

# Molecular detection of virulence genes for multi drug resistance *Klebsiella pneumoniae* in patients with urinary tract infections, Thi-Qar Provenance, Iraq

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## ABSTRACT

An agent that contributes to hospital acquired illnesses is *Klebsiella pneumoniae*. In community-acquired infections and illnesses of the urinary tract, it is the primary and most frequent causal agent. The strains were detected by using Analytical Profile Index (Api-20 E) and 16s rRNA techniques, fifteen strains of *K. pneumoniae* were obtained from eighteen urine samples collected from patients in Al-Hussein Hospital, Thi-Qar Governorate, Iraq. This research sought to identify the antibiotic resistance of *K. pneumoniae* to Aztreonam, Ceftriaxone, Trimethoprim, Amikacin, Ciprofloxacin, Meropenem, and Piperacillin, the resistance was high and ranged between (86.6-100)%. The multidrug resistance percentage was 100%, in addition the investigation aimed to detect some virulence genes, (i.e. HtrA and MrkA gene) in *K. pneumoniae*. The HtrA and MrkA genes were found using the PCR method, which revealed that 6 (40%) of the isolates had the HtrA gene and that all strains under study lacked the MrkA gene.

**Keywords:** MDR, *Klebsiella pneumoniae*, urinary tract infections, HtrA gene, MrkA gene

## INTRODUCTION

In most nations around the world, urinary tract infections rank second to respiratory tract infections in terms of frequency of health issues [1]. These infections are the most often reported bacterial illnesses in the community and have been estimated to impact up to 150 million people worldwide annually [2], affecting both men and women of all ages [3]. UTIs are a group of illnesses that can affect the kidneys, renal pelvis, or lower urinary tract alone (urethritis, cystitis, and urethrocystitis), and are all caused by the presence of microorganisms in the urine [4]. UTIs can also be divided into uncomplicated and complicated infections as a result of the infection, depending on a number of variables that may help to distinguish between these two classifications, such as the type and length of antibiotic

therapy as well as a more thorough examination of the urinary tracts. *Escherichia coli* is one of the most frequent agents (around 80%) of UTI in individuals [5] and one of the most frequent causes of Gram-negative bacteremia in hospitalized patients [6]. Some of the other bacteria involved are *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Enterococcus species, Enterobacter species, group B Streptococcus, and *Staphylococcus saprophyticus* [7]. The second most frequent cause of urinary tract infections after *Escherichia coli* is *K. pneumoniae*, which is frequently brought on by the use of indwelling catheters [8]. It is an important nosocomial pathogen that can lead to potentially lethal septic shock, pneumonia, uti, and other infections [9]. Pathogenic *Klebsiella pneumoniae* is a Gram-negative bacteria that causes wound infections, urinary tract infections, and community-acquired illnesses.

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The emergence of MDR strains of this bacterium is quite alarming [10].

*K. pneumoniae* has the capacity to penetrate the host due to a variety of virulence factors, such as capsular polysaccharide, lipopolysaccharide, siderophores synthesis, fimbriae, and other factors such the development of urease and enterotoxin [11].

The majority of *Klebsiella* strains produce type 3 fimbriae, which are responsible for bacterial adhesion to host mucosal surfaces [12]. Type 3 fimbriae's main structural element is the MrkA protein, while MrkD adhesion determines whether type 3 fimbriae can bind to collagen molecules [13]. High temperature requirement A, or htrA, is a gene that plays a role in the virulence of other species including *Salmonella enterica* serovar *Typhimurium*, *Brucella abortus*, and *Yersinia enterocolitica*. HtrA mutant strains of several gram-negative bacteria have been shown to be attenuated in animal models and can also be used as vaccines [14]. Mice and macrophages with htrA mutations from these species had lower survival rates: Their 50% fatal doses (LD50) plummeted between 2 and 5 log units, likely due to their increased vulnerability to oxidative stress, which makes them more susceptible to the oxygen-dependent host death mechanisms. However, the methods by which the pathogenicity of htrA mutants is reduced are not completely understood [15,16]. In this investigation, we looked for the existence of the mrkA and HtrA genes in *Klebsiella pneumoniae*, a pathogen that is susceptible to the oxygen-dependent death mechanisms of the host.

## METHODS

### The isolation and detection of *Klebsiella pneumoniae*

80 urine samples from patients with UTIs were taken between October and December 2022 when they were enrolled in Al-Hussein Hospital in Thi-Qar Province, Iraq. UTI sufferers sent their urine samples to the relevant hospital laboratory in accordance with their doctor's orders. The bulk of urine samples from adult patients were taken using the clean-catch midstream method. In the morning, the urine was preferred. The samples were maintained in a 4°C refrigerator until processing [17]. Different bacterial strains were identified using various growth conditions, API20 E tests, and 16srRNA studies.

### Antimicrobial susceptibility testing

On Muller-Hinton agar, the identified isolates underwent an antibiotic sensitivity test. Aztreonam (ATM) 30 µg, Ceftriaxone (CRO) 10 µg, Trimethoprim

(TMP) 10 µg, Amikacin (AK) 30 µg, Ciprofloxacin (CIP) 10 µg, Meropenem (MEM) 10 µg, Piperacillin (PRL)100 µg (Bioanalyse, Turkey) were used in the current study. According to CLSI 2020 recommendations, data from the susceptibility assay were interpreted [18].

### DNA extraction and Purification Kit

The genomic DNA was recovered from bacterial growth using the DNA Purification Kit, Transgene/China technique in the following ways:

The bacteria were re-suspended by overtaking after being centrifuged at 12,000 g for one minute with a 1.5 ml tube containing 1 ml of overnight Gram-negative bacteria, 100 µl of LB11, and 20 l of Proteinase K. At 55 oC, it was incubated for 15 minutes.

If the solution isn't clear after incubation, the incubation was extended by 30 minutes with a vortex every five minutes. 400 µl of BB11 (verify that 96-100% ethanol has been supplied) were added and vortexed for 30 seconds after 20 µl of RNase A had been mixed and incubated in the tube at room temperature for 2 minutes. (At this step, there may be white flocculent precipitate or translucent gelatinous materials; this will not affect DNA extraction), The whole thing was moved to a spin column, spun at 12,000 g for 30 seconds, and the flow-through was thrown away. The flow-through was then removed after centrifuging 500 µl of CB11 for 30 seconds at 12,000 g. WB11 was diluted to 500 l, centrifuged at 12,000 g for 30 seconds, and the flow-through was discarded (make sure 96–100% ethanol was supplied).The sample was centrifuged at 12,000 g for 2 minutes to remove any leftover WB11. The spin column was contained in a 1.5 ml microcentrifuge tube that was sterile. 50-200 µl of elution buffer (preheated to 65°C) or sterile, distilled water (pH >7.0) were added to the center of the column. 2 minutes of room temperature incubation. After 1 minute of centrifugation at 12,000 g, genomic DNA was eluted. The isolated DNA was preserved at -20°C. By measuring the absorbance at (260/280 nm) on a Nanodrop spectrophotometer, the purity of the isolated genomic DNA was evaluated [19].

### Primer pairs preparation

The lyophilized primers are dissolved in TE (Tris-EDTA) buffer to create a stock solution with a concentration of 100 pM/l, and the primer working solution is created by dilution with TE buffer, spinning down, and storing it at 4°C for the duration of the following day, as per the manufacturer's instructions (Alph, Canada). For each primer, (10 pM/l) Table 1.

## Preparation of the PCR master mix reaction

Using (one taq quick-load) PCR Kit, a reaction of PCR master mix was created, and this master mix was carried out in accordance with the manufacturer's instructions as given in Table 2. Then, these PCR master mix reaction components that are listed below are added to typical PCR tubes that already have all of the other components required for a PCR reaction, such as Taq DNA Polymerase, dNTPs, 6 mM MgCl<sub>2</sub>, and pH 8.7. For three minutes in the Exispin vortex centrifuge, the tube was then spun. The Multigene PCR thermocycler was then transferred.

## Polymerase Chain Reaction (PCR)

Using particular primer pairs listed in Table 1, conventional PCR was employed to amplify the target DNA. It consists of three sequential processes that are repeated for a predetermined number of cycles (about 35-38 cycles) in order to produce PCR result (amplicon), which can then be seen following agarose gel electrophoresis.

TABLE 1. Primers used in the recent study

Target Gene	Oligonucleotide Sequence (5'-3')	Size (bp.)	References
16srRNA	F: GCAAGTCGAGCGGTAGCACAG R: CAGTGTGGCTGGTCATCCTCTC	216	[20]
HtrA	F: CGTTCTGCCAGGATGGTTCT R: CCCCAATGATGACATCGCCT	1071	[21]
mrkA	F: AGTGCTTTCACCCCCTCT R: GAGTGACTGGGGTGAGCAA	862	[21]

TABLE 2. Components of PCR Master Mix preparation

Component	25µl reaction
10 µM Forward Primer	0.5 µl
10 µM Reverse Primer	0.5 µl
Template DNA	1.5 µl
OneTaq Quick-Load 2X Master Mix with Standard Buffer	12.5 µl
Nuclease-free water	10 µl
Total	25 µl

## RESULTS AND DISCUSSION

The gram-negative, lactose-fermenting bacillus *Klebsiella pneumoniae* is a rod-shaped member of the Enterobacteriaceae family with a distinct capsule. It is a clever pathogen that mostly attacks people with immune systems that are resistant and frequently causes nosocomial infections [22]. After performing the Biochemical and 16srRNA tests Figures 1 and 2.

In the current investigation, a total of 80 samples taken from patients with UTIs at Al-Hussein hospital in Thi-Qar origin yielded fifteen isolates of *Klebsiella*

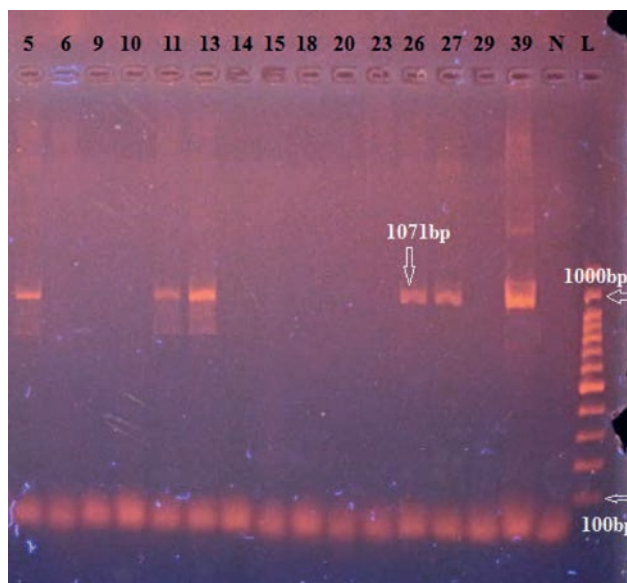


FIGURE 1. Api 20E test for *Klebsiella pneumoniae*

*pneumoniae*. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other species of UTI bacteria were the pathogens that were most frequently found. These findings supported the assertion that *E. coli* is the primary bacterium responsible for UTIs, which was also supported by findings from other research carried out internationally. This outcome is analogous to a study by [23] from Zakho, Iraq, where they discovered that the higher percentage of *E. coli* isolates, and another study by [24] was consistent with our findings. One of the most significant causes of bacteremia, burns, urinary tract infections, fees, and respiratory tract infections is *K. pneumoniae* [25]. *K. pneumoniae* recorded high resistance in all tested antibiotics i.e. Ampicillin (AM), Piperacillin (PRL), Trimethoprim (TMP), Amikicin (AK), Meropenem (MEM), Aztreonam (ATM), Ceftriaxone (CRO), and Ciprofloxin (CIP) the resistance percentage was ranged between 86.6%-100% (Table 3), these results were in line with [26,27]. Since there are many different causes of urinary tract infections and antibiotic resistance patterns evolve over time, evaluating antimicrobial sensitivity is crucial. This will let doctors provide safe and effective empiric treatments to halt the growth and dissemination of bacteria that cause urinary tract infections [28]. Self-medication, noncompliance with therapy, the spread of resistant isolates between people, the selling of faulty medications, and most importantly the fact that antibiotics are affordable and widely available without a prescription may all be factors in the rise in antibiotic resistance. A strain was termed MDR if it demonstrated resistance to at least three different types of antibiotics [29]. *K. pneumoniae* resistance to both classes of antibiotics may be caused by the -lactamase enzymes cephalosporinase and penicilli-





**FIGURE 2.** The 216 bp Primer TM from the *K. pneumoniae* primer was electrophoresed on a gel at 55°C with 1% Agarose for 10 minutes at 100 volts before being decreased to 70 volts for 60 minutes. Following ethidium bromide staining and visualization under ultraviolet light, Lane L represents a DNA ladder (100-1500 bp), Lanes 5-39 indicate positive results, and Lane (N) represents a negative control

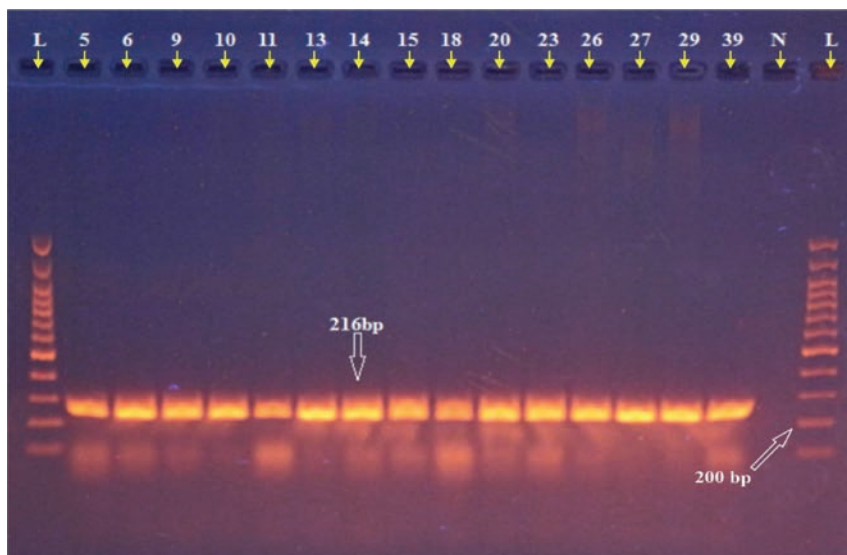
nase, which can inactivate penicillins and cephalosprins by cleaving the drug's -lactam ring [30]. The β-lactamase gene is found on chromosomes or plasmids, the majority of which are self-transmitting plasmids. Additionally, due to a lack of penicillin binding proteins (PBPs), bacteria may change their susceptibility to the antibiotic or acquire a resistance to penicillins [31].

In *Klebsiella* isolates harboring extended spectrum -lactamase enzymes, aminoglycosides resistance was reported, according to [32]. Quinolone resistance is brought on by alterations in the antibiotic-enzyme (GyrA) binding site, while car-

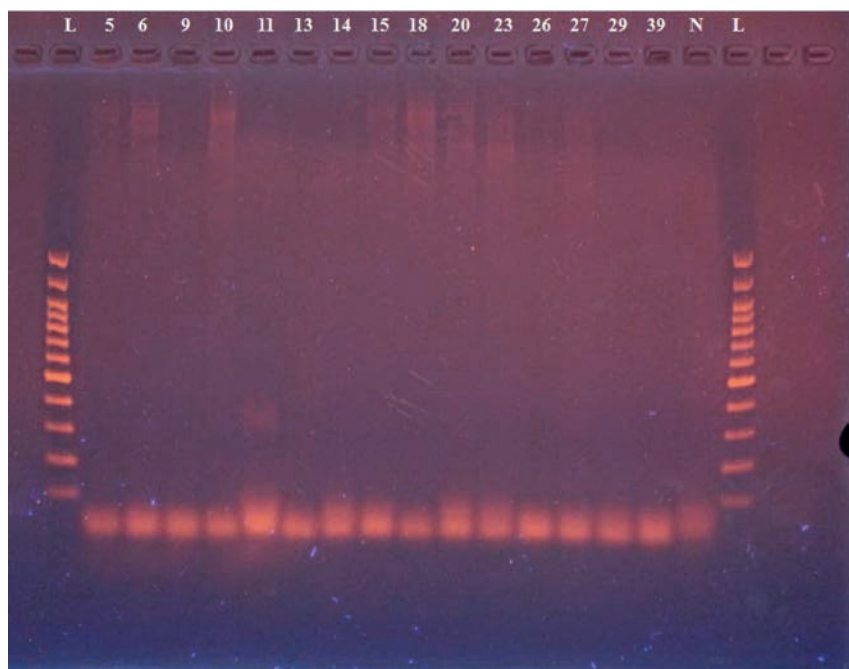
bapenem resistance is brought on by the Carbapenemase enzymes produced by *K. pneumoniae* [33].

**TABLE 3.** Antibiotic Resistance for MDR *K. pneumoniae*

No.	Antibiotics	Resistance NO. (%)
1.	ATM	13 (86.6)
2.	CRO	14( 93.3)
3.	TMP	14 (93.3)
4.	AK	13 (86.6)
5.	CIP	15 (100)
6.	MEM	14 (93.3)
7.	PRL	15 (100)



**FIGURE 3.** 1071 bp are seen on the gel electrophoresis for the PCR product of the (*htrA* primer) at 63°C (Agarose 1%, 10 min. at 100 voltage, decreased to 70 volts, 60 min.). visualized under ultraviolet lighting following ethidium bromide staining. Lane L: DNA ladder (100-1500 bp), good findings were represented by lanes 5, 11, 13, 26, 27, and 39, whereas negative results were represented by lanes 6, 10, 14, 23, and 29. Lane (N) stood for the adverse control



**FIGURE 4.** Gel electrophoresis was performed at 59°C for the PCR product of the mrkA primer, which produced no results (Agarose 1%, 10 min. at 100 volts, followed by 60 min. at 70 volts). After being stained with ethidium bromide and viewed under ultraviolet light, Lane L represents the DNA ladder (100-1500 bp), while Lane (5-39) represents the negative result. Negative control was symbolized by Lane (N)

The MDR resistance percentage in the recent study was 100%. Multidrug-resistant Gram-negative bacterial infection is a widespread issue that is linked to higher rates of morbidity and mortality. Antibiotic overuse and abuse, particularly in patients receiving broad-spectrum or multiple medications, may result in unique changes that make the bacteria resistant to other antibiotics [34].

Recent study recorded that 6 (40%) of *K. pneumoniae* were harbor HtrA gene (Figure 3), this is in agreement with (35), they stated that about 27% of *K. pneumoniae* from urine were carry the HtrA gene whereas all isolates were recorded negative results for MrkA gene (Figure 4) and that's in some text similar to the results reported by [35], that's mean that the studied isolated may contain other genes responsible for attachment or the primer doesn't pick up the presence gene or we must increase the sample size to get positive MrkA gene in *K. pneumoniae*.

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## CONCLUSIONS

The most prevalent isolates from Urinary Tract Infections were Gram Negative bacteria, high resistance to various antibiotics was recorded in the studied isolates due to random use of antibiotics. An acceptable percentage from *K. pneumoniae* contain HtrA genes and none of the studies isolates were contain MrkA gene.

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