

## Genotype distribution of extended Spectrum $\beta$ -Lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*.

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### Abstract

Extended-spectrum beta-lactamase (ESBL) production is the most important cause of beta-lactam resistance in Gram-negative bacteria. Although it may also be found in other Gram-negative bacteria, ESBL is most commonly produced by *Escherichia coli* and *Klebsiella pneumoniae* strains. In this study, we aimed to investigate the distribution of  $\beta$ -lactamase genes in ESBL-producing *E. coli* and *K. pneumoniae* strains. One hundred and twenty isolates of *E. coli* and *K. pneumoniae* isolated from clinical samples were used in this study. The identification and the antibiotic susceptibility tests were performed by VITEK 2 system in accordance with the manufacturer's instructions. ESBL production was determined according to Clinical and Laboratory Standards Institute guidelines. The DNA isolation was performed with a commercial kit following company recommendations. *bla*<sup>TEM</sup>, *bla*<sup>SHV</sup> and *bla*<sup>CTX-M</sup> genes were amplified by multiplex PCR with specific primers. Of the 120 isolates collected, 84 isolates were of *E. coli* and 36 isolates were of *K. pneumoniae*. *bla*<sup>TEM</sup> gene was the most prevalent type (85.8%) followed by *bla*<sup>CTX-M</sup> (83.3%) and *bla*<sup>SHV</sup> (24.2%). No *bla*<sup>SHV</sup> gene was detected in the *E. coli* strains. Among 120 ESBL-producing strains, 10.8% were susceptible to cefepime, 10.0% to ceftazidime, while 5.0% to ceftriaxone. In conclusion, *bla*<sup>TEM</sup> gene was the most frequently encountered ESBL of *E. coli* and *K. pneumoniae* in our hospital. Further molecular surveillance and epidemiological studies of such resistant bacteria are recommended for monitoring and controlling the spread of ESBL producing strains.

**Keywords:** Extended-spectrum beta-lactamase, *Escherichia coli*, *Klebsiella pneumoniae*, TEM, SHV, CTX-M

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### Introduction

Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria due to their outer membrane structure of the cell walls, and acquire the multiple resistances with the transfer of genetic materials and/or the selective pressure of the antibiotics. Extended-spectrum beta-lactamase (ESBL) production is the most prominent cause of beta-lactam resistance in Gram-negative bacteria that mediate a resistance to penicillins, cephalosporins and monobactams [1]. Recently, infections caused by ESBL producers have become an emerging public health concern worldwide because ESBL-producing isolates exhibit multidrug resistant phenotype, including the resistance to aminoglycosides and fluoroquinolones, and limit the treatment options available [2].

TEM, SHV and CTX-M, which are the three basic types of ESBLs, have been isolated worldwide. Of over 300

variants of ESBL have been defined and the strains expressing CTX-M-type ESBLs have rapidly spread over the past decade. The genes encoding ESBLs, usually carried on plasmids, facilitate their spread among Gram-negative bacteria [3,4]. Although it may also be determined in other Gram-negative bacteria, ESBLs are most commonly produced by *E. coli* and *K. pneumoniae* strains.

Detection of ESBL is initially based on phenotypic tests, such as the double-disc synergy test and combined disc method. However, these tests are time-consuming and inhibited by the AmpC  $\beta$ -lactamases. Over the past years, PCR has replaced traditional phenotypic methods [5].

The molecular detection of the common ESBL genes and the antimicrobial resistance can provide reliable information about their epidemiology. In this study, we aimed to investigate the distribution of *bla*<sup>TEM</sup>, *bla*<sup>SHV</sup> and *bla*<sup>CTX-M</sup> genes in ESBL-producing *E. coli* and *K. pneumoniae* strains by multiplex PCR.

## Material and Methods

One hundred and twenty isolates of *E. coli* and *K. pneumoniae* used for this study were isolated from clinical samples in the Microbiology Laboratory of Selcuk University, Faculty of Medicine, in the year 2013. The identification and the antibiotic susceptibility tests were performed by VITEK 2 system (bioMerieux, France) in accordance with the manufacturer's instructions. ESBL production was determined by double-disc synergy test according to Clinical and Laboratory Standards Institute (CLSI) guidelines [6]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the control strains.

The DNA isolation was performed with a commercial kit (Qiagen, Germany) following the company recommendations. Multiplex PCR was carried out to detect *bla*<sub>TEM</sub>,

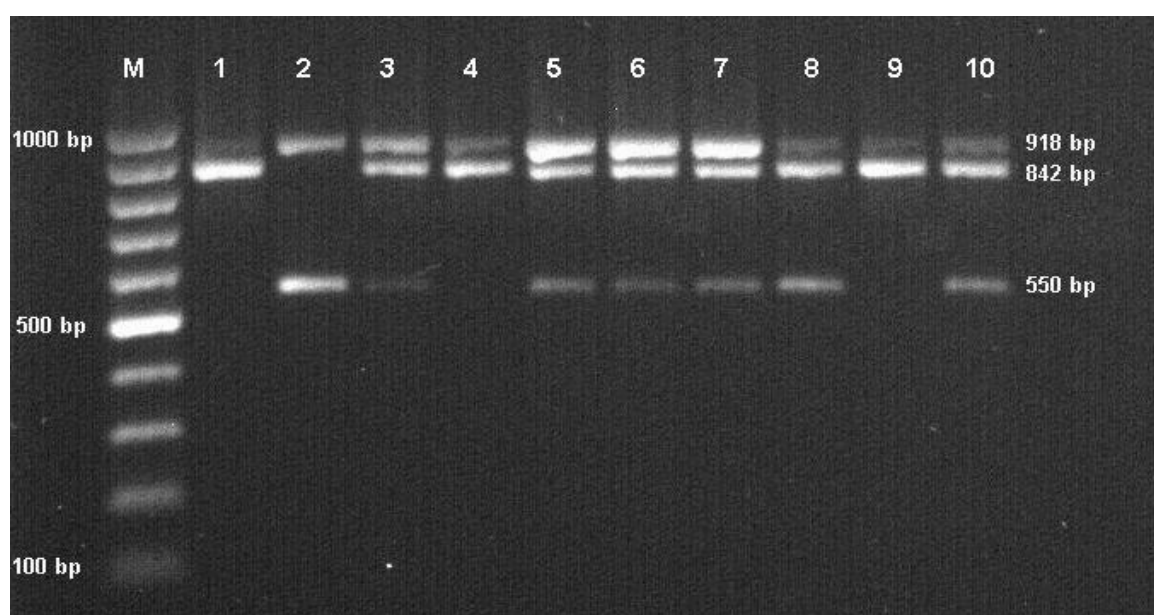
*bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes with specific primers. PCR amplification steps included an initial denaturation at 95°C for two minutes and following 30 cycles at 95°C for 45 sec, annealing at 62°C for 45 sec, elongation at 72°C for one minute and a final elongation at 72°C for five minutes. The PCR products were analyzed on 2% agarose gel and visualized with UV light transilluminator staining 0.5µg/mL ethidium bromide. Presence of bands at 918 bp, 842 and 550 bp was considered positive for the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes respectively [7].

## Results

Of the 120 collected isolates, 84 isolates were of *E. coli*, and 36 isolates were of *K. pneumoniae*. Single gene was detected in 23 (19.2%) isolates, various combinations of the genes were found in 96 (80%) isolates. *bla*<sub>TEM</sub> gene

**Table 1.** Distribution of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes in ESBL- producing *E. coli* and *K. pneumoniae* isolates.

ESBL type	<i>E. coli</i>	<i>K. pneumoniae</i>	Total Number (%)
TEM	12	2	14 (11.7)
SHV	0	2	2 (1.7)
CTX-M	7	0	7 (5.8)
TEM + SHV	0	3	3 (2.5)
TEM + CTX-M	65	4	69 (57.5)
CTX-M +SHV	0	7	7 (5.8)
TEM + SHV+ CTX-M	0	17	17 (14.2)
Negative	0	1	1 (0.8)
Total	84	36	120 (100)



**Figure 1.** Agarose gel electrophoresis of *bla*<sub>TEM</sub> (918bp) *bla*<sub>SHV</sub> (842bp) and *bla*<sub>CTX-M</sub> (550bp) genes: M-Marker (100-1000 bp); Lane 1-SHV positive control strain (*K. pneumoniae* ATCC 700603); Lane 2- TEM and CTX-M positive strain; Lane 3,5,6,7,8,10-TEM, SHV and CTX-M positive strains; Lane 4,9- TEM and SHV positive strains

was detected in 103 isolates (85.8%), *bla*CTX-M in 100 (83.3%), and *bla*SHV in 29 (24.2%). No *bla*SHV gene was detected in *E. coli* strains. All isolates were susceptible to colistin and tigecycline. Among 120 ESBL-producing strains, 98% were susceptible to meropenem, 97% to ertapenem, 76% to amikacin, 52% to piperacillin-tazobactam, 46% to gentamicin, 33% to trimethoprim-sulfamethoxazole, 18% to levofloxacin, 10.8% to cefepime, 10.0% to ceftazidime, and 5.0% to ceftriaxone. However, all strains were resistant to ampicillin and amoxicillin clavulanate.

## Discussion

The ESBL-producing bacteria have been dramatically spreading worldwide, requiring continuous monitoring systems and effective infection control measures. The use of antibiotics, particularly third-generation cephalosporins, and the transfer among hospitals are risk factors for acquisition of ESBL-producing bacteria [2,3]. Treatment options of infections caused by ESBL-producing isolates are becoming increasingly limited because ESBL-producing bacteria are generally resistant to cephalosporins and aminoglycosides [8,9]. In agreement with this, most of the strains are susceptible to meropenem, ertapenem and amikacin, but the resistance rates against third-generation cephalosporins, trimethoprim-sulfamethoxazole, gentamycin and levofloxacin are very high in our study.

The prevalence of ESBL producers and the distribution of ESBL genotypes vary markedly in different geographical areas. Nowadays, the CTX-M type  $\beta$  lactamase is predominant among ESBLs all over the world [10-12]. Similarly, CTX-M-type is the most common  $\beta$ -lactamase found in Turkey too. According to the results of the multicenter 'HITIT Study' in Turkey, during 2004 and 2005, the rate of CTX-M-type  $\beta$ -lactamase was 71.4%, TEM 49.4% and SHV 46.7% in *E. coli* and *K. pneumoniae* isolates [13]. In another study, *bla*CTX-M genes were detected in 167 out of 200 (83.5%) *Enterobacteriaceae* isolates obtained from various clinical samples in Istanbul [14]. In a nationwide study from Turkey, CTX-M1 was the most prevalent (83.18%) gene followed by TEM-1b (44.09%), CTX-M2 (31.81%) and SHV-11 (1.81%) in *E. coli* isolates [15]. In a different study, the percentage of CTX-M, TEM and SHV in *E. coli* were determined to be 93%, 64% and 11%, respectively in urinary isolates acquired from community [16]. In contrast to these studies, among non-duplicate 41 ESBL positive isolates of *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., 35 (85.4%) had *bla*TEM, 33 (80.5%) *bla*SHV and 20 (48.8%) *bla*CTX-M [17]. In another study, SHV (92.9%) was detected as the most prevalent ESBL type, and TEM and CTX-M (64.3%) were equivalent in *K. pneumoniae* strains [18]. In our study, TEM (85.8%) and CTX-M (83.3%) types were approximately at the same rates, while SHV was detected

in 24.2% of the strains and all of them were *K. pneumoniae* strains. These differences may be caused by several factors such as type and number of the samples, number of the isolates studied, genus and species of the isolates.

*bla*TEM is a broad spectrum  $\beta$ -lactamase that is always combined with CTX-M in the same plasmid and the combinations of these genes are frequently seen in the ESBL producing strains [10]. Sharma et al. [19] observed that 77.5% of isolates carried more than one type of  $\beta$  lactamase genes, while 32.5% of isolates harbored three  $\beta$  lactamase genes, 22.5% TEM and CTX-M, 15% SHV and CTX-M, and 7.5% TEM and SHV in ESBL producing *E. coli* and *Klebsiella* spp. isolates. In our study, while single gene was detected in 23 (19.2%) isolates, various combinations of the genes were found in 96 (80%) isolates. The coexistence of the TEM and SHV was 2.5%, TEM and CTX-M 57.5%, CTX-M and SHV 5.8%, and TEM, SHV and CTX-M 14.2%. Alike in a study from Turkey, TEM and CTX-M were found together in 52%, TEM, SHV and CTX-M were 5%, and CTX-M and SHV were 1.8% [16]. On the other hand Bali et al., [20] noticed that most of the ESBL isolates (80.8%) carried one type of  $\beta$  lactamase genes.

In conclusion, *bla*TEM gene was the most frequently encountered ESBL of *E. coli* and *K. pneumoniae* in our hospital. There was no *bla*SHV gene in *E. coli* strains. The detection and identification of beta lactamases are essential for a reliable epidemiological investigation. The molecular surveillance and epidemiological studies of such resistant bacteria are recommended for monitoring and controlling the spread of ESBL producing strains.

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