

# Study of Antibiotic Resistance and Prevalence of bla-TEM gene in *Klebsiella pneumoniae* Strains isolated from Children with UTI in Tabriz Hospitals

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## Abstract

**Introduction:** *Klebsiella pneumoniae* is one of the most important factors in children's urinary tract infections. Resistance to routine antibiotics is a major problem in the therapeutic process. Therefore, this study was conducted to determine the antibiotic resistance and to investigate the prevalence of *bla-TEM* gene in *K.pneumoniae* isolated from urinary tract infections.

**Methods:** This cross-sectional descriptive study was performed on 120 samples of *K.pneumoniae* isolated from children with urinary tract infection referring to hospitals in Tabriz. Samples were isolated and identified using standard laboratory methods and microbiology and culture in specific environments. Antibiotic resistance patterns of bacteria isolated by Kirby-Bauer disc diffusion method were performed. In order to identify the *bla-TEM* gene, the combined disk test was used as a phenotypic method and PCR method was used to identify the genotypes.

**Results:** The highest sensitivity to the antibiotics was Tazocin(88%) and Aztreonam (87%). The combined test results showed that 26 isolates producing Extended-Spectrum beta-lactamases, of which 22 were isolates with the *bla-TEM* beta-lactamase gene

**Conclusions:** By increasing the prevalence of resistance to antibiotics, rapid and timely diagnosis of antibiotic-resistant strains is necessary in order to select appropriate treatments and to prevent the spread of resistance.

## INTRODUCTION

Urinary tract infections are one of the most common infections in outpatient and hospitalized patients and urinary tract infection is the second most commonly occurring infection in humans, and its lack of diagnosis and timely treatment can cause severe complications such as urinary tract disorders, hypertension, Renal Failure, and uremia [1]. In studies in different societies, *Escherichia coli* gram-negative bacilli, *Klebsiella pneumoniae* have been reported as the most important causes of urinary tract infections [2, 3]. Klebsilas are opportunistic pathogens that can ferment glucose and lactose. This microorganism is a normal flora of the human body that causes a wide range of infections including sepsis, pneumonia, meningitis and urinary tract infections [4, 5]. High resistance and rapid spread of these microorganisms in different parts of the hospital cause major problems in treatment and cause septicemia and death [6]. Some organisms are inherently resistant to the number or even of all antimicrobial agents, but some are resistant to organisms resistant to other organisms through mutation or diffusion of genes [7]. The beta-lactamase is an inactivating antibiotic of the  $\beta$ -lactam family, the first identified

beta-lactamase, penicillinase. It was first isolated from *E.coli* in 1940. The first Extended-spectrum beta-lactamases (ESBL) antibiotic in the 1980s in Germany Following extensive use of ESBL antibiotics, it was identified [8, 9]. An antibiotic resistance gene transfer occurs through plasmids, transposons, and integrin [10]. The *bla-TEM* gene encoded by the plasmid in Enterobacteriaceae, which causes multi-drug resistance, entails an increase in the administration of broad-spectrum drugs [11]. *bla-TEM* was first isolated in 1965 from *E.coli*, a disease in Athens, Greece. They then appeared in 1969 in *Paeruginosa*, in 1973 in *Vibrio cholerae*, and in 1974 in *Haemophilus* and *Neisseria* species [12]. *bla-TEM* has the ability to hydrolyze ampicillin more than Carbenicillin, Oxacillin and Cefalotin, and has little activity against broad-spectrum Cephalosporin and inhibited by clavulanic acid. *TEM-1*, *TEM-2* and *TEM-3* have similar hydrolytic profiles [13-17]. Due to the high prevalence of antibiotic resistance genes and the spread of hospital-acquired infections, *K.pneumoniae* was isolated from children's urinary tract infections and the prevalence of *bla-TEM* gene was studied.

## METHODS

This cross-sectional descriptive study was carried out on 276 urine samples of children suffering from urinary tract infections in Tabriz hospitals during the 6 month period from May to October 2017. All specimens were identified using standard laboratory tests, microbiology and differential biochemical tests including TSI, SIM, MR-VP, citrate, urea. Antibiotic resistance pattern of *K.pneumoniae* isolates was carried out using disk diffusion method in agar according to CLSI. The antibiotics used included: Imipenem (10 µg), Cefazidime (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Nalidic Acid (30 µg), Ampicillin (10 µg), Cotrimoxazole (23.55 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Tetracycline (30 µg) and Aztreonam (30 µg) (Himedia Company). The standard strain of *K. pneumoniae* ATCC 700603 was used as a qualitative control. To identify isolates producing ESBLs, Combined Disk Test was performed using ceftazidime (30 µg), cefotaxime (30 µg), and ceftazidime / clavulanic acid (10 µg / µg) and cefotaxime / clavulanic acid (30µg / 10µg) manufactured by Himedia company. Plate temperature of 35° C, were incubated for a period 24 h. If the inhibition zone around the disk containing clavulanic acid, at least 5 (mm) from the disk without clavulanic acid was higher was considered as positive confirmatory tests for ESBLs. In this investigation, extraction of the genome of bacteria was performed by using extraction kit (InvitekStratec Business) (Made in Canada). PCR technique with specific primers (Table 1) was used to identify the *bla-TEM* gene. The reaction was 25 µl in volume, including 2.5 µl PCR Buffer 10X, 1 µl MgCl<sub>2</sub>, 0.75 µl dNTP

Mix, 1 µL Primer, 0.2 µl Taq Polymerase Enzyme and 3 µl DNA from each sample. The thermosecler thermal program was performed as follows: Initial denaturation at 94°C for 4 minutes, denaturation at 94°C for 46 seconds of 30 cycles, annealing at 59°C for 45sec of 30cycles, extension at 72°C for 45 seconds of 30 cycles and final extension at 72°C for 2 minutes. PCR product was electrophoresed on 2% agarose gel and the results were recorded.

After PCR testing, the PCR products in the 1.5% agarose gel in a TBE buffer were performed at 90 volts for 60 minutes. The gel was stained for 15 minutes in an Ethidium bromide tank and then the results were observed by geldocument with UV light. We used the strain of *E. coli* ATCC35218 as a positive control for the *bla-TEM* gene. The results were analyzed using SPSS software (version 19) and chi square test. In all cases, P < 0.05 was considered significant.

## RESULTS

Of the 276 urine samples of children, 120 isolates were identified as *K. pneumoniae*. 46 (38.33%) samples were female and 74 (61.67%) samples were male. 84 (70%) of samples were collected from hospitalized sector and 36 (30%) of them were collected from outpatients. The average age of the patient was 8.64±2.89, varying from at least 4 years to a maximum of 13 years. There was no significant relationship between age, sex, and *K. pneumoniae* infections (P > 0.05). The highest resistance was ampicillin 90% and ceftriaxone 64.2%, and the highest sensitivity was related to imipenem 90.8% and ciprofloxacin 84.16% (Table 2).

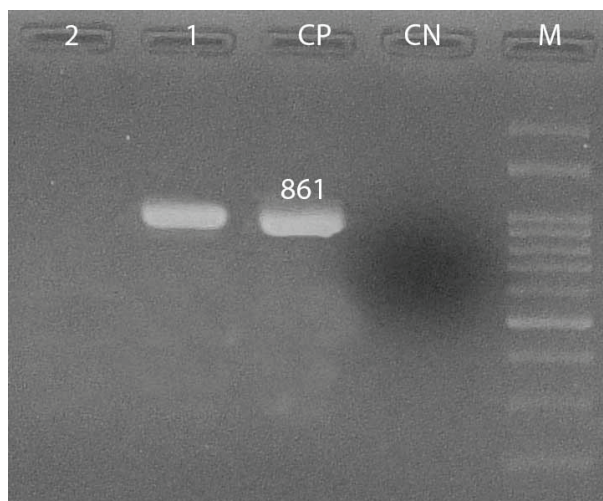
**Table 1:** Sequence of the Oligonucleotide Primers used for Detection of *bla-TEM* Gene

Primers	Nucleotide Sequences (5'-3')	Amplicon size (bp)	References
<i>bla-TEM</i>	F-GAGTATTCAACATTTCCGTGTC	861	[18]
	R-TAATCAGTGAGGCACCTATCTC		

**Table 2:** Antimicrobial Resistance Pattern of Strains isolated from Antibiotics used (%)

Antibiotics	sensitive	Intermediate	Resistant
Imipenem	90.8	0	9.2
Ceftazidime	38.3	0	61.7
Chloramphenicol	70.8	9.2	20
Ciprofloxacin	84.16	4.2	11.64
Gentamicin	67.5	1.7	30.8
Amikacin	56.7	11.7	31.6
Nalidic Acid	75	12.5	12.5
Ampicillin	4.2	5.8	90
Cotrimoxazole	42.5	3.3	54.2
Cefotaxime	40	2.5	57.5
Ceftriaxone	35.8	0	64.2
Tetracycline	57.5	6.7	35.8
Aztreonam	30.9	8.3	60.8

Combined disk test results for the phenotypic identification of ESBL producing isolates showed that 79 (65.8%) samples were positive ESBLs, but after performing PCR, 61 (50.8%) isolate containing *bla-TEM* gene were identified. The highest antibiotic resistance was found in ESBLs producing specimens.



**Figure 1:** Electrophoresis of the Products of PCR in Agarose Gel 1%, DNA Ladder: M, CP: Positive Control Strain (*E. coli* ATCC 35218), CN: Negative Control, 1: isolates containing *blaTEM* Gene

## DISCUSSION

Urinary tract infection is one of the most important human infections being ranked as the second after respiratory infection [19]. Along with *E. coli*, *K. pneumoniae*, *Proteus* and *Enterobacter* species are regarded as the most important causes of urinary tract infection [20]. However, Shahcheraghi et al. reported that the prevalence of *Klebsiella pneumoniae* -isolated from urinary samples- is more than other urinary pathogens [21]. The results of the present study indicate that girls, compared to boys, are more likely to develop urinary tract infection such as *K. pneumoniae*. There are several reasons for this issue; one of the reasons may be the shortness of the urethra and the proximity of its opening to the anus in women. In contrast, the anatomical system in male urinary tract is different having prostate secretions which contain bactericidal, zinc and cationic proteins. These factors, together, can play an important role in fighting the attack of urinary pathogenic bacteria [22]. Nowadays, extended-spectrum beta-lactamases are known to be a problem for patients admitted to hospital all over the world. The prevalence of extended-spectrum beta-lactamases varies among clinical species in different countries. Bacteria generating extended-spectrum beta-lactamases are often resistant to several antibiotic classes, leading to treatment failure and serious problems. The bacteria producing extended-spectrum beta-lactamases have been considered as a clinical threat and have caused physicians to be concerned about the treatment of infections caused by these organisms [23]. In this study, 120 isolates of *K. pneumoniae* were isolated from the 276 urine samples of children with UTI. Ramazanzadeh et al., Jarsiah et al., and Molazade et al. reported the frequency of *K. pneumoniae* in urinary tract infections to be 5.3%, 6.7% and 23.8%, respectively [24-26].

The results of the recent studies indicate the presence of ESBLs and *blaTEM* gene in all studied isolations. Several studies have reported the prevalence of ESBLs in hospital infections; a high percentage of these strains has had *blaTEM* gene [27]. According to Mirsalehian et al., Mehragan et al. and Riyadhani et al. 50%, 77.7% and 62.15% of isolates, respectively, were producing extended-spectrum beta-lactamases [18, 28, 29]. Tasli et al. (2005), Perilli et al. (2002) reported the prevalence of *blaTEM* gene in the hospital environment to be 52.7% and 56.4%, respectively [30, 31]; this is consistent with the findings of the present study. Shahcheraghi et al. (2007), reported that 69.6% of 50 isolates of *K. pneumoniae* generating ESBLs, had TEM genotypes [32]. Izadin et al. and Khosravi et al. reported that out of 43% and 47.27% of isolates of extended-spectrum beta-lactamase-generating *K. pneumoniae*, 87% and 34.6% had *blaTEM* gene [33, 34]. In the present study, resistance to amikacin and gentamicin was 31.6% and 30.8%, respectively. Kong et al. reported the resistance to amikacin and gentamicin to be 18.8% and 56.82% respectively [35]. Another study conducted by Nevine et al., in Egypt in 2008 showed that 90-100% of all isolates of *E. coli* and *K. pneumoniae* were resistant to aztreonam [36]; however, this rate was reported in the present study as 60.8%. Tsering et al. reported an antibiotic resistance of *Klebsiella pneumoniae* to CO-trimoxazole 75%, ciprofloxacin 52% and gentamicin 45% [37]. The least resistance in this study was to imipenem by 9.2%. Similar studies also showed the lowest resistance of *K. pneumoniae* strains to Imipenem [38-40]. Amin et al., Ishii et al. and Al-shara et al., introduced imipenem antibiotics as an effective drug in the treatment of *K. pneumoniae* [41-43]. Jazayeri from Semnan, Iran reported resistance to anti-biotics of ciprofloxacin (92.6%) and gentamicin (93%) [44]. Taslima et al. In 2007, in Bangladesh, reported the resistance of this bacterium to ceftazidime (36%), gentamicin (27%), tetracycline (27%), and ciprofloxacin (45%) [22].

## CONCLUSIONS

The findings indicated the highly reflective state of antibiotic resistance of *Klebsiella pneumoniae* beta-lactamases strains in the study area, which, if not sufficiently considered, will have irreparable health and therapeutic consequences in the upcoming years.

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## CONFLICTS OF INTEREST

There is no conflict of interest to declare.

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## AUTHORS' CONTRIBUTIONS

All authors worked equally in this work.

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