

## Original Article

### Prevalence of TEM and SHV Genes in Clinical Isolates of *Klebsiella Pneumonia* From Mashhad, North- East Iran

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#### ABSTRACT

**Background & Objectives:** Extended-spectrum-B-lactamase (ESBL)-producing strains of *Klebsiella Pneumonia* are an important cause of many serious infections in hospitalized and nonhospitalized patients and delayed treatment of these infections in crease chance of death in patients. This study was performed to determine the prevalence of ESBL-producing *K. Pneumonia* and to evaluate the frequency of TEM and SHV genes among the clinical samples.

**Methods:** One hundred and thirty isolates of *K. Pneumonia* were collected at Imam Reza Hospital in Mashhad (Iran) from May 2011 to July 2012. ESBL production was determined by the double disk diffusion (DDs) test. PCR method was used to detect TEM and SHV genes.

**Results:** Of 130 patients with *K. pneumonia* infection 28 were out-patients and 102 hospitalized patients. The most specimens was urine samples (n=25 in out-patients, n=39 in hospitalized patients, totally 49.2%) followed by wound samples (n=3 in out-patients, n=21 in hospitalized patients, totally 21.5%), blood samples (n=19 in hospitalized, 14.6%). The prevalence of ESBL producing *K. pneumoniae* was estimated 43% (n=56) including three of ESBLs positive isolates from out-patients and 53 from hospitalized patients. Of 56 ESBLs positive isolates, 44(87.54%) TEM, 39(69.64%) SHV and in 27 cases (48.21%) both TEM and SHV were detected.

**Conclusion:** A high prevalence of ESBL-producing *K. Pneumonia* among the hospital isolates obtained of urinary followed by blood and wound samples were documented. The majority of them carried both TEM and SHV genes. Results of this study alarm for the physicians because treatment and control nosocomial infections for them were difficult.

**Keywords:** Extended-spectrum B-lactamases, *Klebsiella Pneumonia*, Cephalosporin

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## Introduction

Extended-spectrum-B-lactamase (ESBL)-producing strains of *Klebsiella Pneumonia* are an important cause of many serious infections such as urinary tracts infections (UTIs), pneumonia, liver abscess and wound infections in hospitalized and nonhospitalized patients. Infections with *K. pneumonia* are mostly acquired in a hospital or are happened in patients with diverse underlying diseases such as diabetes mellitus (1).

*K. Pneumonia* is one of the most common organisms which carries plasmids encoding extended-spectrum B-lactamases (ESBLs). Bacteremia with such strains is associated with higher rates of treatment failure and death (2). ESBLs producing *K. Pneumonia* are susceptible to carbapenems such as imipenem and meropenem. Both antibiotics are administered for the treatment of ESBLs producing *K. Pneumonia*. Recent concern is the emergence of strains which are resistant to imipenem(3). Acquiring the plasmid type of beta-lactamases TEM<sub>1,2</sub> and SHV1 genes and chromosomal type of SHV1 gene in *K. Pneumonia* with low level resistance to penicillins may cause high level resistance to these antibiotics (4) which these plasmids are easily transferred between bacteria. In a study in Turkey, prevalence of TEM and SHV genes in *Enterobacteriaceae* was reported 52.7% and 74.3%, respectively (5). In a similar study in India in 2010, of 25 ESBL-producing *K. Pneumonia* isolates 15 case (60%) had TEM gene and 18 case (72%) had SHV gene(6). In another study in Venezuela in 2011, the prevalence of ESBL-producing *K. Pneumonia* in hospital infections was reported 56.6% (7). The aim of this study was to evaluate the prevalence of ESBL-producing *K. Pneumonia* and to determine the frequency of TEM and SHV genes among the clinical samples.

## Materials and Methods

### Isolation Specimens

This cross-sectional study was carried out at the Imam Reza Hospital in Mashhad (Iran). Proposal has been approved by the regional Ethics Committee. One hundred and thirty samples of *K. Pneumonia* were collected from the hospital and its associated clinic from May 2011 to July 2012. Presence of *K. Pneumonia* species were confirmed by gram staining growth on blood agar, Mac conky agar oxidase test, catalase and growth on TSA, SIM (sulfide-in dole-motility), LIA, urea and Simon citrate media (8).

### Detection of ESBL Production

Phenotypic production ESBL was determined by the double disk diffusion (DDS) test according to clinical and Laboratory Standards Institute (CLSI) guideline. Briefly bacterial suspension in concentration of 0.5 MF (Mac Farland standard  $1.5 \times 10^8$  cfu/ml) was prepared in Brain-Heart broth media. Bacterial suspension was spread with sterile swab on a Muller-Hinton agar plate. Cefotaxim (30 µg) disk and cefotaxim (30µg)/ clavulanate (10µg) disk 25-30 mm apart (Center to Center) was placed on the media for ceftazidime (30µg); ceftazidime/ clavulanate (30/10 µg) and cepodoxim (30 µg); cepodoxime plus clavulanic acid (30/10µg) disks (Mast.uk) are acting similarly. The plates were incubated for 18-24 h at 37 °C. A  $\geq 5$ -mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone indicated ESBL production (9).

### DNA Extraction

2-3 colonies of the bacteria were suspended in 500µL of double distilled water (DDW). Suspensions were boiled for 15 min and were then centrifuged at 5000 rpm for 10 min at room temperature. Supernatant was transferred to a new micro tube and were stored at 20°C (10).

### Multiplex PCR

A fragment of 471bp SHV gene was amplified by Specific Primer: Forward 5'-TCA GCG AAA AAC ACC TTG-3' and Reverse 5'- CCC GCA GAT AAA TCA CCA -3' and 861bp TEM gene was amplified by forward primer 5'-GAG TAT TCA ACA TTT CCG TGT C-3' and Reverse 5'-TAA TCA GTG AGG CAC CTA TCT C-3'. In our previous study, the PCR primers were designed by aligning nucleotide sequences of TEM and SHV variants downloaded of the NCBI/GenBank database in mega5 software for finding conserved sequences; then the conserved sequences were used in GenRunner & DNAMAN softwares for designing the primers. For evaluating the designed primers, the amplified fragments were sequenced and registered to GenBank (Accession: GU338981.1; Accession: GU338980.1; Accession: GU338979.1; Accession: GU338986.1; Accession: GU338985.1; Accession: GU338984.1; Accession: GU338983.1; and Accession: GU338982.1). The blaTEM and SHV genes were amplified under the following conditions: initial denaturing of 95°C for 2 min followed by 35 cycles including denaturing of 91°C for 1 min, annealing of 52°C for 30 s, and extension of 72°C for 1 min; the cycles were followed by a final extension of 72°C for 5 min. The amplification fragments were visualized on 1.5% agarose gel stained with ethidium bromide. They were photographed using an ultraviolet transilluminator. The reaction volume was 25 µl and contained 2.5 µl of 10X PCR reaction buffer with 1.5 µl MgCl<sub>2</sub> (25 mM), 0.8 µl (320 µM) deoxynucleoside triphosphates mix (dNTPs, 10 mM), 1 µl of each primers (10 pm/µl) with 0.1 µl (5 U/µl) Taq DNA polymerase. One microlitre of the prepared template DNA was added to the reaction mixture. PCR quality control was performed using *K. Pneumonia* GenBank Accession no: GU338979-84 that in our previous study was sequenced.

### Statistical Analysis

Descriptive statistics were used to plot graphs and tables.

### Results

A total of 130 patients with *K. Pneumonia* infection were included. This sample included 28 out-patients and 102 hospitalized patients. Among 130 patients with *K. Pneumonia* infection, the most common specimen with this infection was urine samples (n=25 in out-patients, n=39 in hospitalized patients, totally 49.2%) followed by wound samples (n=3 in out-patients, n=21 in hospitalized patients, totally 21.5%), blood samples (n=19 in hospitalized, 14.6%). The prevalence of ESBL producing *K. Pneumonia* was estimated 43% (n=56). Characteristics of patients with infections caused by ESBLs producing *K. Pneumonia* and non-ESBLs producing *K. Pneumonia* are shown in Table 1.

**Table 1-** Characteristics of patients with infections caused by ESBL-positive *K. Pneumonia* and ESBL-negative *K. pneumonia* isolates

Patient characteristics	ESBL <sup>+</sup> N=56	ESBL <sup>-</sup> N=74	Total N=130
<b>Gender</b>			
Female	23	42	65
Male	33	32	65
<b>Referral</b>			
Out-Patients	3	25	28
Hospitalized	53	49	102
<b>Source samples</b>			
Urine	24	40	64
Wound	8	15	23
Blood	12	7	19
<b>Respiratory Secterctions</b>	6	1	7
<b>Other samples</b>	6	11	17
<b>Average age</b>	34	43	39

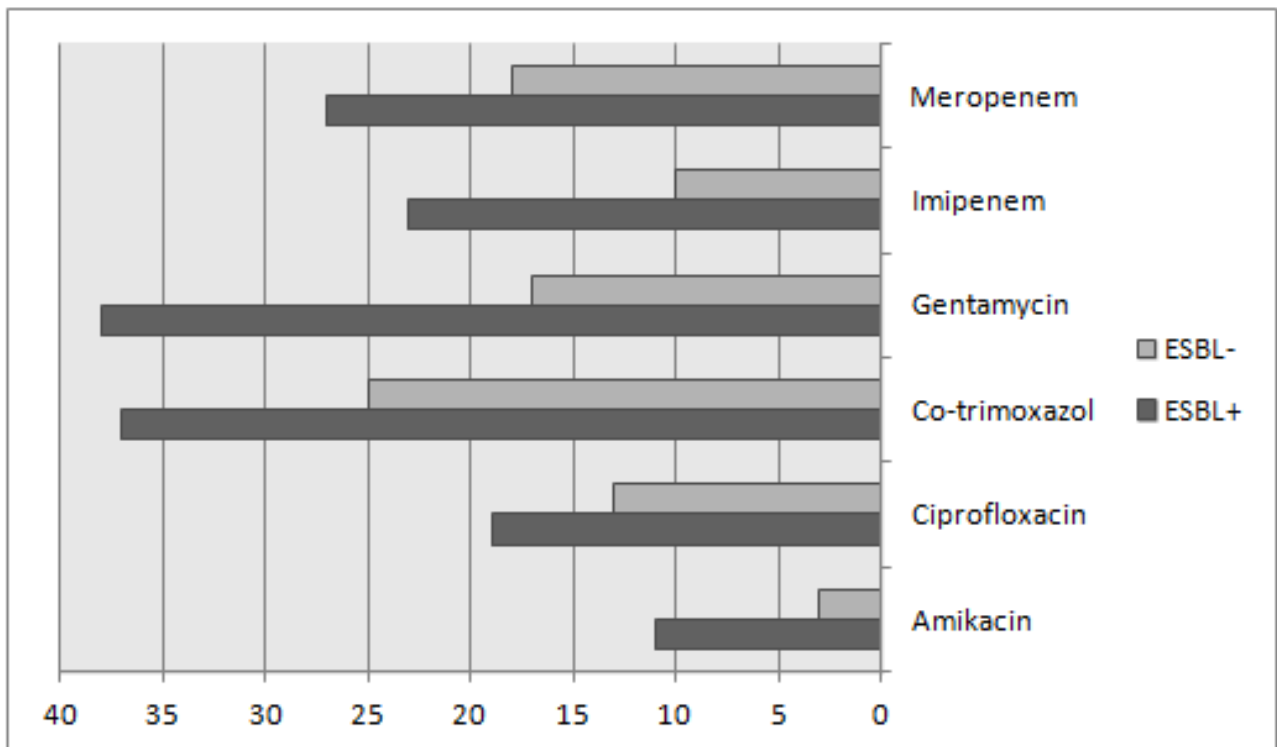
\*ESBL: Extended-spectrum-B-lactamase

Our findings showed that of the total of 56 ESBL positive samples, 17 (30.4%) had TEM and 12 (21.4%) had SHV genes. While 27 (48.2%) carried both gene TEM and SHV (Fig. 1). Comparison patterns of resistance to antibiotics

amikacin, ciprofloxacin, co-trimoxazol, gentamicin, meropenem and imepenem among ESBLs producing *K. Pneumonia* and non- ESBLs producing *K. Pneumonia* are shown in Fig. 2.



**Fig. 1:** PCR results for SHV and TEM genes. Lane numbers 1-4, 6, 8, 9, 12-14 and 16 showed a 471bp fragment of SHV gene. Lane numbers 1, 2, 4-12, 14-16 showed a 861bp fragment of TEM gene. Lane L represents 100bp DNA Ladder. Lane C- : negative control. Lane C+: positive control



**Fig. 2:** Antibiotic resistance profile of ESBL- *K. Pneumonia* and non ESBL- *K. Pneumonia* isolates.



## Discussion

*K. Pneumonia* is important pathogen acquired in hospitals and has the potential of causing sever disease. B-lactamase production is the most critical antibiotic resistance mechanism in the pathogen (11). The first paper of plasmid-encoded B-lactamase was published in 1983 (12). Broad-spectrum beta-lactamase is able by hydrolyzing expanded-spectrum cephalosporins lead to resistance to these drugs. Production of ESBL was initially reported from Germany and France but it was later on discovered in other regions of the world (13). This increasing trend is a major threat because use of cephalosporins for treating infections caused by these strains is not satisfactory as a result of the failure-treatment and high mortality to follow also in the case of epidemic infection control it will be problematic. Our study estimated the prevalence of ESBLs

producing *K. Pneumonia* to be 43%. The prevalence of ESBLs producing *K. Pneumonia* was reported from other cities of Iran such as Ilam, Tehran and Tabriz as 39.4%, 50.7% and 45.8%, respectively (14). A study by Riyahi Zaniani *et al.* in Mashhad in 2010 showed the prevalence of 20% for ESBLs producing *K. Pneumonia*(10). According to our results, the prevalence of ESBLs producing *K. Pneumonia* has been increased in recent years and for the screening of these strains has not been properly monitored. In other countries such as Saudi Arabia, India, Venezuela, Skopje and South Korea a rate of 55%, 66.7%, 47.6%, 31% and 16.8% was reported, respectively these differences are probably due to differences in the geographic area (7, 15-18).

Prevalence of ESBLs producing *K. Pneumonia* isolated from various specimens is summarized in Table 2.

**Table 2-** Prevalence of ESBLs producing *K. Pneumonia* isolated from various specimens

Place of Research	References	Specimens (%)					
		Aspirates	Blood	Sputum	Purulent	Urine	Wound
Korea	19	57.1	14.8	-	-	35.4	-
Saudi Arabia	20	-	7	-	-	57.5	17
	21	-	19.5	-	-	51.2	22
Thailand	22	-	18.68	30	-	23.78	-
Venezuela	7	-	30.1	-	43.3	-	-
Iran	Current Study	10.7	21.4	-	-	42.9	14.3

In our study 51.96% of ESBL+ specimens were related to hospitalized patients while only 10.7% of ESBL+ specimens belonged to out-patients. In Saudi Arabia, similar to our study, ESBLs producing *K. Pneumonia* had the highest prevalence in urine samples (20, 21). However, in Thailand, Korea and Venezuela, sputum, aspirates and purulent samples, respectively, had the highest frequency of ESBLs producing *K. pneumonia* (22, 19, 7). In our study, similar

to the study performed in Venezuela, ESBLs producing *K. Pneumonia* in blood samples had high prevalence while in Korea, Saudi Arabia and Thailand, blood samples were third rank (Table 2). Detection of ESBLs in these samples should be considered further.

The prevalence of TEM and SHV genes was reported in studies performed in different regions of the world are summarized in Table 3.

**Table 3-** Prevalence of TEM and SHV genes in ESBLs producing *K. Pneumonia* reported in studies performed in different parts of the world

Place of Research	Reference	Prevalence (%)		
		TEM Gene	SHV Gene	TEM & SHV
Skopje	17	16	19.5	-
Malaysia	23	90.2	7.8	-
Brazil	24	89.1	79.7	-
India	16	54.9	32.9	-
	25	48.4	20.3	26.5
Turkey	5	52.7	74.3	32.4

In our study, 44 samples out of 56 ESBL-positive cases were positive by PCR for TEM genes, 39 samples were SHV gene positive and in 27 samples, both TEM and SHV genes were positive. These results were similar to studies in Malaysia, Brazil and India (16, 23- 25). In our study, TEM genes among ESBLs producing *K. Pneumonia* showed a higher prevalence than SHV genes while in Skopje and Turkey, SHV genes were more prevalent. Therefore, the distribution pattern of ESBL genes in ESBLs producing *K. Pneumonia* is varies in different geographical regions.

### Conclusion

A high prevalence of ESBL-producing *K. Pneumonia* among the hospital isolates obtained of urinary followed by blood and wound samples was documented and the majority of them carried both TEM and SHV genes. Almost more than 50% of ESBL+ samples were resistant to impenem and meropenem. Such strains are a threat to the lives of patients. By detection of genes encoding ESBLs, drug resistance was much faster than methods based culture in order to assist the clinician in selecting appropriate treatment. It also shows the multi-drug resistance (MDR) among *K. Pneumonia*, which encourages the proper administration and use of antibiotics. It also raises this issue among hospital personnel to be aware of the possibility of the transmission of such bacterial strains.

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### References

1. Chuang YP, Fang CT, Lai SY, Chang SC, Wang JT. Genetic determinants of capsular serotype K1 of *Klebsiella Pneumonia* causing primary pyogenic liver abscess. *J Inf Dis* 2006;193(5):645-54.
2. Tumbarello M, Spanu T, Sanguinetti M, Citton R, Montuori E, Leone F, *et al.* Bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Klebsiella Pneumonia*: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob Agents Chemother* 2006;50(2):498-504.
3. Woodford N, Tierno PM, Jr., Young K, Tysall L, Palepou MF, Ward E, *et al.* Outbreak of *Klebsiella Pneumonia* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. *Antimicrob Agents Chemother* 2004; 48(12):4793-9.
4. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988;10(4):867-78.
5. Tasli H, Bahar IH. Molecular characterization of TEM- and SHV-derived extended-spectrum beta-

- lactamases in hospital-based Enterobacteriaceae in Turkey. *Jpn J Infect Dis* 2005;58(3):162-7.
6. Sharma J, Sharma M, Ray P. Detection of TEM & SHV genes in *Escherichia coli* & *Klebsiella Pneumonia* isolates in a tertiary care hospital from India. *Indian J Med Res* 2010;132:332-6.
  7. Tedesco Maiullari RM, Guevara A. Enterobacteria producers of extended-spectrum beta-lactamase in a hospital from Venezuela. *Infez Med* 2012;20(2):93-9.
  8. Enterobacteriaceae. In: Connie R. Mahon DCL, George, Manuselis., editors. *Textbook of Diagnostic Microbiology*. Fourth ed: W.B. Saunders Company; 2011. p. 427-62.
  9. d'Azevedo PA, Goncalves AL, Musskopf MI, Ramos CG, Dias CA. Laboratory tests in the detection of extended spectrum beta-lactamase production: National Committee for Clinical Laboratory Standards (NCCLS) screening test, the E-test, the double disk confirmatory test, and cefoxitin susceptibility testing. *Braz J Infect Dis* 2004;8(5):372-7.
  10. Ziani FR, Meshkat Z, Naderi Nasab M, Khaje-Karamadini M, Ghazvini K, Rezaee A, et al. The Prevalence of TEM and SHV Genes among Extended-Spectrum Beta-Lactamases Producing *Escherichia Coli* and *Klebsiella Pneumonia*. *Iran J Basic Med Sci* 2012;15(1):654-60.
  11. Ramdani-Bouguessa N, Manageiro V, Jones-Dias D, Ferreira E, Tazir M, Canica M. Role of SHV beta-lactamase variants in resistance of clinical *Klebsiella Pneumonia* strains to beta-lactams in an Algerian hospital. *J Med Microbiol* 2011;60(Pt 7):983-7.
  12. Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella Pneumonia* and *Serratia marcescens*. *Infection* 1983;11(6):315-7.
  13. Valverde A, Coque TM, Garcia-San Miguel L, Baquero F, Canton R. Complex molecular epidemiology of extended-spectrum beta-lactamases in *Klebsiella Pneumonia*: a long-term perspective from a single institution in Madrid. *J Antimicrob Chemother* 2008;61(1):64-72.
  14. Ghafourian S, Sekawi Z, Neela V, Khosravi A, Rahbar M, Sadeghifard N. Incidence of extended-spectrum beta-lactamase-producing *Klebsiella Pneumonia* in patients with urinary tract infection. *Sao Paulo Med J* 2012;130(1):37-43.
  15. Al-Agamy MH, Shibl AM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum beta-lactamase-producing *Klebsiella Pneumonia* in Riyadh, Saudi Arabia. *Ann Saudi Med* 2009;29(4):253-7.
  16. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A. Extended spectrum beta-lactamases in *Escherichia coli* & *Klebsiella Pneumonia* & associated risk factors. *Indian J Med Res* 2009 Jun;129(6):695-700.
  17. Kaftandzieva A, Trajkovska-Dokic E, Panovski N. Prevalence and molecular characterization of Extended Spectrum Beta-Lactamases (ESBLs) producing *Escherichia Coli* and *Klebsiella Pneumonia*. *Prilozi* 2011;32(2):129-41.
  18. Lee KM, Yoo JI, Yoo YS, Yoo JS, Chung GT, Ahn TI, et al. Investigation of *Klebsiella Pneumonia* isolates producing SHV-12 and SHV-11 beta-lactamases in Korean hospitals. *J Microbiol Biotechnol* 2009;19(9):1065-9.