

PCR detection of spreading TEM and CTX-M gene in *Klebsiella pneumoniae* isolated from urinary tract infection

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Abstract. Mostly, urine is devoid of bacteria, viruses, and fungi, making it sterile. However, an infection may arise when minuscule organisms, mostly bacteria from the digestive system, attach to the urethral opening and proliferate, leading to infections. This research seeks to identify the β -lactam genes responsible for antibiotic resistance in *Klebsiella pneumoniae* linked with infections, as well as their resistance to treatments. This inquiry specifically focuses on this aspect. The primary focus of the study was identifying bacterial isolates by established diagnostic methods. The secondary objective was to assess the resistance and sensitivity of antibiotics. Lastly, the study used the Polymerase Chain Reaction (PCR) method to ascertain the presence of the *bla*CTX-M and *bla*TEM genes. Within the period from November 2022 to June 2023, a 540 clinical samples were gathered from patients with urinary tract infections at Al-Hakim General Hospital, Al-Sadr City Hospital, Al-Furate Al-Awsat Hospital, and Al-Zahra Maternity and Children in AL-Najaf governorate. A total of 120 samples were found to be tainted with *Klebsiella pneumoniae*. This study assessed the sensitivity of the test using the disk diffusion method. The findings indicated that *Klebsiella pneumoniae* exhibited high levels of resistance to a broad scope of antibiotics, namely Ampicillin/sulbactam, Ampicillin, and Ticarcillin/Clavulanic acid. Additionally, some isolates showed resistance to Cefotaxime. The experiment yielded diverse outcomes for the quinolone, aminoglycoside, and macrolide groups. Among the 120 samples that were examined, 87 of them showed positive results for *K. pneumoniae* when the PCR technique was used to detect the presence of the *bla*CTX-M gene. On the other hand, 36 samples tested positive for *K. pneumoniae* when the *bla*TEM gene was used. The specimens were obtained from the urine of individuals diagnosed with urinary tract infections.

1 Introduction

Bacterial urinary tract infection (UTI) is the third most prevalent infection in humans, following respiratory and gastrointestinal infections. It affects individuals of all age groups and has a higher incidence in females compared to males. Each year, roughly 150 million

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people worldwide suffer from UTIs, which are infections that affect a specific part of the urinary tract and it's widely happen [1]. *Klebsiella pneumoniae* is an opportunistic pathogen that is recognized for causing a variety of human ailments, such as pneumonia, bacteremia, ulcers, infections of the urinary tract (UTIs), and digestive tract infections. These infections may manifest in both hospitals and community contexts [2]. Approximately *K. pneumoniae* isolates with a ratio of 30% exhibit resistance to antibiotics with broad-spectrum, and several virulence factors were found in these strains. Fimbrial adhesins are crucial for the pathogenicity of bacteria since they enable the germs to attach to certain tissue surfaces. The FimH component of Type 1 fimbriae, which is present in several Enterobacteriaceae species, has a significant impact on urinary tract infections [3]. The Fim gene cluster produces fimbriae, containing most of the genes that are responsible for constructing it. A repeated subunit called FimA and a tip adhesin molecule called FimH are encoded by these genes [4]. The capsular polysaccharide is considered crucial for the pathogenicity of *K. pneumoniae* because it shields the microorganism from phagocytosis and inhibits antibacterial serum components from eliminating this bacterium [5]. The presence of capsular type K-1 was detected in isolates obtained from patients diagnosed with pneumonia and has more recently been associated with the occurrence of pyogenic liver abscesses [6]. *Klebsiella pneumoniae* is often found in the human digestive system and nasopharynx as a saprophytic organism. This bacteria is regarded as the primary cause of nosocomial infections compared to infections obtained in the community. Nosocomial infections caused by *K. pneumoniae* remain very widespread and potentially more hazardous owing to the rapid development and dissemination of antibiotic resistance within hospital environments [7]. Therefore, the objective of this investigation is to identify the presence of the *blaCTX-M* gene and *blaTEM* genes in *K. pneumoniae* isolates obtained from patients with urinary tract infections (UTIs). The hypothesis posits that the presence of these two genes may be seen in *K. pneumoniae* strains obtained from patients with urinary tract infections (UTIs) and that these genes have a role in bestowing resistance to antibacterial therapies.

2 Materials and Methods

2.1 Moral Consideration

Permission for this study was granted by both the Scientific Research Committee of Najaf Health Department and the Scientific Research Ethics Committee at Kufa Science College-Kufa University.

2.2 Patients

This research included the enrollment of 540 patients, both male and female, who had urinary tract infections (UTIs). There were a wide range of ages among the patients (i.e., 1-60 years). The recruitment period for the study was from November 2022 to June 2023. Participants were enlisted from AlSader Medical City, Al-Hakeem General Hospital, Hospital of Al-Furate Al-Awsat, and Al-Zahra Maternity and Children in Najaf City, Iraq. Specimens were obtained from the middle section of the urine and placed in a sanitized, sterile receptacle.

2.3 Identification and Isolation of the Bacterial Isolates

Following collection of the samples, they were conveyed to the lab so they could be cultured on MacConkey's and blood agar plates, respectively. Afterward, we transferred them to an incubator maintained at a temperature of 37°C for 18-24 hours. The primary identification of the isolates was conducted utilizing Gram staining, analysis of colony shape on diverse media, and biochemical testing. An overall of 540 isolates were found and verified utilizing the Vitek2 technology. Bacteria with Gram-positive are identified using GP-ID cards, while bacteria with Gram-negative are identified using GN-ID cards in this method. The identification procedure assigns confidence levels to the ID messages, which range from very excellent to exceptional. These confidence levels are represented by probability percentages between 95 and 99. In addition, the VITEK2-automated system was used to biochemically confirm the ultimate bacterial isolates identification. The Clinical and Laboratory Standards Institute (CLSI) provided us with guidelines in 2023 to evaluate antibiotic resistance in *K. pneumoniae* isolates using the disc diffusion method. Figure 1. Twenty antibiotics were employed to conduct susceptibility profiles, including Amoxiclav with Ampicillin, Ticarcillin /Clavulanic acid, Ampicillin /sulbactam, Cefotaxime, Cefepime, Ceftazidime, Aztreonam, Co- Trimoxazol, Gentamycin, Amikacin, Tobramycin, Ciprofloxacin, Cefoxitin, Ceftriaxone, Meropenem, Kanamycin, Nalidixic acid, Norfloxacin, and Chloramphenicol.

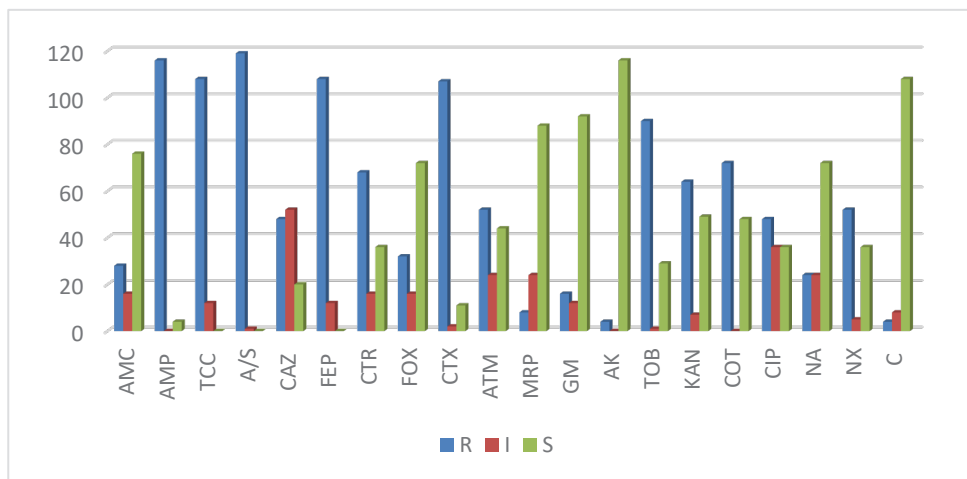


Fig. 1. Susceptibility of isolates *K. pneumoniae* to antibiotics

2.4 DNA Extraction

The DNA extraction technique included the following steps: a) A volume of a single milliliter of the bacterial growth in brain heart infusion (BHI) broth was obtained and transferred into a microcentrifuge tube with a capacity of 1.5 ml. The mixture was subjected to centrifugation at a velocity of 10000 r.p.m for 1 minute. b) Genomic DNA was gained from the bacterial cell pellets after discarding the supernatant. c) Genomic DNA was isolated using a commercially available extraction kit (Genomic DNA Promega Kit) according to the manufacturer's instructions. The DNA yield and integrity from bacterial cultures were assessed utilizing UV spectroscopy. A Shimadzu ultraviolet (UV)-1800 double-beam UV/Visible Scanning Spectrophotometer was used to measure the Optical Density (OD) for evaluating the purity of DNA at 260/280 nm.

2.5 PCR Amplification

As mentioned earlier [9], DNA samples (5 µL) were amplified using a PCR instrument in a 25µL reaction mixture utilizing the Sure Cycler 8800 (Agilent Technologies, Inc. USA). The *blaCtxm* gene was amplified using a specific thermal cycling pattern: The PCR technique comprises 40 cycles, beginning with an initial denaturation phase lasting 5 minutes at a temperature of 94°C. Afterward, denaturation is carried out for 1 minute at 94°C, annealing for 1 minute at 57°C, stretching for 1 minute at 72°C, and a final stretching was performed at the last 10 minutes. Gene of the *blaTEM* was amplified per the prescribed requirements: a primary step of denaturation at 94°C for 5 minutes, trailed by 35 denaturation cycles at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1.5 minutes, and a final stretching step at 72°C for 7 minutes.

Table 1 Alpha DNA Company, situated in Canada, offers an exhaustive compilation of the specific sequence primers used. The DNA fragments underwent electrophoresis on a 1% agarose gel at a voltage of 70 V for 1.5 hours. The gel was produced by combining TBE 1X buffer with 4 µL of ethidium bromide solution, which had a concentration of 10 mg/mL. The size of the DNA fragments was determined utilizing a 100-bp DNA ladder obtained from Bioneer, Korea. A solitary band was seen precisely at the designated position on a UV light transilluminator manufactured by Cleaver, a company based in the United Kingdom. The bands were acquired utilizing a gel documentation device manufactured by Cleaver in the United Kingdom.

Table 1. Primers utilized in the present study

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Stretching	Cycle
blaCTX-M	94°C / 5min	94°C / 1min	57°C/1min	72°C/1min	72°C/10min	40
blaTEM	94°C / 5min	94°C / 30sec	56°C/30sec	72°C/1.5min	72°C/7min	35

3 Results

3.1 Characteristics and Distribution of Patients

Table 2 reveals how the patients are dispersed and their demographic features among the four hospitals that supplied the samples. The majority of patients came from the Najaf province's AL-Sadar Medical City. Our study determined that 175 specimens, or 32.4% of the total, were acquired from males, whilst 365 specimens, equivalent to 67.6% of the total, were gathered from females. Depending on the place of the patient, 48.5% of the samples were obtained from urban patients, while 51.5% were obtained from rural patients. Table 3 presents the categorization of patients according to their gender and the geographical region of their home. Our observation revealed that persons between the ages of 1 and 20 had the greatest occurrence rate, while those between the ages of 21 and 40 had the lowest incidence rate, irrespective of gender.

Table 2. Gender and position of their Residence distribution of the patients.

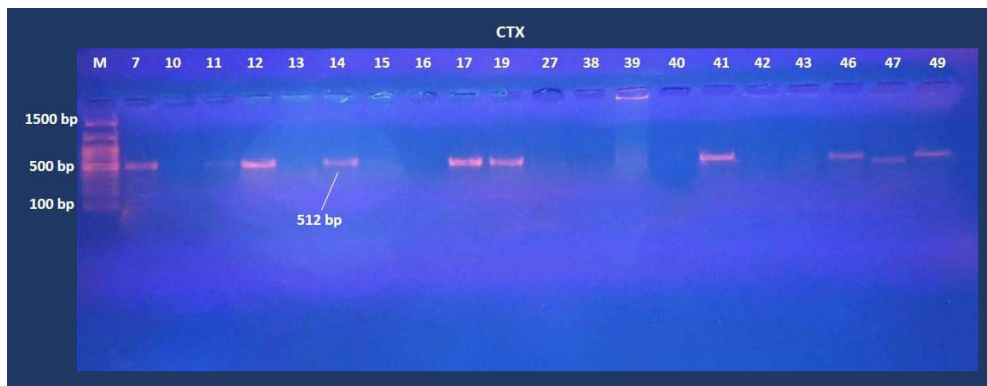
Hospital	Male	Female	Rural	Urban
Al-Sadr Medical City	64	128	78	81
Al-Hakeem General Hospital	58	102	69	76
Al-Furate Al-Awsat Hospital	32	56	52	45
Al-Zahra for Maternity and Children	21	79	63	76
Total	175	365	262	278
	540		540	

Table 3. Demographic breakdown of the patients by age and gender

Age(year)	Male	Female	Total %
1-20	68	118	186(34.4%)
21-40	25	64	89(16.5%)
41-60	38	78	116(21.5%)
>60	44	105	149(27.6%)
Total	175(32.4%)	365(67.6%)	(100%) 540

3.2 Detection of the blaCTX-M Gene

The CTX-M gene of *K. pneumoniae* was discovered in the isolates utilizing the PCR procedure, 87(72.5%) of *K. pneumoniae* isolates gave positive results with the blaCTX-M gene as in Figure 2.



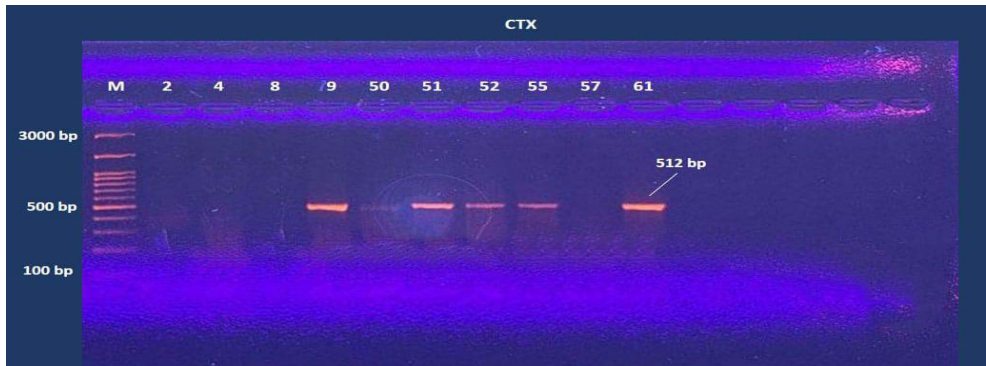


Fig. 2. PCR amplification products of *K. pneumoniae* isolates that amplified with blaCTX-M gene primers with product 512 bp. Lane (L), DNA molecular size marker (3000-bp ladder).

3.3 Detection of the blaTEM gene

According to the data shown in the Figure 3, the blaTEM resistance gene was found in 36 out of 120 (30%) *K. pneumoniae* isolates.

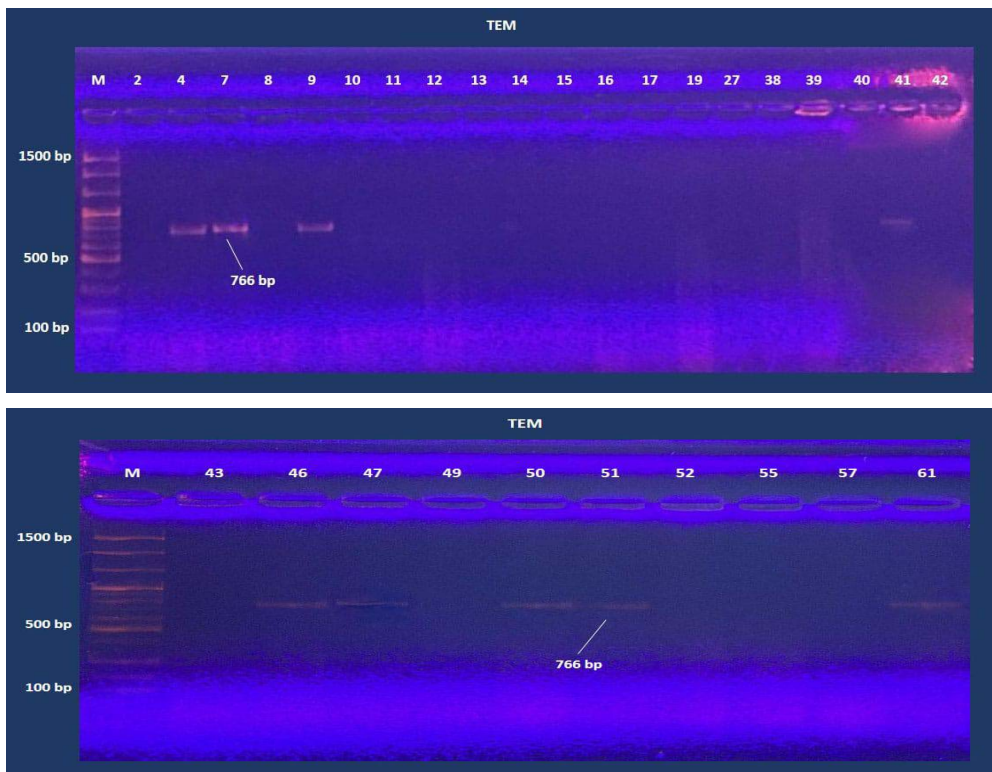


Fig. 3. PCR amplification products of *K. pneumoniae* isolates that amplified with blaTEM gene primers with product 766 bp. Lane (L), DNA molecular size marker (1500-bp ladder).

4 Discussion

Gram-negative bacteria such as *Klebsiella pneumoniae* have rod-like structures and are rod-shaped. It is not capable of movement and is surrounded by a protective layer called a polysaccharide capsule. This capsule plays a crucial role in the bacterium's ability to cause disease and is responsible for the colony's shiny and slimy look on agar plates [8]. The organism is a chemoorganotrophic bacterium that can function both aerobically and anaerobically. It exhibits both fermentative and respiratory metabolic processes [9]. An investigation was performed to recognize the presence of *bla*TEM and *bla*CTX-M genes in 120 isolates of *K. pneumoniae*. Of these isolates, 36 (30%) included *bla*TEM genes and 87 (72.5%) carried *bla*CTX-M genes, which are responsible for β -lactamases. As seen in Figures (2) and (3), the PCR products for *bla*TEM and *bla*CTX-M were 512 bp and 766 bp, respectively. The TEM-1 plasmid-mediated beta-lactamase was identified in the early 1960s. This enzyme exists in many variants known as TEM-type extended-spectrum beta-lactamases (ESBLs). Over time, microorganisms have acquired resistance to several antibiotics that are widespread [10]. The worldwide dissemination of *K. pneumoniae* strains that produce CTX-M enzymes is a significant issue in several nations, including Iraq [11]. The CTX-M was a novel enzyme of plasmid-mediated ESBLs that has emerged as the primary ESBL reported in the majority of global regions over the past decade. Its prevalence has increased significantly in numerous countries, including Iraq [12]. In a previous study, [13] The research revealed that 92% of *Klebsiella* isolates had the *bla*CTX-M gene, whereas 76% of bacterial isolates have the *bla*TEM genes of ESBL strains. Research conducted in the Eastern Province of Saudi Arabia found that *bla*CTX-M was present in 97.4% of Enterobacteriaceae strains, while *bla*SHV was discovered in 23.1% of isolates. None of the isolates were found to carry the *bla*TEM gene. The Brazilian analysis revealed that TEM and CTX-M type ESBL genes had the greatest occurrence rates among bacteria that produce ESBLs [14]. During this study, 64 *K. pneumoniae* isolates producing ESBLs, including *bla*TEM and *bla*CT-M, were found to harbor the *bla*TEM and *bla*CT-M genes, where TEM and CTX-M-type ESBLs represented the two primary ESBL families, respectively [15]. CTX-M-generating *Klebsiella pneumoniae* isolates are often seen in Iraq [16]. Identification of CTX-M enzymes in 8 isolates of *E. coli* and 4 clinical isolates of *K. pneumoniae* in Najaf was first reported in 2010 [17]. The prevalence of CTX-M-generating *K. pneumoniae* isolates in various countries is as follows: 61.8% in Turkey [18], 74.1% in Saudi Arabia [19], 54.5% in Kuwait [20], and 48.9% in the United Arab Emirates [21].

5 Conclusions

Utilizing the VITEK-2 system is essential to accurately verify the diagnosis of *K. pneumoniae* pathogens in urinary tract infections. The high incidence of *bla*TEM and *bla*CTX-M genes among *K. pneumoniae* isolates is a worrisome issue that requires effective management techniques.

Acknowledgments

The authors express their gratitude to the personnel of Al-Sader Medical City, Al-Hakim General Hospital, Al-Furate Al-Awsat Hospital, and Al-Zahra Maternity and Children in AL-Najaf city, Iraq, for their assistance in gathering urine samples from patients.

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