



## Investigation of Extended-Spectrum Beta-Lactamase (Esbl) in Nosocomial Gram-Negative Bacterial Isolates by Different Test Methods

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### Abstract

*The aim of this study is to investigate the existence of extended-spectrum beta-lactamase (ESBL) in nosocomial Gram-negative bacteria isolates and to compare different test methods that detecting ESBL. The strains producing the largest quantities of these enzymes are Klebsiella pneumoniae (K. pneumoniae), Klebsiella spp., and Escherichia coli (E. coli).*

**Keywords:** Extended spectrum beta-lactamase, gram-negative bacteria, nosocomial infection

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## Introduction

The increase in morbidity and mortality due to hospital infections, coupled with the rising cost of treatment, render the application of infection control strategies necessary [1]. Resistance to antibiotics has become a major problem due to their widespread and uncontrolled use in hospitals. The issue of resistance is becoming particularly crucial in intensive care units, where high-dose parenteral broad-spectrum antibiotic use is necessary. Gram-negative bacteria play an important role in the etiology of nosocomial infections [2].

The development in gram-negative bacteria of resistance to antibiotics and the importance of the nosocomial infections of which these microorganisms are the cause are both on the rise, and treatment with existing antibiotics is becoming difficult. The production of beta-lactamase is the most important mechanism for the development of beta-lactam resistance in nosocomial Gram-negative bacteria [3]. ESBL are beta-lactamase enzymes with a broad spectrum that are found in many Enterobacteriaceae. The strains producing the largest quantities of these enzymes are *Klebsiella pneumoniae* (*K. pneumoniae*), *Klebsiella* spp., and *Escherichia coli* (*E. coli*). When these enzymes are produced, the microorganism enters a state in which it is highly effective at inactivating various beta-lactams [4].

ESBL production in Turkey ranges from 11% to 86%. The twofold reason for such a huge difference is socio-economic and cultural variations, and dissimilar and widespread antibiotic use among patient groups.

Several methods of detecting ESBL-producing bacteria have been propounded, including such tests as: the DDST, the CDT, the three-dimensional test, the E-test, observing the resistance to Ceftazidime, using a higher density of bacteria, disk diffusion in clavulanic acid medium, automated Vitek, and Microscan [5,6].

The aim of this study is to investigate the frequencies of extended-spectrum beta-lactamase (ESBL) nosocomial gram-negative bacterial isolates by different test methods that detecting ESBL-producing bacteria. In this study the presence of ESBL was detected with; E-test, the three synergy tests - the combined disk test (CDT), the double disk synergy test (DDST) and the disk diffusion test (DDT) and the results were compared for there sensitivity.

## Materials and Methods

The study interpreted the presence of ESBL in 68 strains of *E. coli* and 47 strains of *Klebsiella* spp. responsible for hospital infections in the Cukurova University School of Medicine's Balcali Hospital between 29 May 2003 and 30 September 2004. Bacteria from the Central Laboratory isolated as infectious agents were identified using the automated Vitek2 (BioMerieux, France) system. For all tests, the cultures were introduced into Mueller-Hinton agar and interpreted the following day.

In this study, we considered the ESBL test based on the determination of the E-test as a reference method. The results obtained from the disk diffusion test (DDT), the combined disk test (CDT), and the double-disk synergy test (DDST) were compared with those of the E-test.

In the DDT, suspected ESBL isolates were identified according to the CLSI criteria for four antibiotics (Ceftazidime zone  $\leq 22$ mm, cefotaxime zone  $\leq 27$ mm, ceftriaxone zone  $\leq 25$ mm, and Aztreonam zone  $\leq 27$ mm) and by evaluation of others (amikacin, piperacillin/tazobactam, imipenem, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid) (CLSI) (Figure 1).

In the CDT, both Cefotaxime and Ceftazidime were tested alone and with clavulanic acid, and the result was judged to be ESBL-positive in the event of a  $\geq 5$ mm diameter increase obtained with clavulanic acid (CLSI).

In the DDST, the presence of ESBL was evaluated by depositing amoxicillin-clavulanic acid in the middle of the plate and Ceftazidime, Cefotaxime, Ceftriaxone, and Aztreonam at 20 mm distances around the perimeter of the disks.

The E-test was deemed to be ESBL positive in the event of a Ceftazidime/Ceftazidime-clavulanate or Cefotaxime/Cefotaxime-clavulanate rate  $\geq 8$  (Figure 2).

Statistical analysis was conducted using SPSS software version 20.0 package program. A value of  $p < 0.05$  was considered statistically significant. McNemar Test was used to compare the groups. To evaluate the tests; sensitivity, specificity, positive predictive value and negative predictive value was calculated.

## Results

Of the 47 *Klebsiella* spp. strains in this study, ESBL production was detected in 21 (44%) using the CDT, 27 (57%) with the DDST, and 30 (63%) with the E-test ESBL strips.

The figures for the 68 *E. coli* strains' test results were: CDT 34 (50%), DDST 36 (52%), and E-test strips 42 (61%).

The zone diameters with the DDT were interpreted, ESBL production was considered to be present in 73 (63%) of the total 115 strains. With the E-test, the existence of ESBL was observed in 72 of these 73 (Table 1).

Zone diameters of the disk diffusion test (DDT) (73% patients or 63% ESBL positive) were found to be similar to the E-test results.

The sensitivity of the DDT was 100% and specificity 97.7%, the sensitivity of CDT was 80.8% and specificity 100%. The sensitivity of the DDST was calculated as 88.8% and the specificity as 100% (Table 2).

The rates of resistance to third-generation cephalosporins were, for *Klebsiella* spp. strains: Ceftriaxone 65.9%, Cefotaxime 61.7%, Ceftazidime 53.2%, and Aztreonam 59.6%; and for *E. coli* strains: Ceftriaxone 61.8%, Cefotaxime 57.4%, Ceftazidime 51.4%, and Aztreonam 58.8%. The *Klebsiella* spp. and *E. coli* strains were determined to be most resistant to Trimethoprim/Sulfamethoxazole — 85.10% and 80.88%, respectively — whereas they were most susceptible, at 95.74% and 100%, to Imipenem.

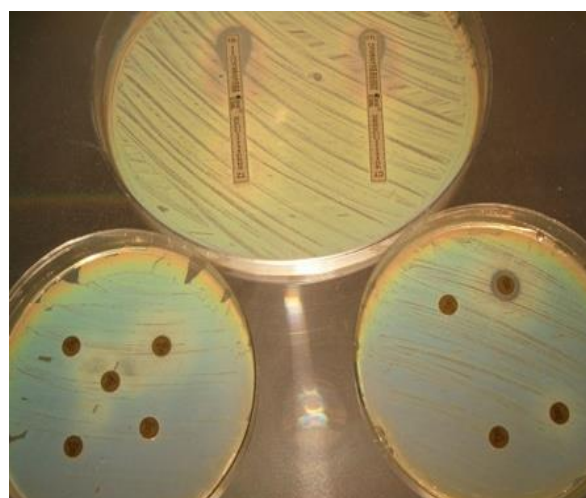
**Table 1:** MIC values according to the E-test interpretation.

| Enzim Grubu<br>(İzolat sayısı) | Seftazidim |       | Seftazidim/klavulanat |              | Sefotaksim |              | Sefotaksim/klavulanat |              |
|--------------------------------|------------|-------|-----------------------|--------------|------------|--------------|-----------------------|--------------|
|                                | MIC50      | MIC90 | MIC50                 | MIC90(µg/ml) | MIC50      | MIC90(µg/ml) | MIC50                 | MIC90(µg/ml) |
| ESBL pozitif(n=72)             | > 32       | >32   | 0.094                 | 0.380        | >16        | >16          | 0.032                 | 0.094        |
| ESBL negatif(n=43)             | < 0.50     | >32   | 0.094                 | >4           | <0.25      | >16          | 0.023                 | >1           |

**Table 2:** Detection of ESBL; comparison of other tests with E-test

|                |                   | E TEST                 |                        | Sensitivity | Specificity | NPV*  | PPV** | P***   |
|----------------|-------------------|------------------------|------------------------|-------------|-------------|-------|-------|--------|
| TESTS          |                   | ESBL                   | ESBL                   |             |             |       |       |        |
|                |                   | Positive (+)<br>strain | Negative (-)<br>strain |             |             |       |       |        |
| DISK           | ESBL Positive (+) | 72                     | 1                      | 100.0       | 97.7        | 100.0 | 98.6  | 1,000  |
| DIFFUSION TEST | ESBL Negative (-) | 0                      | 42                     |             |             |       |       |        |
| E TEST         |                   |                        |                        | 80.8        | 100         | 71.6  | 100   | 0,0001 |
| COMBINED       | ESBL Positive (+) | 55                     | 0                      |             |             |       |       |        |
| DISK TEST      | ESBL Negative (-) | 17                     | 43                     | 88.8        | 100         | 82.6  | 100   | 0,004  |
| E TEST         |                   |                        |                        |             |             |       |       |        |
| DOUBLE-DISK    | ESBL Positive (+) | 63                     | 0                      |             |             |       |       |        |
| SYNERGY TEST   | ESBL Negative (-) | 9                      | 43                     |             |             |       |       |        |

\*negative predictive value    \*\*positive predictive value    \*\*\*Mc Nemar test

**Figure 1.** ESBL positive specimen. Left, DDST. Right, DDT**Figure 2.** ESBL positive specimen with E-test, ESBL negative specimen with DDST and CDT

## Discussion

Studies conducted at various centers in our country report that the rate of nosocomial infections is between 2.4% and 11.8%, while the incidence of the Gram-negative bacteria that cause these infections is 40.8%<sup>6</sup>. Köseoglu Ö, Gür D. And Akova M. in a study at the University of Hacettepe, identified *E. coli* (33%) and *Klebsiella* spp. (22.1%) as the most common among such microorganisms in patients with bacteremia [7].

B.S. Kocazeybek and Arabacı Ü. found *E. coli* (31%) and *K. pneumonia* (22%) to be the causative agents in the nosocomial Gram-negative bacteria isolated from the intensive care units of four separate hospitals. The same study found no significant difference between ESBL-positive and -negative isolates in terms of the sensitivity to Imipenem, Amikacin, and Ciprofloxacin [8].

The *Klebsiella* spp. and *E. coli* strains in our study were determined to be most resistant to Trimethoprim/Sulfamethoxazole — 85.10% and 80.88%, respectively — whereas they were most susceptible, at 95.74% and 100%, to Imipenem.

In our study, we found that both ESBL-positive and -negative isolates were susceptible to Imipenem, and that this was due to the occurrence of different mechanisms of resistance to carbapenems. G. Sener and A. Yüce, in their study concerning strains of *K. pneumoniae*, found that while it had an ESBL production rate of 39%, it was 100% sensitive to Imipenem and Meropenem [9].

B.S. Kocazeybek and U. Arabaci used the E-test method in their study to identify the inducible beta-lactamase (IBL) using the ESBL double disk test. They discovered that Imipenem and Meropenem had sensitivities of 89.7% and 95.1%, respectively. They found slightly lower carbapenem activity in IBL-producing strains [8].

ESBLs are typically encoded in large plasmids of 80-300 KB and inactivate oxyimino-cephalosporins including penicillins and first-generation cephalosporins, as well as such broad-spectrum antibiotics as Cefotaxime, Ceftazidime, and Aztreonam<sup>10</sup>. However, in many cases these plasmids carry other resistance genes. For this reason, resistance to aminoglycosides, Trimethoprim/Sulfamethoxazole, and tetracycline in bacteria that have ESBL is normal [8,10-13]. In our study, we connect the development of resistance against Trimethoprim/Sulfamethoxazole to the frequent use of this drug in our hospital. In fact, prior use of Ciprofloxacin and/or Trimethoprim/Sulfamethoxazole was shown to be one of the independent factors in the occurrence of the colonization of multidrug-resistant strains of *Klebsiella* spp. and *E. coli* in 24 patients in a nursing home [14].

In Malaysia, the E-test was used to determine the existence of ESBL. The rates in *E. coli* and *Klebsiella* spp. were 36.7-38% and 5.6-7.0%, respectively. The same strains were found to be susceptible to Cefepime and Imipenem [15].

In a study performed in Bangladesh using DDST, the rates of ESBL in *E. coli* and *K. pneumoniae* were determined to be 43.2% and 39.5%, respectively [16].

From the point of view of being able to identify ESBL, the E-test and DDST were seen to have high sensitivity (98.2%) when taken as a reference test according to CLSI criteria. When we compared the four tests, 18 strains found to be negative with the combined disk test, nine strains with the E-test, and 18 with the DDST tested positive with the DDT. This suggests false positive or false negative results.

In England a study similar to ours was done using the MAST DD test(MAST Laboratories LTD. uses only two discs), the DDST, and the E-test. The sensitivity of the E-test and the MAST DD test were determined to be 93% [17].

The E-test has a sensitivity of 100% in SHV and TEM-type ESBLs [18].

Methods such as the E-test and the Vitek were inadequate to distinguish real ESBLs from false positive results in strains of *E. coli* and *K. oxytoca* that produce excessive K1 [19-21].

The E-test in our study had different results for ESBL in three specimens with two different strips. No one of the third-generation cephalosporins was able to detect all of the ESBLs by itself. This seems to have been caused by the ESBL substrate showing differences in spite of the high rate of similarities. It is accepted that for different types of ESBLs, application of the routine test with a single beta-lactam agent will prove insufficient in detection of these enzymes because of the changes in the optimal substrate profiles [22]. In addition, the reason we preferred the E-test over the microdilution test as an MIC test was the substantial influence of such factors as the E-test's ease of application, the inoculum density of its results, its prediffusion period, and phase of bacterial growth [23]. On the other hand, from a technical aspect, use of the E-test is exacting and a more expensive method than other tests.

The fact that the CDT, DDST, and E-test sensitivity was high in our study suggests that the production of TEM- or SHV-type enzymes in our hospital's strains was greater.

The sensitivity of the DDT was 100% and specificity 97.7% ,the sensitivity of CDT was 80.8% and specificity 100%. The sensitivity of the DDST was calculated as 88.8% and the specificity as 100%.

The three-dimensional test and dilution methods are time-consuming and difficult to implement in practice. The E-test is materially expensive, frequently used in laboratories, and occasionally creates difficulties in reading the results of beta-lactamase inhibitor diffusion towards beta-lactam antibiotics. As a conclusion; all the three test can be used and as phenotypic confirmatory tests both the DDST and the CLSI-recommended CDT are methods that are practical and easily applied in all laboratories. One advantage of the CDT is that it provides the convenience of using only two disks.

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## References

1. Uzun Ö. Hastane infeksiyonları: Tanımlar. In: Doğanay M, Ünal S, eds, Hospital Infections, 1. Baskı. Ankara: Bilimsel Tıp Yayınevi, 2003;1(4):3.
2. The Turkish Antimicrobial Resistance Study Group, Pfaller MA, Korten V, Jones RN, Doern Gary. [Multicenter evaluation of the antimicrobial activity for seven broad-spectrum beta-lactams in Turkey using the E-test method]. Diagn Microbiol Infect Dis. 1999;35(1):65-73.
3. Bilgehan A, Kayabaş Ü. Infection problems in intensive care units. Klimik Derg. 2001;14(2): 83-7
4. Murray BE. New aspects of antimicrobial resistance and the resulting therapeutic dilemmas. J Infect Dis. 1991;163(6):1185-94
5. Legrand P, Fournier G, Bure A, Jarlier V. Detection of extendedbroadpectrum beta-lactamases in Enterobacteriaceaein fourfrenchhospitals. Eur J Microbiol Infect Dis. 1989;8(6):527-9.
6. Gül M, Şensoy A, Çetin B, Korkmaz F, Seber E. Investigation of ticarcillin-clavulanic acid with E-test in nosocomial gram-negative bacills. Cukurova Med J. 2003;28:60-4.
7. Köseoğlu Ö, Gür D, Akova M. Nosocomial bloodstream infections in a Turkish university hospital: Study of Gram-negative bacilli and their sensitivity patterns. Inter J Antimicrob Agents. 2001;17(6):477-81.
8. Kocazeybek BS, Arabacı Ü. Use of E-tests with carbapenems for gram-negative rods producing beta-lactamases. Inter J Antimicrob Agents. 2002;19(2):159-62.

9. Şener GA, Yüce A. [Efficacy of Imipenem, meropenem and Cefaperazone-sulbactam in extended spectrum beta-lactamase producing *Klebsiella pneumoniae*]. DEU Tıp Fakültesi Dergisi. 2001;15:325-9.
10. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of the strains expressing an extended-sepectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. Clin Infect Dis. 2001;32(2):94-103.
11. Decre D, Gachot B, Lucet JC, Arlet G, Berezin EB, Regnier B. Clinical and bacteriologic epidemiology of extended-spectrum beta-lactamase producing strains of *K. pneumoniae* in a medical intensive care unit. Clin Infect Dis. 1998;27(4):834-44.
12. Shen D, Biedenbach DJ, Winokur PL, Pfaller MA, Jones RN. Phenotypic and genotypic characterizations of chinese strains of *E. coli* producing ESBL. Diagn Microbiol Infect Dis. 1999;34(3):159-64.
13. Palucha A, Mikiewicz B, Hyniewicz W, Gniadkowski M. Concurrent outbreaks of ESBL producing organisms of the family enterobacteriaceae in a Warsaw hospital. J Antimicrob Chemother. 1999;44(4):489-99.
14. Wiener J, Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K, Weinstein. Multiple antibiotic-resistant *klebsiella* spp. and *E. coli* nursing homes. JAMA. 1999;281(6):517-23.
15. The Malaysia/Singapore Antimicrobial Resistance Study Group, Biedenbach D, Lewis MT, Jones R. In vitro evaluation of cefepime and other broad-spectrum beta-lactams for isolates in Malaysia and Singapore medical centers. Diagn Microbiol Infect Dis. 1999;35(4):277-83.
16. Rahman MM, Haq JA, Hossain MA, Sultana R, Islam F, Islam S. Prevalence of ESBL producing *E. coli* and *K. pneumoniae* in a urban hospital in Dhaka, Bangladesh. International Journal of Antimicrobial Agents. 2004;24(5):508-10.
17. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of ESBL in members of the family Enterobacteriaceae: Comparison of the MAST DD test, the double disc and the Etest ESBL. Antimicrob Chemother. 2000;45(6):881-5.
18. Brown DFJ, Andrews J, King A, MacGowan P. Detection of ESBL's with E-test and double disc potentiation methods. Antimicrob Chemother. 2000;46(2):323-42.
19. Hall MAL, Fluit AC, Paauw A, Box ATA, Brisse S, Verhoef J. Evaluation of the E-test ESBL and the BD Phoenix, VITEK 1, and VITEK 2 automated instruments for detection of ESBL's in multiresistant *e. coli* and *klebsiella* spp]. J Clin Microbiol. 2002;40(10):3703-11.
20. Carter MW, Oakton KJ, Warner M, Livermore DM. Detection of ESBL in *klebsiellae* with the oxoid combination disc method. J Clin Microbiol. 2000;38(11):4228-32.
21. Sanguinetti M, Posteraro B, Spanu T, Cicaglione D, Romano L. Characterization of clinical isolates of Enterobacteriaceae from Italy by the BD Phoenix ESBL detection method. J Clin Microbiol. 2003;41(4):1463-68.
22. Cormican MG, Marshall SA, Jones RN. Detection of ESBL producing strains by the E-test ESBL screen. J Clin Microbiol. 1996;34(8):1880-84.

23. Kotapati S, Kuti JL, Nightingale CH, Nicolau DP. Clinical implications of ESBL producing *Klebsiella* species and *E.coli* on cefepime effectiveness. *J Infect.* 2005;51(3):211-17.