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Detection of virulence iroN gene in Klebsiella pneumoniae in urinary tract infections patients from Iraq

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ABSTRACT

Klebsiella pneumoniae belongs to the family Enterobacteriaceae, and it is the most clinically pertinent species, also it causes both nosocomial and communities-acquired infections, such as liver abscesses, urinary infections, pneumonia, and bacteremia. The current study aimed to detect the virulence iroN gene in Klebsiella pneumoniae for patients with urinary tract infections. One hundred fifty collected 75 samples of urine and 75 samples of blood samples from patients with urinary tract infections from Ibn Al-Baladi Hospital/ Baghdad -Iraq from November 2023 to February 2024. The results showed the diagnosis of 60 isolates of bacteria with an 80% prevalence of Klebsiella pneumoniae observed percentage for the age (more than 60 years) was 80% higher than for age (less than 60 years) was 20%. Moreover, females recorded 55% and males 45%. Also, blood group O has a percentage (33.3%) more than other groups A (23.3%), AB(30%), and B (13.3%). The results of antibiotic resistance of Klebsiella pneumoniae showed 100% resistance toward Cefoxitin Screen, Amoxicillin/ Clavulanic acid, Ticarcillin/ Clavulanic Acid, Piperacillin, Piperacillin/Tazobactam, Cefmetazole, Ceftazidim, Cefepime, Aztreonam, Amikacin, Ciprofloxacin). In contrast, it was 63.3 % for (Meropenem, Gentamicin, Tobramycin),66.7%for Trimethoprim / Sulfamethoxazole, and 61.6% for Imipenem. Moreover, these isolates were sensitive to Ticarcillin (100%), Minocycline (83%). Besides, the results of the virulence gene showed iroN gene was found in 38(63.3%) isolates out of 60 isolates. In conclusion**, t**his study appeared to the prevalence resistance of K. *pneumoniae* in urinary infections with the presence of *iroN* gene.

1. Introduction

Klebsiella pneumoniae belongs to Enterobacteriaceae, and is the most species clinically pertinent, also it causes both nosocomial and communities-acquired infections, involving liver abscess, urinary

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infections, pneumonia, and bacteremia [1]. In past decades, a distinguished hypervirulent difference of *K. pneumoniae*, was described via the phenotype of hypermucoviscous. it was first isolated from abscesses liver in Asia and appeared as an important clinical pathogen accountable for extremely invasive infection [2]. Urinary tract infections are considered multi-microbial infections, which occur via various strains of bacteria and fungi. The members of *Enterobacteriaceae* especially *Klebsiella pneumoniae* and Escherichia *coli* are considered the most bacteria prevalent in urinary infections [3].

The main *K. pneumoniae* pathogenicity is established from different virulence agents that permit it to attack innate immunity and preserve infections in a human host. The major virulence agents that have a significant role in pathogenesis include capsular polysaccharides, lipopolysaccharides, pili, and siderophores [4].

The factors of virulence in hypervirulent *K. pneumoniae* (hvKp) are represented by lipopolysaccharides, capsules, siderophores, and fimbriae [5]. Interestingly, siderophores and small metabolites of iron-binding have specific importance. Iron is considered a necessary mineral ion of growth bacteria and its availability is usually below the demanded concentration [6]. Iron (Fe3+) free is rare in physiological situations because it binds to heme and transferrin through infection. The absorption of iron in vivo via bacteria demands a system of siderophore -dependant iron acquisition [7]. These molecules are produced via bacteria, chelate iron in the environmental surrounding, and enter into cells. Therefore, siderophores are considered frequently as one of the main virulent factors for survival and pathogenesis of bacteria.

Salmochelin (iroN) is one of the siderophores in *K. pneumoniae* [8]. *Klebsiella pneumoniae* strains differ in virulence as a result of the existence of mobile genes. Particularly, gene encoding siderophores biosynthesis such as salmochelin is related to invasive infection and is common amongst *K. pneumoniae* hypervirulent that cause acute community-associated infection [9]. Consequently, the properties of *K. pneumoniae* virulence understanding and taking suitable measures are considered important matters for clinical evaluation, controlling, and reducing the risk of death rate in *K. pneumoniae* infection patients [8]. Consequently, the current study aimed to detect the virulence *iroN* gene in *Klebsiella pneumoniae* for patients with urinary tract infections.

2.Materials and Methods

2.1. Ethical consent

It was obtained consent from the patients and sample collection was approved by Ethics Committee in the Iraqi Ministry of Health.

2.2. Sample collection

It was collected 150 samples of urine and blood from patients (40-80 years) with urinary tract infections from Ibn Al-Baladi Hospital/ Baghdad -Iraq from November 2023 - February 2024. Urine (75 samples) was collected in sterile containers and the blood sample (75 samples) was collected in EDTA tubes to conduct ABO blood groups.

2.3. *Klebsiella pneumoniae* isolates

The samples were cultured by streaking a sterile loop of sample urine on Blood agar and McConkey agar, incubated for 24 hours at 37 ºC. The bacterial colonies on MacConkey agar were isolated and cultured in the nutrient broth for genetic detection.

2.4. Identification bacteria and antibiotics sensitivity detection by Vitek 2 compact

Vitek 2 Compact was used for the identification and antibiotics sensitivity detection (Identification card: GN ID card 21341) and AST-gram negative (AST card: AST-GN76 ID 413433) to detect the sensitivity of *Klebsiella pneumoniae*.

2.5. Genetic detection

Bacterial genomic DNA was extracted using Mini prep DNA Extraction Zymo research Kit /USA (Catalog: D6005) and primers used from (Alpha DNA/USA) as in table (1). Gene amplification was conducted by PCR at a final volume of 25µl including DNA template (1.5 µl without diluted), forward10 picomols/µl and reverse 10 picomols/µl primer (1 µl for each them), 5µl of Taq PCR

premix (ID Catalog:25025 Intron, Korea) and added (16.5 µl) free nuclease water. PCR condition's reaction were shown in Table (2). PCR product was detected by agarose gel electrophoresis with Red safe stain.

Table1. Primer sequences of *iroN* gene

Primer	Sequence	Tm (°C)	GC	Product size
Forward	5′- CTGTCGGCATCGGTTTTATT -3′	49.73	45%	556 bp [10]
Reverse	5′ - TGGCGTGTCGATTATTACCA -3′	49.73	45%	

Table 2. PCR programs for *iroN* gene

Stage	Temp (°C)	Time	Cycles
Initial Denaturation	94	5 min.	1 cycle
Denaturation 2	94	40sec	
Annealing	52	40sec	35 cycle
Extension1	72	1min	
Extension 2	72	5 min.	1 cycle

2.6. Statistical analysis

MedCalc Software 2023 was applied to conduct statistical analysis using chi-square. P-value < 0.01 means significant differences and P value > 0.05 means nonsignificant differences.

3.Results

3.1. *Klebsiella pneumoniae* identification

The results showed that 60 samples out of 75 samples were diagnosed as *Klebsiella pneumoniae* by Viteck 2 system with a probability of 99%. While the rest of samples (15 samples) were diagnosed with other species of bacteria.

3.2. Distribution of *Klebsiella pneumoniae* isolates

The results distribution of *Klebsiella pneumoniae* observed percentage for the ages more than 60 years was 80% higher than for age less than 60 years was 20%, Moreover, females recorded 55% and males 45% with significant differences (P<0.05). Also, patients with blood group O were a percentage (33.3%) more than other groups A (23.3%), AB (30%), and B (13.3%) with nonsignificant P>0.05 (Table 3).

Table 3. Distribution of according to age, sex, and ABO groups

Parameters	Number 60 samples	P value
Age < 60 years	12(20%)	<0.0001
>60 years	48(80%)	
Sex		
Male	27(45%)	0.03
Female	33(55%)	
ABO groups		
A	14(23.3%)	0.6
AB	18(30%)	
B	8(13.3%)	
O	20(33.3%)	

3.3. Antibiotics resistance of *Klebsiella pneumoniae* isolates

The results of antibiotic resistance of Klebsiella pneumoniae showed 100% resistance toward Cefoxitin Screen, Amoxicillin/Clavulanic acid, Ticarcillin/Clavulanic Acid, Piperacillin, Piperacillin / Tazobactam, Cefmetazole, Ceftazidime, Cefepime, Aztreonam, Amikacin, Ciprofloxacin). In contrast, it was 63.3 % toward (Meropenem, Gentamicin, and Tobramycin), 66.7%for Trimethoprim/ Sulfamethoxazole, and 61.6% for Imipenem. Moreover, these isolates were sensitive to Ticarcillin (100%), and Minocycline (83%) as shown in table (4).

Table 4. Antibiotic resistance of *Klebsiella pneumoniae* isolates

Antibiotic	Resistant	Sensitive	P value
Cefoxitin Screen	60(100%)	0(0%)	P < 0.001
Amoxicillin/Clavulanic acid	60(100%)	0(0%)	P < 0.001
Ticarcillin	60(100%)	0(0%)	P < 0.001
Ticarcillin / Clavulanic Acid	0(100%)	60(100)	P < 0.001
Piperacillin	60(100%)	0(0%)	P < 0.001
Piperacillin / Tazobactam	60(100%)	0(0%)	P < 0.001
Cefmetazole	60(100%)	0(0%)	P < 0.001
Ceftazidime	60(100%)	0(0%)	P < 0.001
Cefepime	60(100%)	0(0%)	P < 0.001
Aztreonam	60(100%)	0(0%)	P < 0.001
Imipenem	37(61.6%)	23(38.3%)	P < 0. 01
Meropenem	38(63.3%)	22(36.6%)	P < 0. 01
Amikacin	60(100%)	0(0%)	P < 0.001
Gentamicin	38(63.3%)	22(36.6%)	P < 0.01
Tobramycin	38(63.3%)	22(36.6%)	P < 0.01
Ciprofloxacin	60(100%)	0(100%)	P < 0.001
Minocycline	10(16.7%)	50(83.3%)	P < 0.002
Trimethoprim/ Sulfamethoxazole	40(66.7%)	20(33.3%)	P < 0.003
Cefoxitin Screen	60(100%)	0(0%)	P < 0.001

3.4. Detection *iroN* gene

The results showed *iroN* gene was found in 38(63.3%) isolates out of 60 isolates (Figure1).

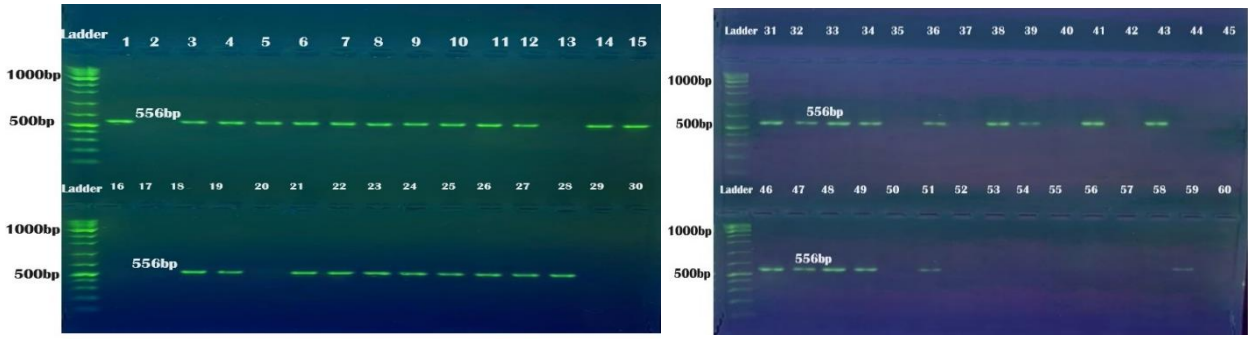

Fig1. Electrophoresis of PCR amplicons for *iroN* gene (556bp) for 38 *Klebsiella pneumoniae* isolates on 2% Agarose gel. 1x TBE buffer at 1 hour. Ladder DNA (1500 bp). Whereas other isolates (22 isolates including number 2,13,16,17,20,29,30,35,37,40,42,44,45,50,52-58,60) did not contain *iroN* gene

4. Discussion

At present time, antibiotic resistance increases for uropathogenic bacteria causing complicated important issues in controlling urinary tract infections over time [11]. Different studies have been

conducted in previous years to determine the antibiotics resistance for uropathogenic bacteria in patients, all results indicated an increase in resistance through the years [12,3].

In one Iraqi study conducted by Al-Gasha'a et al. [13], they observed that there is a relationship between sex, age, and urinary tract infection. The prevalence of bacteria that cause infection was higher at age (30-39) at 25.40%. Jameel and Artoshi [14] observed in their study the more frequent bacteria were *E. coli* (43.20%) and, *K. pneumoniae* (19.90%), and urinary infections in females were (68.04%) whereas in males (31.96%). Also, another Iraqi study indicated that the rate of urinary infections in women (53.2%) is higher than in men (30.2%) [15].

Kakde et al. [16] observed that urinary tract infections in women are related to the anatomy and physiological structure nature of their urinary tract system; while the length of urethra is lower than in men. On the other hand, hormonal changes prompt infections. While males are protected as a result of the anatomical structure of the urinary tract system.

The current study accordance with another study which showed due to age, urinary tract infection is commonly prevalent in elderly patients and increased during the frequency of risk and infection complications. Thus, urinary tract infection in patients with age (60 years) was 50-60 % and 38.55% for patients (40-59) years. This increasing prevalence in the elderly may be explained by several factors the urinary system secretion senility and a reduction of contraction muscular for bladder which plays a role in bacteria growth by a decrease in the rate of excretion of urine [17].

The current study agrees with other research that studied ABO blood groups in urinary infections. Najari *et al.* [18] noticed in their study no relationship between urinary infections and blood group A. Mahmoudian et al. [19] indicated group O for pregnant suffering from urinary infections was more frequent, followed by group B. This study conflicts with research that noticed a relationship between urinary tract infection and blood group. Safarkar et al. [20] detected in their study group O was prevalent in urinary tract infection. Benli *et al*. [21] showed a correlated urinary tract infection with A group. One of the reasons for the differences in the studies' findings is due to the paradigms and the patients characteristics [22].

In the current study was estimated resistance against antibiotics. One study displayed that the beta-lactam group of antibiotics includes Penicillin, Carbapenem, Cephalosporin, and Monobactam, while a group of Beta-lactamase inhibitors includes Clavulanic acid, Sulbactam, and Tazobactam [22]. Roshan *et al.* [23] showed in their study that resistance is often related to extended spectrum of beta-lactamases. Some studies showed that the resistance in amoxicillin/clavulanic acid was 97.67% higher than in other antibiotics [24]. Pfeifle *et al.* [25] showed in their study *Klebsiella* spp. was resistant to β-lactam antibiotics due to the synthesis of β-lactamase enzyme which destroyed β-lactam rings before connecting to the target.

The current study was convenient with one study performed by Kot *et al*. [26] they noticed resistance of K. *pneumonia* toward amoxi/clav (75%); piperacillin/ tazobactam (76.2%); cefotaxime (81%); and cefuroxime (81%). Alsanie [27] showed resistance *K. pneumoniae* against Ampicillin at (96%); Cephalothin at (90%); Ceftriaxone at (90%); Amox/Clav at (90%); Cefuroxime at (85%); Aztreonam at (87%); Cefepime at (80%); Ceftazidime at (80%); Trimthoprim-Sulfamethoxazole at (82%).

Concerning the presence of *iroN* gene in *K. pneumonia* there are no previous Iraqi studies about this virulence gene. The current study agreed with global other studies. Daoud *et al*. [28] showed the presence *iroN* gene and iron uptake gene that are associated with the resistance of bacteria to antibiotics. The system of iron acquisition has an important effect on Cefiderocol activity, and their change expression represents a factor that reduces the susceptibility. One study found that 55% of K. pneumoniae hypervirulent have iroN gene were multidrug resistance and produce ESBL, consequently, hospital K. pneumoniae isolates cause a menace to the healthcare systems [29].

Recent research has focused on the significance of iron, its processes of absorption, and its relationship to K. pneumoniae's pathogenicity. Chen et al.[8] examined the growth of *K. pneumoniae* strains that produce liver abscesses versus ones that do not, about varying iron concentrations. They showed that the highest tested concentration of iron (50 μM) was associated with the maximum bacterial growth in iron-rich media. The siderophore expression rates were one of their study's other key discoveries. They found that the expression of all four siderophores:aerobactin, salmochelin,

yersiniabactin, and enterobactin were higher in iron-depleted conditions than in iron-rich environments.

6.Conclusion

The current study showed the distribution of K. *pneumoniae* in elderly patients with urinary tract infection and there is no correlation with ABO groups but an increase in the sample size may lead to show significant correlation among ABO groups. Moreover, in female was more prevalent than males. *K. pneumoniae isolates* showed resistance to most antobiotics studied. Also, the prevalence resistance of K. *pneumoniae* in urinary infections with the presence of *iroN* gene.

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الكشف عن جين الضراوة ***iroN*** في بكتيريا ***Klebsiella pneumoniae*** للمرضى العراقيين المصابين بالتهاب المسالك البولية

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الملخص

تنتمي *Klebsiella pneumoniae* إلى عائلة Enterobacteriaceae، وهو أكثر الأنواع أهمية سريرياً، كما أنه يسبب عدوى المستشفيات المكتسبة ، كذلك خراج الكبد، والتهابات المسالك البولية، والالتهاب الرئوي، وتجرثم الدم. هدفت الدراسة الحالية إلى الكشف عن جين الضراوة *iroN* في بكتيريا *Klebsiella pneumoniae*. لمرضى التهابات المسالك البولية.تم جمع مائة وخمسين عينة بول ودم من مرضى التهابات المسالك البولية من مستشفى ابن البلدي/بغداد-العراق للفترة من تشرين الثاني 2023 - شباط 2024. اظهرت النتائج تشخيص 60 عزلة بنسبة انتشار 80% للبكتيريا *Klebsiella pneumoniae* وكانت النسبة للعمر (أكثر من 60 سنة) 80% اعلى من العمر (أقل من 60 سنة) كانت 20%.. كما بلغت نسبة الإناث 55% والذكور 45%. كما أن فصيلة الدم O لديها نسبة (33.3%) أكثر من فصائل الدم الأخرى AB (23.3%)، A (30%)، وB (13.3%). وأظهرت نتائج مقاومة بكتيريا *Klebsiella pneumoniae* للمضادات الحيوية مقاومة 100% تجاه السيفوكسيتين و أموكسيسيلين / حمض كلافولانيك، تيكارسيلين / حمض كلافولانيك، بيبيراسيلين، بيبيراسيلين / تازوباكتام، سيفميتازول، سيفتازيديم، سيفيبيم، أزتريونام، أميكاسين، سيبروفلوكساسين) في حين كانت 63.3% ضد (ميروبينيم، جنتاميسين، توبراميسين)، 66.7% تريميثوبريم / سلفاميثوكسازول، و 61.6% لـلامبينبم. كما كانت هذه العزلات حساسة(100%) للتيكارسيلين و للمينوسايكلين (83%). كما أظهرت نتائج جين الضراوة وجود جين *iroN* في 38 عزلة (63.3%) من أصل 60 عزلة. أظهرت هذه الدراسة مدى انتشار مقاومة بكتيريا *K. pneumoniae* في الالتهابات البولية مع وجود جين *iroN*.

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