academicJournals

Vol. 7(32), pp. 4091-4095, 9 August, 2013
DOI: 10.5897/AJMR2013.5794
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Comparison of conventional method and automatized vitek sysytem in the detection of extended-spectrum beta-lactamase in *Escherichia coli* and *Klebsiella pneumoniae* isolates

**Gulfem Ece**

Department of Medical Microbiology Izmir University School of Medicine Medicalpark Hospital Yeni Girne Boulavard 1825th Street No:12 Karsiyaka/Izmir Turkey.

Accepted 28 July, 2013

**Beta-lactamase production is the most important mechanism in resistance development of Gram negative bacteriae. Extended spectrum beta-lactamases (ESBL) enzymes hydrolyse penicillin and cephalosporins. Many ESBLs are derived by genetic mutation from naturally occuring enzymes. ESBL can be detected by phenotypic and genotypic methods. In our study, we compared the ESBL detection performance of automatized Vitek version 2.0 and double disk synergy among *Escherichia coli* and *Klebsiella pneumoniae* isolates which were previously detected as ESBL (+) by combined disk method at Izmir University School of Medicine Department of Medical Microbiology. The comparison of double disk synergy (DDS) and automatized Vitek system (Biomerieux, France) in ESBL positivity detection of *E. coli* and *K.pneumoniae* strains which were isolated between 30.05.2012 to 23.01.2013 at Izmir University School of Medicine Medical Microbiology Laboratory (Izmir, Turkey) was evaluated. The ESBL detection of the strains was previouly studied by combination of ceftazidime/clavulanic acid (30/10 µg) and cefotaxime/clavulanic acid (30/10 µg). 117 isolates consisted of ESBL(+) 106 *E.coli* and 11 *K. pneumoniae*. Ten (%8.5) *E. coli* isolates were Vitek (-) and double disk synergy (+) for ESBL detection. Three *E. coli* isolates were Vitek (+) and DDS (-) for ESBL detection. All of the *K. pneumoniae* isolates were detected positive for ESBL by both Vitek system and DDS. There are various methods in ESBL detection. Although Vitek automatized system reported false negative results in our study, it can provide rapid data for routine laboratories. We think that further studies with greater number of isolates are necessary.**

**Key words:** ESBL, cephalosporin resistance, Vitek 2, Gram negative bacteria.

## INTRODUCTION

Extended spectrum beta-lactamases (ESBL) are an enzymes that are transmitted by plasmids and hydrolyse penicillin and cephalosporin group antibiotics. Many ESBL enzymes are derivatives of natural beta-lactamases like TEM-1, TEM-2 and SHV-1. These basic enzymes exist in Gram negative bacteriae especially in members of *Enterobacteriaceae* (Paterson et al., 2005; Bradford, 2001). ESBL was first defined in Germany in 1983; and then in France in 1985 from *Klebsiella* spp. strains. ESBL producing isolates are sensitive to carba-penems, and hydrolyse third generation cephalo-sporins. They are inhibited by beta-lactamase inhibitors like

E-mail: gulfem.ece@izmir.edu.tr; gulfem.ece@gmail.com. Tel: 902323995050-2135.

**Table 1.** The distribution of the methods DDS and Vitek system in ESBL detection of *K.pneumoniae* and *E.coli* strains.

| ESBL detection method | *E. coli* (n=106) | *K. pneumoniae* (n=11) |
|---|---|---|
| Vitek(+) | 96 (90.5%) | 11 (100%) |
| DDS(+) | 103 (97.1%) | 11(100%) |
| Vitek(+)/DDS(+) | 93 (87.7%) | 11(100%) |
| Vitek(+)/DDS(-) | 3 (2.8%) | 0 (0%) |
| Vitek(-)/DDS(+) | 10 (9.4%) | 0 (0%) |
| Vitek(-)/DDS(-) | 0 (0%) | 0 (0%) |

clavulanic acid (Perez et al., 2007).ESBL can be detected by phenotypic and genotypic methods (Tsering et al., 2009). Specific phenotypic methods to show ESBL have been defined since 1980s. E-test, double disk synergy (DDS), three dimentional test, dilution methods and automatized systems are routine phenotypic methods to detect ESBL. DDS is the first described method in ESBL detection. 'Key hole' appearance towards cefotaxime disk from 30 mm clavulanic acid disk indicates ESBL positivity.

Clavulanic acid containing E-tests are another method to show ESBL. Eliptic zone (phantom zone) indicates positivity. Combined disk method involves clavulanic acid contaning disks and zone diameter difference more than 5 mm detects ESBL positivity. Vitek ESBL test (Biomerieux,France) is an automatized method to detect ESBL. Turbidity reading after bacteria inocualtion to Vitek cards is done to show ESBL positivity (Drieux et al., 2008; Al-Jasser, 2006).

In our study, we compared the ESBL detection performance of automatized Vitek version 2.0 and double disk synergy among *E.coli* and *K.pneumoniae* isolates which were previously detected as ESBL (+) by combined disk method at our recently opened University Hospital.

## MATERIALS AND METHODS

117 ESBL(+) *E.coli* and *K.pneumoniae* strains isolated between 30.05.2012 to 24.01.2013 at Izmir University School of Medicine Medical Microbiology Laboratory (Izmir, Turkey) were included in this study. The comparison of double disk synergy (DDS) and automatized Vitek system (Biomerieux, France) in detection of 117 ESBL positive *E.coli* and *K.pneumoniae* strains was studied. The ESBL detection of the strains was previously studied by combination disk method.

### Combination disk method

0.5 McFarland turbid bacterial suspensions were prepared and streaked on Mueller Hinton agar. The combined disk of ceftazidime/clavulanic acid (30/10 µg) and cefotaxime/clavulanic acid (30/10 µg) were placed on Mueller Hinton agar. The ESBL positivity was evaluated as difference between zone diameters which were more than 5 mm.

### Double disk synergy method

0.5 McFarland turbid bacterial suspensions were prepared and streaked on Mueller Hinton agar to show DDS. Ceftriaxone, ceftazidime, and cefepime disks were placed 2.5 cm away from the center and amoxicillin-clavulanic acid disk was placed in the middle. Synergy between amoxcillin-clavulanic acid and other disks was considered as positive for ESBL positivity (CLSI 2005).

### Automatized Vitek version 2.0 system

Vitek cards included 1.0 mg/L cefepim, 0.5 mg/L cefotaxime or ceftazidim accompanying 10 or 4 mg/L clavunate including cards. After inoculation to the cards, trubidity reading was made. Proportional decrease in clavulanic acid containing and not containing cards indicated ESBL positivity. Quality control strains *E.coli* ATCC 25922 and *K.pneumoniae* 700603 were used.

## RESULTS AND DISCUSSION

A total of 117 ESBL positive strains isolated from urine specimens were included in this study. 106 strains were *E.coli* and 11 were *K. pneumoniae*. Ten (9.4%) *E.coli* isolates were Vitek (-) and double disk synergy (+) for ESBL detection. Three (2.8%) *E.coli* isolates were Vitek (+) and DDS (-) for ESBL detection. All of the *K. pneumoniae* isolates were detected positive for ESBL by both Vitek system and DDS (Table 1). ESBL(+) *E.coli* and *K. pneumoniae* antibiotic susceptibility results are shown in Tables 2 and 3. ESBL negative control was *E.coli* ATCC 25922. *K. pneumoniae* ATCC 700603 was used as the positive control. The combined disk method and DDS are shown consequently in Figures 1 and 2.

Beta-lactamase production is the most important mechanism for resistance against beta-lactam antibiotics in Gram negative bacteriae. Prevalent clinical use of extended spectrum cephalosporins led to production of these enzymes and they are called extended spectrum due to broad spectrum of these antibiotics (Akova, 2004).

Penicilin, cephalosporin, monobactam and carba-penems are frequently used worldwide. ESBL enzymes have hydrolytic activity over these antibiotics and this is the main reason of growing resistance problem. The recent increase in these enzymes cause trouble in treatment of infections due to these ESBL positive strains. E-test, DDS test, three dimesional test, dilution methods and automatized system are phenotypic methods in ESBL detection (Yurtman et al., 2009).

In our study, ten (9.4%) *E.coli* isolates were Vitek (-) and double disk synergy (+) for ESBL detection. Three (2.8%) *E.coli* isolates were Vitek (+) and DDS (-) for ESBL detection. All of the *K. pneumoniae* isolates were detected positive for ESBL by both Vitek system and DDS. The sensitivity for *E. coli* was 91.1% and specificity was 97.1% for Vitek system. *K. pneumoniae* strains performed 100% sensitivity and specificity with Vitek system in ESBL detection.

False negative ESBL results of the Vitek system is the

**Table 2.** The antimicrobial susceptibility of ESBL(+) *E.coli* isolates.

| ESBL(+) *E. coli* | AMP* | AMC** | CAZ*** | CTX& | GNµ | AK# | TZP± | SCF₸ | IMP¶ | MEM¥ | CİP€ | TG## |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n=106 | 0% | 0% | 0% | 0% | 38.9% | 50.4% | 23.3% | 14.3% | 100% | 100% | 100% | 100% |

AMP*, Ampsilin; AMC**, Amoksisilin/Klavulanik asit; CAZ***, Seftazidim; CTX&,Sefotaksim; TZP±, Tazobaktam/piperasilin; AK#, Amikasin; SCF₸, Sulbaktam/sefoperazon; IMP¶, İmipenem; MEM¥, Meropenem; GNµ, Gentamisin CİP€, Siprofloksasin; TG##, Tigesiklin

**Table 3.** The antimicrobial susceptibility of ESBL(+) *K. pneumoniae* isolates.

| ESBL(+) *K.pneumoniae* | AMP | AMC | CAZ | CTX | GN | AK | TZP | SCF | IMP | MEM | CİP | TG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n=11 | 0% | 0% | 0% | 0% | 45.4% | 54.5% | 27.2% | 27.2% | 100% | 100% | 100% | 100% |

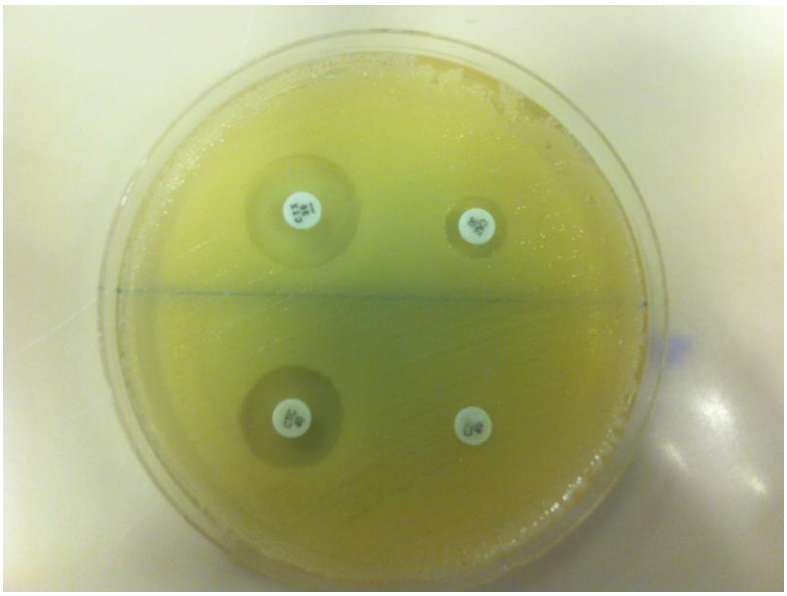

**Figure 1.** Combined disk method.

outcome of existence of *bla* $_{CTX-M}$ or *bla*$_{SHV}$ type beta-lactamases. Spanu et al. (2006) investigated ESBL existence in 1219 strains by Vitek system and molecular methods. Out of 312 isolates; 306 were ESBL positive and detected by Vitek system. Six strains had false negativity and *bla* $_{SHV-2}$ .[10] This similar situation could also have taken palce in our study; but we could not perform molecular genotypic studies to confirm this data (Spanu et al., 2006).

Dashti et al. (2006) compared the ESBL detection methods (Vitek, E-test, and DDS) in 251 *Enterobacteriaceae* members isolated in Scotland, Edinburgh and Kuwait. The strains consisted of *K. pneumoniae* (123), *E. coli* (114), *Klebsiella oxytoca* (7), *Enterobacter cloacae* (5) and *Citrobacter freundii* (2). The isolates were studied by automatized Vitek system (GNS-526 card) for ESBL detection. Fifteen (15) out of 101 strains from Edinburgh were ESBL negative by E-test; but positive with Vitek system. The two of the remaining 236 isolates were false negative by E-test, and 38 strains were also false negative with DDS test (Dashti et al., 2006). The authors concluded that E-test is the most reliable and expensive method. Vitek system can provide valuable and fast data although it has false negative ESBL(+) isolates. In our study we also detected false negative results similar to the above study.

Donaldson et al. (2008) compared Vitek AST N054 card and MASTDISCS ID ESBL detection disk diffusion to detect 137 ESBL(+) *E.coli* strains isolated from fecal samplesof a nursing home residents. Vitek AST N054 detected ESBL positivity in 93 isolates and MASTDISCS among 135 strains (Donaldson et al., 2008). The authors thought that AST N054 card was less reliable in detecting isolates with *bla*$_{CTX-M}$ . In our study, we showed the

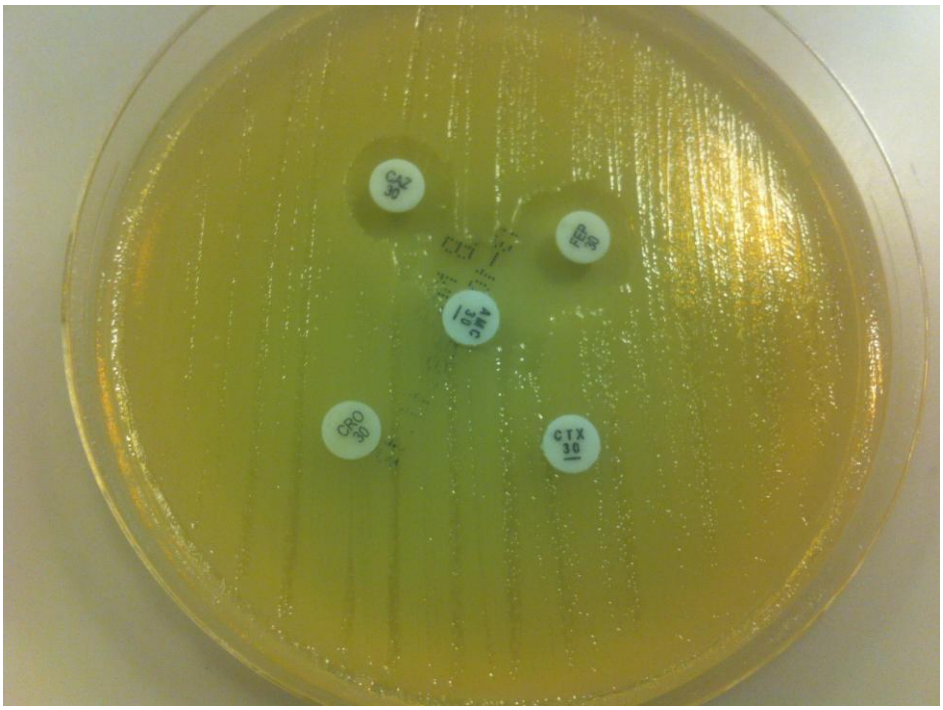


**Figure 2.** Double disk synergy method.

isolates that were ESBL positive with DDS; but negative by Vitek system and also we had Vitek(+) and DDS (-) results.

Thomson et al. (2007) compared Phoenix and Vitek 2 automatized systems for ESBL detection in ESBL positive and negative *E. coli*, *K. pneumoniae* isolates. Seventy six (76) isolates were ESBL positive and 26 were ESBL negative. Phoenix system showed 96% sensitivity in ESBL detection and Vitek system established 89% sensitivity. The specificity was 85% for Vitek system and this was higher for Phoenix system (Thomson et al., 2007). In our study we showed that 10 strains were positive with DDS and negative with Vitek system. We did not perform a comparison with Pnoenix system. The sensitivity in *E.coli* isolates in our study was higher for Vitek system.

Sorlozano et al. (2005) compared DDS, E-test and Vitek system in detection of 115 ESBL positive and 284 ESBL negative *E.coli* isolates. The three methods indicated similar sensitivity, specificity and positive predictive values (Sorlozano et al., 2005). The authors stated that automatized system is an accaptable assay in ESBL detection of *E.coli* strains. In our study, we also found Vitek system as a reliable method for ESBL detection.

Valenza et al. (2011) studied 51 ESBL (+) and 50 ESBL (-) *E. coli*, *K. pneumoniae*, and *K. oxytoca* isolates by \ Vitek AST N-111 and molecular methods. The sensitivity was 92.1% and specificity was 90%. The authors emphasized the use of combined disk method and Vitek system in ESBL detection (Valenza et al., 2011). We also performed combined disk method and Vitek system in detection of ESBL together with DDS method.

Cost effective, rapid and reliable methods are necessary for ESBL diagnosis. In our study, we compared automatized Vitek system and DDS in ESBL detection. Vitek system could not detect 10 ESBL(+) *E.coli* isolates. Three strains were DDS(-) and Vitek (+). All the ESBL *K. pneumoniae* strains were detected positive by both methods.

ESBL producing bacteria, are important in multidrug resistance issue. Increased duration at intensive care unit, catheter use, surgical procedures, prevalent use of aminoglycosides, and cephalosporins are risk factors for ESBL producing bacteriae (Kuzucu et al., 2011). Vitek automatized system is a reliable method in ESBL detection although false negative results are reported. It produces rapid data in routine Clinical Microbiology Laboratories. CLSI referred phenotypic and confirmation ESBL detection methods take approximately 18 h; but Vitek system can be performed in 7.5 h. In our study, we concluded that automatized Vitek system can report ESBL results rapidly in routine Clinical Microbiology Laboratories; and further studies with higher number of isolates are necessary to confirm this data. This is our data extracted from a recently developed University Hospital in Western Turkey. This data can be evaluated further as the patient admissions are increased. Besides, there may be need for improvements of the device in the

near future.

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