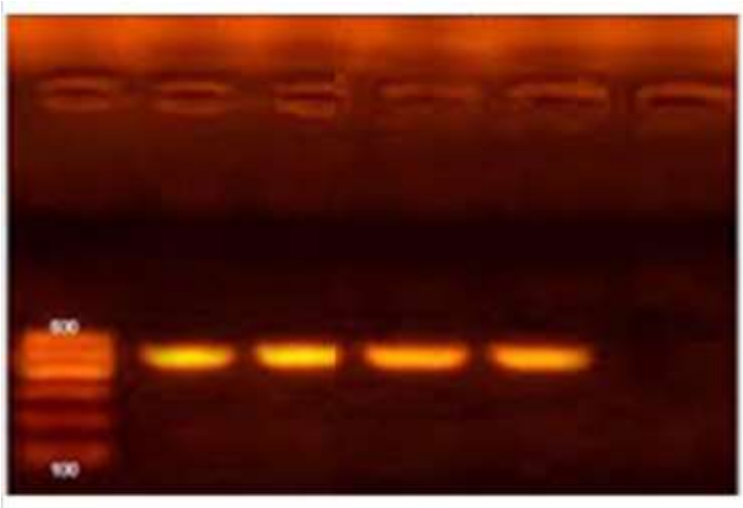
Molecular Detection of *fimH, rmp* and *bla-TEM* Genes of *K. pneumoniae*

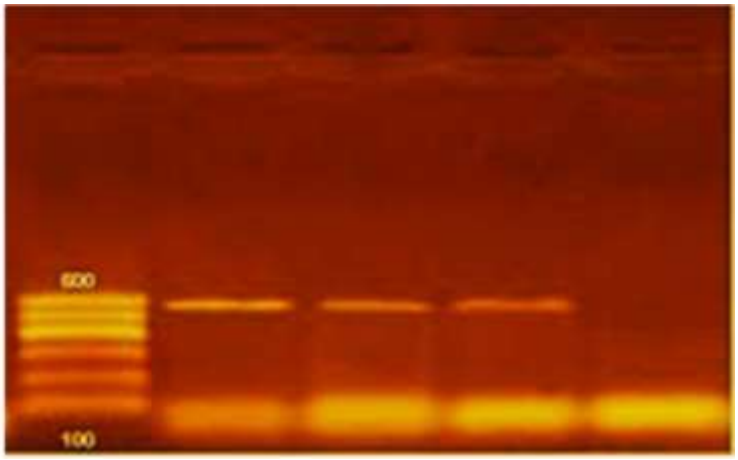
Some virulence genes for the *K.pneumoniae* isolates were investigated using single-plex PCR by using a specific primer to each gene. The polymerase chain reaction (PCR) technique was employed to identify the presence of the *fimH, rmpA*, and *bla-TEM* genes. The resulting amplicons were determined to have 550bp, 535bp, and 296bp, respectively. This investigation was conducted using the singleplex PCR. As depicted in under figures, it was observed that all isolates exhibited the presence of *fimH* at a rate of 40.2%. Additionally, *rmpA* was detected in isolates, accounting for 76.6% of the total, while bla-TEM was identified in isolates, representing 66.6% of the sample. The present study had results that many clinical *K. pneumoniae* isolates typically possess both type 1 and type 3, one of the most crucial steps in the development of *K. pneumoniae* infection is related to its capacity to adhere to host surfaces and demonstrate persistent colonization [60]. About 90 percent of the environmental isolates of K. show type 1 fimbriae [61]; nevertheless, it is unclear exactly how these fimbriae contribute to the formation of biofilms [12]. Urinary tract infections are mostly brought on by type 1 fimbriae expressed by *K. pneumoniae* [62].



***K. pneumoniae bla-TEM* 296 bp**



K. pneumoniae rmpA535 bp



***K. pneumoniae fimH* 550bp**

The genes fimH in *K. pneumoniae* encode type 1 fimbriae adhesins that facilitate adhesion to the extracellular matrix, support biofilm production, and possibly play a significant role in colonization, invasion, and pathogenicity [63]. The majority of the MRD *K. pneumoniae* isolates in the current study had both *fim*H-1. Although many clinical *K. pneumoniae* isolates typically express both type 1 and type 3 fimbrial adhesins, one of the most crucial steps in the development of *K. pneumoniae* infection is related to its capacity to adhere to host surfaces and demonstrate persistent colonization [60]. 90% of clinical and environmental *K. pneumoniae* isolates show type 1 fimbriae [12], nevertheless, it is unclear

exactly how these fimbriae contribute to the formation of biofilms [12]. Type 1 fimbriae expressed by *K. pneumoniae*, in particular, cause urinary tract infections [62, 64]. However, their precise role in the production of biofilms remains unclear [12]. The expression of Type 1 fimbriae by *K. pneumoniae* is specifically associated with the occurrence of urinary tract infections [62]. The capsule is considered a significant virulence component [41], since it provides protection of *K. pneumoniae* from fatal serum factors and phagocytosis [65]. According to [66], the capsular serotypes K1 and K2 in *K pneumoniae* have been identified as the prevailing virulent strains. Previous research conducted on clinical samples has suggested that KPC-producing isolates lack virulence factors such as K1, K2, K5, *rmpA*, and the aerobactin gene [67]. Contrary to the findings of prior investigations, our research revealed that rmpA was present in all isolates, which were classified as KPC-producing *K. pneumoniae*. It is noteworthy that the genes responsible for encoding *rmpA* have exhibited a strong correlation with the hypervirulent (hypermucoviscous) strain of *K. pneumoniae* (hvKP) [41, 68]. This particular strain is known to cause severe infections within the community. It has recently emerged as a carbapenem-resistant hypervirulent variant of K. pneumoniae (CR-HvKP), observed in various clinical settings[69, 70]. Hence, the findings of this investigation indicate that the *K. pneumoniae* strains examined exhibited molecular attributes consistent with those of hypervirulent (hypermucoviscous) *K. pneumoniae*.

Association of detected Genes with Presence of some virulence factors in *K. pneumoniae*

This study's results showed that the most common genes were *fim, rmp,* and *bla*. To assess the relationship between biofilm generation and *VFG*, the *K. pneumoniae* isolates demonstrated a high biofilm formation capacity, with a prevalence of 100%. The study's findings indicated that all isolates exhibited the presence of the *fim* H gene at a rate of 42.2%, while the *rmpA* gene was detected in 76.6% of the isolates, and *blaTEM* gene was observed in 66.6% of the isolates. Table 6 displays the distribution of various VFG among isolated *K. pneumoniae* strains, their propensity for biofilm formation and additional characteristics such as capsule presence, siderophors production, and ESBL production.

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Table 6: Association of genes with the presence of some virulence factors of *K. pneumoniae* isolates

Isolate	Genes			Biofilm production	ESBL	Capsule	Siderophore
	*fim*H	rmpA	*bla*-TEM				
K1	+	+	+	+	+	+	+
K2	+	+	-	+	+	+	+
K3	+	+	-	+	+	+	-
K4	-	+	+	+	+	+	+
K5	+	+	+	+	+	-	+
K6	+	+	-	+	+	+	+
K7	+	+	-	+	+	+	+
K8	-	+	-	+	+	+	+
K9	-	+	+	+	-	+	+
K10	-	-	+	+	+	+	+
K11	+	-	-	+	-	+	+
K12	-	+	-	+	-	+	+
K13	+	-	+	+	+	+	+
K14	+	+	+	+	-	+	+
K15	+	+	+	+	-	-	+
K16	-	-	+	+	+	+	+
K17	-	+	+	+	+	+	+
K18	-	+	-	+	-	+	+
K19	-	+	+	+	+	+	+
K20	+	+	-	+	+	+	+
K21	-	-	+	+	+	+	+
K22	-	+	+	+	-	+	+
K23	-	+	+	+	+	+	+
K24	-	+	+	+	-	+	+
K25	-	-	+	+	+	+	+
K26	-	+	-	+	-	+	-
K27	-	+	+	+	-	+	+
K28	-	+	+	+	-	+	-
K29	+	-	+	+	-	+	-
K30	-	+	+	+	+	+	+
%	42.2%	76.6%	66.6%	100%	60%	93.3%	86.6%

+ = positive, - = negative

Conclusions

The current study was done in Baqubah city supplied a basic database for hyper virulence and multidrug resistance prevalence among patients infected with UTI and were often caused by K. pneumoniae these isolates possessed virulence factors including biofilm formation, hemolysis and capsule as well as Extended spectrum Beta-lactamase (ESBL). Molecular detection by PCR showed; the genes *fimH*, *rmpA*, *blaTEM* were the most prevalent in the study's *K. pneumoniae* isolates which were in different proportions.

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