

Prevalence of Extended-spectrum Beta-lactamases (ESBL) Types *bla_{TEM}* and *bla_{SHV}* in *Klebsiella pneumoniae* Strains Isolated from Clinical Samples by PCR in Miandoab, West Azerbaijan

Mehdi Roshdi Maleki^{1*}  Javid Taghinejad¹ 

1. Department of Microbiology, Malekan Branch, Islamic Azad University, Malekan, Iran

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ABSTRACT

Background and Aim: Beta-lactamases are the most important factors in the resistance to beta-lactam antibiotics among gram-negative bacteria, especially *Klebsiella pneumoniae*. Nowadays, the prevalence of infections caused by extended-spectrum β -lactamases (ESBLs)-producing *K. pneumoniae* is increasing, as one of the emerging health problems throughout the world. This study aimed to investigate the prevalence of *bla_{TEM}* and *bla_{SHV}* genes in *K. pneumoniae* isolated from the clinical specimens in Miandoab in West Azerbaijan province.

Materials and Methods: In this study, 120 *K. pneumoniae* strains which were isolated from the clinical specimens in Miandoab hospitals were used. Then, an antibiotic susceptibility test was performed to determine ESBL-producing *K. pneumoniae* isolates using the combined disk method. The presence of *bla_{TEM}* and *bla_{SHV}* genes was detected by the polymerase chain reaction (PCR) technique.

Results: in the combined disk method, of 120 strains of *K. pneumoniae*, 71 (59.2%) were positive for ESBL. The *bla_{TEM}* and *bla_{SHV}* ESBLs were detected in 35 (49.3%) and 31 (43.7%) strains respectively. Eventually, the co-existence of *bla_{TEM}* and *bla_{SHV}* was detected in 5 (7%) isolates.

Conclusion: *bla_{TEM}* was the most common gene with a frequency of 49.3% in *K. pneumoniae* isolates.

Keywords: *bla_{TEM}*, *bla_{SHV}*, *K. pneumoniae*, Miandoab

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Corresponding Information: Mehdi Roshdi Maleki, PhD, Department of Microbiology, Malekan Branch, Islamic Azad University, Malekan, Iran.
Email: mehdiroshdi@gmail.com



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Introduction

In 1882, Carl Friedlander described *Klebsiella pneumoniae* for the first time. He described it as an encapsulated bacillus after isolating the bacterium from the lungs of those who had died from pneumonia. (1). *Klebsiella* spp causes a wide range of healthcare-related infections, including pneumonia, urinary tract infections (UTIs), bloodstream infections, wound or surgery infections, and sepsis (2, 3). It is, therefore, not surprising to state that this pathogen has

been identified by several organizations as an "immediate threat to human health" (4). Disproportionate and wrong use of antibiotics leads to the rapid expansion of antibiotic-resistant bacteria and antibiotic resistance genes (5, 6). *K. pneumoniae* has undoubtedly become the most common pathogenic bacterium in nosocomial infections due to its high virulence factor and general resistance to most antibiotics (7).

bla_{TEM} and *bla_{SHV}* are the 2 main types of ESBL genes. *bla_{TEM}* (found and isolated in the early 80s from Teminora who was a Greek patient), and *bla_{SHV}* (for a variable sulphhydryl which was first observed in a single *Klebsiella ozaenae* strain retrieved in Germany). These genes which are mediated by transposons, plasmids, or chromosomes are all sporadically described all over the world. Nowadays as one of the most mechanisms of resistance against beta-lactam antibiotics, it is considered amongst gram-negative bacteria (8). Beta-lactamase SHV was first discovered in 1972 by Pitton namely PIT-2. Later, because of its sulphhydryl, the name of SHV was replaced. The SHV precursor gene probably appears as a chromosomal gene in *Klebsiella* strains that is genetically transmitted to the plasmid and then spread in its way to other *Enterobacteriaceae* spp (9).

Many beta-lactamases are transported by mobile genetic elements such as plasmids, transposons, and integrons, resulting in the widespread diffusion of resistance factors from one bacterial strain to another. Therefore, identification of this type of antibiotic resistance gene is very important to implement infection control programs and prevent the spread of resistant strains. Because *K. pneumoniae* is the most common bacterium producing ESBLs, the major aim of this study was to determine the frequency of *bla_{TEM}* and *bla_{SHV}* types in *K. pneumoniae* strains isolated from clinical specimens in Miandoab by using PCR.

Materials and Methods

Collection and identification of bacterial isolates:

This descriptive-analytical cross-sectional study was conducted during 8 months from April to December 2019. A total of 120 bacterial isolates of *K. pneumoniae* were collected from clinical specimens in infectious wards of Miandoab hospitals. These specimens were examined considering *bla_{TEM}* and *bla_{SHV}* production after they were confirmed by implementing biochemical tests.

Antibiotic susceptibility test:

Turbidity equivalent to 0.5 McFarland was obtained from *K. pneumoniae* colonies and then an antibiogram was performed by disk diffusion agar or Kirby-Bauer

method to determine the patterns of antibiotic susceptibility of the isolates in Müller-Hinton agar (MHA) medium (10). Antibiotic susceptibility to 9 antibiotic discs including gentamicin (10 µg), cotrimoxazole (1.25 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), cefotaxime (30 µg), imipenem (10 µg), ceftazidime (30 µg), amoxicillin (30 µg) prepared from Padtan Teb Company and the sensitivity or resistance to antibiotics was verified according to the standard Clinical and Laboratory Standards Institute (CLSI) method (11).

To isolate ESBL producing strains, the combined disk method was used (12). Isolated strains were cultured on MHA and cefotaxime disk (30 µg) versus cefotaxime-clavulanic acid disk (30 µg/10 µg), ceftazidime disk (30 µg) versus ceftazidime-clavulanic acid (30 µg/10 µg), also the cefepime disk (30 µg) were placed in front of the cefepime-clavulanic acid disk (30 µg/10 µg) (Mast company) at a distance of 30 mm from each other. After incubating for 24 hours at 37 °C, the production of ESBLs was identified by measuring the diameter of the zone of inhibition (ZOI), which was demonstrated to be 5 mm or more, considering the combined disks (antibiotics with clavulanic acid) and concerning the single antibiotic discs.

DNA extraction and molecular analysis:

DNA extraction was performed by boiling method (13). For this purpose, a loop of the bacterial colony was transferred to a sterile micro tube containing 300 µl of sterile TE (1X) buffer and boiled for 10 min at 100 °C. At that time, it was centrifuged at 13,000 ×g for 10 min. After that, it was transferred from the supernatant to another micro tube for PCR. PCR reactions were performed for each sample in volumes of 25 µl using a thermal cycler with a temperature gradient (Eppendorf, Germany) and primers of Takapo Zist Company (Iran). The composition and schedule of PCR are shown in Tables 1 and 2. Analysis of 3 µl of the PCR products (amplicon) was performed by electrophoresis on 1.5% gel agarose. After electrophoresis and gel staining with GelRed™ DNA stains, the fragments were visualized under UV light in the gel documentation system (Gel Doc, ATP Co).

Table 1. Primer sets for amplification of the *bla_{TEM}* and *bla_{SHV}* genes and PCR reactions

Gene	Primer (5'-3')	Amplicon size (bp)	PCR Volume (25 µL)	Ref
<i>bla_{TEM}</i>	F: ATGCACCACCTGCACACAGG R: GGTGGTTTGTCCGGTTGTTC	861	Taq DNA Polymerase 2x Master Mix MgCl ₂ 4 mM (12.5 µL), Template DNA (variable), Forward and Reverse primer (0.5 µL for each), Nuclease-free water (10.5 µL)	(8)
<i>bla_{SHV}</i>	F: TCAGCGAAAAACACCTTG R: TCCCGCAGATAAATCACCA	475		

Table 2. PCR program.

Steps	Temperature (°C)	Time	Number of Cycles
Initial denaturation	95	4 min	1
Denaturation	94	30 sec	35
Annealing	60	30 sec	
Extension	72	60 sec	
Final extension	72	10 min	1

Results

Antibiotic susceptibility test:

The results of the evaluation of antibiotic susceptibility testing of *K. pneumoniae* strains which were isolated from clinical specimens are presented in [Table 3](#). In the antibiogram test by disk diffusion method, from 120 isolates of *K. pneumoniae*, the highest resistance were detected to cefotaxime, amoxicillin,

nalidixic acid, and cotrimoxazole. Moreover, an extreme sensitivity was found against imipenem, ciprofloxacin, ceftazidime, and cefepime.

In the combined disk method, of 120 strains of *K. pneumoniae*, 71 (59.17%) were positive for ESBL. In this method, if the diameter of the ZOI around the disk was 5 mm or more, it was considered as positive ESBL.

Table 3. Antibiogram test results.

Antibiotic	Sensitive		Intermediate		Resistant	
	Number	Percentage	Number	Percentage	Number	Percentage
Amoxicillin	56	46.67	-	-	64	53.33
Imipenem	83	69.17	7	5.83	30	25
Ceftazidime	60	50	5	4.17	55	45.83
Cefotaxime	50	41.67	5	4.17	65	54.17
Ciprofloxacin	69	57.50	11	9.2	40	33.33
Co-trimoxazole	50	41.67	10	8.33	60	50
Nalidixic acid	53	44.17	5	4.17	62	51.66
Gentamicin	55	45.83	15	12.50	50	41.67
Cefepime	58	48.34	16	13.33	46	38.33

PCR amplification of *bla*_{TEM} and *bla*_{SHV} genes

The presence of *bla*_{TEM} in the isolates was almost slightly higher than *bla*_{SHV} ([Table 4](#)). While the percen-

tage of the presence of both genes in an isolate was minimum (7%). The images of the electrophoresis test are presented in [Figure 1](#).

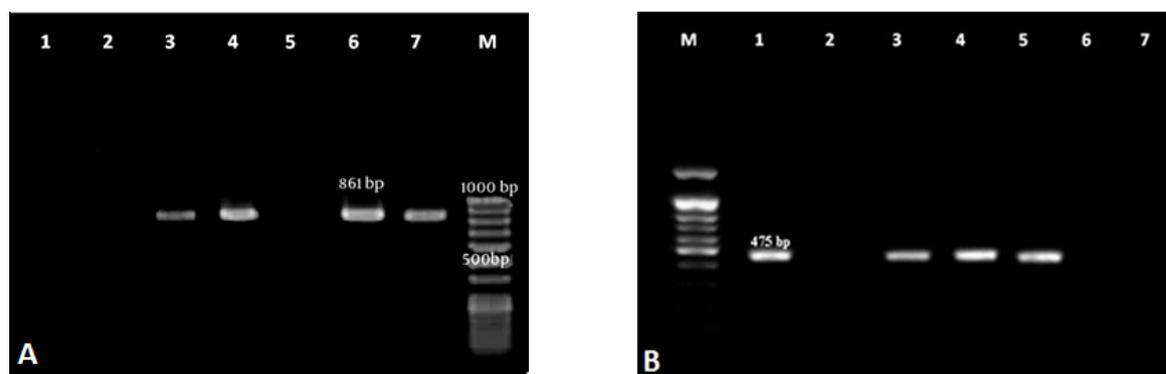
**Figure 1.** Electrophoresis results of PCR products. A) *bla*_{TEM}, B) *bla*_{SHV}, M: DNA ladder (100 bp).

Table 4. Frequency of studied genes based on PCR results.

Genotype	Abundance (%)
<i>bla</i> _{TEM}	35 (49.30)
<i>bla</i> _{SHV}	31 (43.70)
<i>bla</i> _{TEM} + <i>bla</i> _{SHV}	5 (7)
Total	71 (100)

Discussion

It is critically important to be aware of the trend of regional antimicrobial resistance among clinical isolates to make evidence-based recommendations and implement infection control programs and prevent the spread of resistant strains. In this study, the most resistance antibiotics to *K. pneumoniae* isolates were cefotaxime, amoxicillin, nalidixic acid and cotrimoxazole. The obtained results are consistent with several studies (14-16). This may be due to the publicity and familiarity of these drugs as well as their availability and utility. In the present study, imipenem from carbapenems, ciprofloxacin from fluoroquinolones, ceftazidime and cefepime from the 3rd and 4th generation cephalosporins were active and effective antimicrobial agents against isolated strains of *K. pneumoniae*, respectively. In a study conducted by Talebi et al. (16), the lowest resistance was reported to imipenem, which is consistent with the results of the current study. In this study, after imipenem, ciprofloxacin with 57.50% was the most effective drug against *K. pneumoniae*, which is consistent with the results of Mobin et al. (14), Ghane et al. (15) and Talebi et al. (16) investigations. The results were not in accordance with Archin et al. (17) and Derakhshan et al. (18) studies, which confirmed that more than 98% of *Klebsiella* strains showed sensitivity to imipenem. In recent years, ESBL-producing bacteria, especially *K. pneumoniae*, have emerged as a serious pathogen in hospitals and the community, causing various infections such as pneumonia, urinary tract infections (UTIs), and bloodstream infections (2, 4, 7). The incidence of the ESBL in the specimens of clinical isolates is extremely different throughout the world and is rapidly changing over time. In this study, it was found that ESBL phenotype was observed to be positive in 71 isolates (59.17%). Similar results have been reported in various countries such as Sudan (19), Iran (20), India (21) and Turkey (22). However, the results of the present study are more valuable than the similar studies conducted in Germany (23), Switzerland (24) and Saudi Arabia (25). On the other hand, other studies in China (26), Nigeria (27) and India (28) showed that the results considering the prevalence of *K. pneumoniae*-causing strains of ESBL are different from the outcomes of the present study.

In this study, imipenem with 69.17% was identified as the most effective drug against *K. pneumoniae* in vitro. Imipenem has also been introduced as an effective antibiotic in the treatment of *K. pneumoniae* in Ishii et al. (29) in Japan, Bratu et al. (30) in the United States, Soltan Dalal Mohammad et al. (31) in Iran, Amin et al. (32) in Jordan, Al-shara et al. (33) in Saudi Arabia and Tawfik et al. (34).

Molecular studies showed that *bla*_{TEM} and *bla*_{SHV} genes were present in ESBL-producing strains with 49.30% and 43.70% frequency, respectively. In the study of Feizabadi et al. (35), the prevalence of the above genes in *K. pneumoniae* strains were 54% and 67.4%, which contradicts the results of the present study. The frequency of the *bla*_{TEM} gene in *K. pneumoniae* strains studied by Archin et al. (17) in Shiraz, Iran was 38.34%. Likewise, the frequency of *bla*_{TEM} and *bla*_{SHV} genes in *K. pneumoniae* strains performed by Ghane et al. (15) in Tehran, Iran were reported to be 41.5% and 10.7%, respectively. In Ranjbar et al. (36) study, the prevalence of *bla*_{TEM} and *bla*_{SHV} genes in *K. pneumoniae* strains were 41.9% and 54.8%. The frequency of the *bla*_{SHV} gene in their study was higher than the results of the current study. In a comprehensive study conducted in Pakistan by Samyiy et al. (37) in 2019, the frequency distribution of *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV} genes in *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter baumannii* was examined. In their study, the frequency of *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV} genes in *K. pneumoniae* were 67%, 34% and 89% respectively.

The frequency of these genes in *K. pneumoniae* were 67%, 34%, and 89%, in turn. The prevalence of these genes in Doha, Qatar has been reported by Sid Ahmed et al. (38) as 53.2%, 40.4% and 64.1%.

Effendi et al. (2018) examined the presence of *bla*_{TEM} gene as well as drug resistance in *K. pneumoniae* isolates by sampling some foods such as beef, chicken, and fish. The researchers report that the highest drug resistance was to amoxicillin and that 9 of the 10 isolates identified had the *bla*_{TEM} gene (39). Ugbo et al. (2020) also studied 267 bacterial isolates of *E. coli* and *K. pneumoniae* from patients with wound and urinary tract infections to evaluate antibiotic resistance and identify beta-lactamase genes. Similarly, of 89 isolates producing beta-lactamase, 5 strains were *K. pneumoniae* and the prevalence of *bla*_{TEM} and *bla*_{SHV} genes in both bacteria were 55% and 35% (40). Zhong et al. (2020), likewise, described that of 465 fecal samples examined, about 66% of healthy adults carry *K. pneumoniae*. Furthermore, the frequency of *bla*_{TEM} and *bla*_{SHV} genes were 66.27% and 17.02%, sequentially (41).

The comparison of the results of various studies shows that the prevalence of *bla*_{TEM} and *bla*_{SHV} genes in *K. pneumoniae* strains isolated from clinical speci-

mens in different countries and within a country varies from region to region. This may be due to differences in antibiotic protocols or host genetic diversity in each region.

Conclusion

The *bla_{TEM}* and *bla_{SHV}* were highly prevalent in ESBL isolates, and these enzymes played an important role in resistance to beta-lactam antibiotics. Moreover, comparing the results of the present study with studies conducted in other countries as well as different regions in Iran indicated that the prevalence of drug-resistant and ESBL-producing strains varies in

different regions. This was dependent on the infection control system in the area and how patients were treated. Generally, imipenem is known in most countries as an effective drug in the treatment of infections caused by *K. pneumoniae* strains.

Acknowledgment

None.

Conflict of Interest

The authors declared no conflict of interests.

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