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Original Article / Orijinal Araştırma

A comparative study of VITEK-2, Double Disc Synergy and Combined Disc Methods for detection of ESBL (Extended Spectrum Beta-Lactamase) production in *Escherichia coli* and *Klebsiella pneumoniae* strains

Escherichia coli ve *Klebsiella pneumoniae* suşlarında ESBL (Genişletilmiş Spektrum Beta-Laktamaz) üretiminin saptanması için VITEK-2, Çift Disk Sinerjisi ve Kombine Disk Yöntemlerinin karşılaştırmalı bir çalışması

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Abstract

Aim: In this prospective study we aimed to compare the effectiveness of VITEK-2 (bioMérieux, France) automated system, double disc synergy test (DDST) versus combined disc test (CDT) in detecting the Extended Spectrum Beta-Lactamase (ESBL) positivity in *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from various clinical samples.

Material and Method: *E. coli* and *K. pneumoniae* strains inoculated on Mueller Hinton Agar plate. Susceptibility tests were performed with the VITEK 2 (BioMérieux, France) system before. Afterward, EBSL positivity was investigated manually DDST and CDT. Minimal inhibitor concentration (MIC) results of three tests were compared with each other according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

Results: 184 *E. coli* and *K. pneumoniae* strains, elevuated. 92.9% of 98 patients with VITEK 2 positive results were positive with combined disc and DDS method, 100% of the 86 patients with negative results of VITEK 2 were negative with combined disc and DDST.

Conclusion: VITEK 2 was found to have a sensitivity of 100%, a specificity of 92.4%, a positive predictive value of 92.8% and a negative predictive value of 100%. VITEK 2 was found to be compatible with validation tests for ESBL positivity.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, VITEK-2, Double Disk Synergy (DDS) Test, Combined disc test, Extended-Spectrum Beta-Lactamase (ESBL)

Öz

Amaç: Bu prospektif çalışmada, çeşitli klinik örneklerden izole edilen *Klebsiella pneumoniae* ve *Escherichia coli* suşlarında Genişletilmiş Spektrum Beta-Laktamaz (ESBL) pozitifliğini saptamada VITEK-2 (bioMérieux, Fransa) otomatize sistem, çift disk sinerji testi (ÇDST) ve kombine disk testinin (KDT) etkinliğini karşılaştırmayı amaçladık.

Gereç ve Yöntem: *E. coli* ve *K. pneumoniae* suşları Mueller Hinton Agar plağına inokule edildi. Duyarlılık testleri önce VITEK 2 (BioMérieux, Fransa) sistemi ile değerlendirildi. Sonrasında EBSL pozitifliği manuel olarak ÇDST ve KDT ile araştırıldı. Üç testin minimum inhibitör konsantrasyonu (MIC) sonuçları, Avrupa Antimikrobiyal Duyarlılık Testi (EUCAST) kriterlerine göre birbirleriyle karşılaştırıldı.

Bulgular: 184 *E. coli* ve *K. pneumoniae* suşu değerlendirildi. VITEK 2 pozitif sonucu olan 98 hastanın %92,9'u kombine disk ve DDS yöntemi ile pozitifti, VITEK 2 negatif sonucu olan 86 hastanın %100'ü kombine disk ve DDST ile negatifti.

Sonuç: VITEK 2'nin %100 duyarlılık, %92,4 özgüllük, %92,8 pozitif öngörü değeri ve %100 negatif öngörü değerine sahip olduğu bulundu. VITEK 2'nin ESBL pozitifliği için doğrulama testleri ile uyumlu olduğu bulundu.

Anahtar Kelimeler: *Escherichia coli*, *Klebsiella pneumoniae*, VITEK-2, Çift Disk Sinerjisi (DDS) Testi, Kombine disk testi, Genişletilmiş Spektrumlu Beta-Laktamaz (ESBL)

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INTRODUCTION

Extended Spectrum Beta-Lactamases (ESBL) were first reported in Germany in 1983, just after the introduction of broad-spectrum beta-lactam antibiotics against *Klebsiella pneumoniae* species.[1] The most crucial mechanism for developing resistance to beta-lactam antibiotics in gram-negative bacteria is beta-lactamase synthesis. Today, approximately 600 beta-lactamase enzymes have been identified. The most important beta-lactamase enzyme groups are cephalosporinase which is genetically encoded by plasmids, metallo-beta-lactamase and ESBL. ESBLs are enzymes that can cause resistance to penicillin, all cephalosporins except cephamycins (cefoxitin, moxalactam) and aztreonam, be inactivated with beta-lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam, and generate different enzymes as a result of different amino acid changes in TEM and SHV enzymes.[2,3] Plasmids that encode ESBL, also contain genetic material against many antibiotics other than beta-lactams in their genetic structure. As a result, the bacteria that can synthesize ESBL can be simultaneously resistant to fluoroquinolone, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and especially aminoglycosides.[4-6]

Today, ESBL screening is recommended for research purposes in infection control and epidemiological studies. Screening and verification tests are used to determine the presence of ESBL. Inhibition diameter is determined by disk diffusion test performed with cefotaxime, ceftriaxone, ceftazidime, cefpodoxime as screening test or the minimum inhibitory concentration (MIC) is determined by the liquid dilution method. If the zone diameters from the test are lower than the limit values specified in international sources for the tested antibiotics, or the MIC values are greater than the limit values, a verification test should be performed. Verification tests consist of phenotypic tests such as combination disk test, double disk synergy test and microdilution test, and genotypic tests such as PCR (polymerase chain reaction). ESBL can be found in various commercial kits and automated systems.[7-9]

The aim of this study was to investigate whether there is a difference between the VITEK 2 (BioMérieux, France) fully automated system and double disc synergy (DDST) versus combined disc test (CDT) in detecting the presence of ESBL.

MATERIAL AND METHOD

In this prospective study, 131 *E. coli* and 53 *K. pneumoniae* strains isolated from various clinical samples as an infectious agent between November 2016 and January 2017, were included. Susceptibility tests were performed with the VITEK 2 (BioMérieux, France) system before, and after DDSTs and CDTs were applied to ESBL positive or negative *E. coli* or *K. pneumoniae* strains. For this test bacterial suspension which prepared in 0.5 McFarland turbidity was inoculated to Mueller Hinton Agar (MHA) plate. *E. coli* ATCC 25922 used as a positive and negative control group. *E. coli* and *K. pneumoniae* strains inoculated on Mueller Hinton Agar plate. Susceptibility tests were performed with the VITEK 2 (BioMérieux, France) system before, and after Minimal inhibitor concentration (MIC) results of three tests were compared with each other according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

a.Double Disc Sinergy Test: After placing an amoxicillin-clavulanic acid (AMC) (20/10 µg) disc in the centre of the petri dish and placing ceftazidime (CAZ) (30 µg), ceftriaxone (CRO) (30 µg), cefoxitin (FOX) (30 µg), cefotaxime (CTX) (30 µg) radially at a distance of 25 mm from AMC's disc circumference, an expansion towards the AMC disk in the inhibition zones around the CAZ, CRO, FOX or CTX discs or the presence of a non-bacterial synergy area in between was evaluated as ESBL production.

b.Combine Disc Test: In this method ceftazidime (CAZ) (30 µg), ceftazidime-clavulanic acid (CCA) (30/10 µg), cefotaxime (CTX) (30 µg), cefotaxime-clavulanic acid (CCT) (30/10 µg) was used. Bacterial suspension which prepared in 0.5 McFarland turbidity was spread with sterile swab to Mueller-Hinton Agar medium. CAZ and CCA discs were placed in the petri dish with 30 mm between them and same procedure was applied to CTX and CCT disks. Petri dishes was incubated at 35°C for 18 hour and results was evaluated according to EUCAST criteria. 5mm or difference more than that between cephalosporin disc and cephalosporin -clavulanate disc was evaluated as ESBL production.

Ethical approval: In order to conduct the study, Ethical approval was taken from Clinical Researches Ethics Committee of Dr. Lütfi Kırdar Training and Research Hospital (Date: 29.11.2016, Decision No: 2016/514/96/2). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Statistical analysis: Statistical Packages for the Social Sciences (SPSS) for Windows 22.0 package program was used for statistical analysis.

RESULTS

In our study, 131 *E. coli* and 53 *K. pneumoniae* strains isolated from various clinical samples were evaluated. Of the patients from whom the isolates were obtained, 126 (68.5%) were female, 58 (31.5%) were male and the age range was 6 months-92 years, the average age was 42. The most common comorbidities were hypertension (21.2%), Diabetes Mellitus (DM) (16.3%) and malignancy (13.6%)(**Table 1**). The antibiotics used by the patients in the last three months were questioned in terms of ESBL positivity (**Table 2**). The distribution of the samples included in the study is as follows; 162 (88.0%) were urine, 9 (4.9%) were wound, 5 (2.7%) were trachea and sputum and 3 (1.6%) were tissue culture. The antibiotic susceptibility results of *E. coli* and *K. pneumoniae* strains with VITEK-2 are shown in **Table 3**.

In this study, ESBL positivity was detected by VITEK 2, the confirmation tests were performed on these strains with the DDSTs and CDTs to 63 (48.1%) 131 of *E. coli* strains and 35 (66%) 53 of *K. pneumoniae* strains. The verification tests gave positive results methods in 91 (92.9%) of 98 strains that VITEK 2 gave positive results, 86 (100%) of 86 strains that VITEK 2 gave negative results were found negative by CDTs and DDS method. DDST and CDTs results were consistent with each other in terms of positivity and negativity. Evaluated only for *E. coli*; the confirmation tests gave positive results in 57 (90.5%) out of 63 strains that VITEK 2 gave positive results, 68 (100%) of 68 strains that gave negative results with VITEK 2 were also found negative by combined disc and double disc synergy method. Evaluated only for *K. pneumoniae*; the confirmation tests gave positive results in 34 (97.1%) out of 35 strains that VITEK 2 gave positive results, 18 (100%) of 18 strains that gave negative results with VITEK 2 were also found negative by DDSTs and CDTs method.

Thus, when the VITEK 2 results were compared with the validation tests that studied, the sensitivity was 100%, specificity 92.4%, PPD 92.8%, NPD 100% for all strains. For *E. coli*, sensitivity 100%, specificity 91.9%, PPD 90.5%, NPD 100%; For *K. pneumoniae*, the sensitivity 100%, specificity 94.7%, PPD 97.1% and NPD 100% was found (**Table 4**).

DISCUSSION

The most important mechanics in gram negative bacteria for developing resistance against beta-lactam antibiotics is beta-lactamase synthesis, and approximately 600 beta-lactamase enzymes have been identified since today. Because enteric bacteria that can synthesize this enzyme can easily transfer these enzymes to other bacteria via plasmids, the number of bacteria that can synthesize this enzyme is increasing day by day.[1-3] Microorganisms that synthesize ESBL, can transfer these enzymes between species and could cause epidemic in hospitals. In bacterial infection that can synthesize ESBL, should be investigated whether the factor causes ESBL due to the insufficiency of many antibiotics in the treatment, prolonged hospitalization stay, increased morbidity and mortality rates, and serious economic losses.[5,6]

Various studies have been conducted in the literature on methods that detect ESBL production.[10-22] In a comparative study which done with various automatize systems, DDS test and E-test on 150 enteric bacteria, VITEK 2 (BioMérieux, Fransa), Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD, ABD), MicroScan WalkAway-96 System (Dade Behring, Inc., West Sacramento, CA, ABD), DDS test and E-test methods for the ESBL detection sensitivity for *E. coli* (n=61) respectively 81.4%, 100%, 100%, 97.7%, 97.7%, specificity 100%, 72.2%, 72.2%, 100%, 94.4%, PPD 100%, 89.6%, 89.6%, 100%, 94.4%, NPD 69.2%, 100%, 100%, 94.7%, 97.7%, The ESBL detection sensitivity for *K. pneumoniae* (n=29) 95.7%, 100%, 95.7%, 91.3%, 100%, and the specificity 83.3%, 66.7%, 50%, 100%,83.3%, PPD 95.7%,

Table 1. Demographic information, factors and underlying diseases

	n	%
Gender (Female)	126/58	68.5
Isolated bacteria		
E. coli	131	71.2
K. pneumoniae	53	28.8
Underlying diseases		
Hypertension	39	21.2
Diabetes mellitus	30	16.3
Malignancy	25	13.6
Coronary artery disease	15	8.2
Chronic kidney failure	13	7.1
Chronic obstructive pulmonary disease	9	4.9
Cerebrovascular Disease	2	1.1

Table 2. Antibiotics Used by Patients in the Last Three Months

Antibiotic	Exist	(%)	Non-exist	(%)
Aminopenicillin	24	13	160	87
Phosphomycine	20	10.9	164	89.1
2nd generation cephalosporin	19	10.3	165	89.7
Fluoroquinolone	16	8.7	168	91.3
3rd generation cephalosporin	11	6.0	173	94.0
Trimethoprim-sulfamethoxazole	7	3.8	177	96.2
Nitrofurantoin	3	1.6	181	98.4
1st generation cephalosporin	3	1.6	181	98.4
Aminoglycoside	3	1.6	181	98.4
Other antibiotics	3	1.5	181	98.5

*Other antibiotics: (tetracycline, clindamycin, fusidic acid)

Table 3. VITEK-2 antibiotic susceptibility results.

Antibiotic	Susceptible	(%)	Resistant	(%)
Ampicilline	45	24.5	139	75.5
Amoxicilline-clavulanic acid	90	48.9	94	51.1
Cefuroxime	81	44.0	103	56.0
Cefuroxime axetil	81	44.0	103	56.0
Ceftazidime	86	46.7	98	53.3
Ceftriaxone	86	46.7	98	53.3
Cefixime	83	45.1	101	54.9
Piperacillin-tazobactam	114	62.0	70	38.0
Imipeneme	176	95.7	8	4.3
Meropeneme	180	97.8	4	2.2
Ertapenem	178	96.7	6	3.3
Amikacin	139	75.5	45	24.5
Gentamicin	139	75.5	45	24.5
Ciprofloxacin	118	64.1	66	35.9
Trimethoprim-sulfamethoxazole	116	63.0	68	37.0
Tigecycline[1]	13	7.1	1	0.5
Nitrofurantoin[2]	146	79.3	18	9.8
Phosphomycine[2]	150	81.5	14	7.6

[1]Non-urinary samples have been studied, [2]Only studied in urine samples.

Table 4. Comparison of VITEK 2 and verification tests for *E. coli* ve *K. pneumoniae* strains

Test VITEK 2	n	Susceptibility %	Specificity %	PPD %	NPD %
All strains	184	100	92.4	92.8	100
E. coli	131	100	91.9	90.5	100
K. pneumoniae	53	100	94.7	97.1	100

PPD: positive predictive value, NPD: negative predictive value

92%, 88%, 100%, 95.8%, NPD 83.3%, 100%, 75%, 75%, 100%, The ESBL detection sensitivity for *E. coli*, K. oxytoca ve *K. pneumoniae* (n=104) 84.5%, 100%, 98.6%, 94.4%, 98.6%, and the specificity 93.9%, 51.5%, 51.5%, 97%, 72.7%, PPD 96.8%, 81.6%, 81.4%, 98.5%, 88.6%, NPD 73.8%, 100%, 94.4%, 88.9%, 96.0 was found when when molecular methods were taken as a reference.[10] Fincancı et al.[11] was found screen test that based on zone diameter measurements is significantly sensitive compared to DDS and E-test methods for the ESBL detection sensitivity, similarly Oztürk et al.[12] was found screen test that based on zone diameter measurements is concordant as E-test and more efficient than DDS. There are also studies reporting that there is no difference between DDST test and E-test methods in detecting the presence of ESBL. For example, Yavuz et al.[13] compared the ESBL production in Enterobacteriaceae strains with the DDS test and E-test methods and reported that there was no significant difference between the methods. Yurtman et al.[14] and Akçam et al.[15] did not detect a difference between the DDST test and E-test methods. Genç et al.16 in a study that compared VITEK-2 and DDS which investigated the presence of ESBL for 95 *E. coli* and 61 *K. pneumoniae* strains and reported that VITEK 2 sensitivity was 93.3%, specificity 81.8%, false-positivity ratio 18.1%, false-negativity ratio 6.6% and positivity ratio 86.4%.

In a study which conducted with 117 enteric bacteria that ESBL positivity was determined by combination disk diffusion test, 91% of the strains with VITEK-2 and 97% of the strains were found to be ESBL positive with the DDS test, although VITEK 2 could give false negative results It is stated that it can be used routinely in laboratories.[17]

Another study which conducted with 94 ESBL positive and 71 ESBL negative enteric bacteria that were studied with molecular methods, VITEK 2 sensitivity 91.5%, specificity 100%, DDS test sensitivity 97.9%, specificity 97.2%, combined disc test sensitivity 93.6%, specificity 100% was detected on all strains.[17] Focusing only *E. coli* (n=79), VITEK 2 sensitivity 89.8%, specificity 100%, DDS test sensitivity 98%, specificity 100%, combined disc test sensitivity 89.8%, specificity 100% was detected. Focusing only *K. pneumoniae* (n=23), VITEK 2 sensitivity 95.7%, specificity 100%, DDS test sensitivity 95.5%, specificity 100%, combined disc test sensitivity 95.5%, specificity 100% was detected.[18]

Mehli et al.[10] was found in their study conducted with 321 enteric bacteria, when DDS test and VITEK 2 was compared in ESBL detection sensitivity and specificity was respectively detected as 100%, 94.1%. In another study which conducted with 1123 enteric bacteria with molecular methods as a reference, VITEK-2'nin sensitivity 98.1%, specificity 99.7%, PPD 99.3%, NPD 99.3% was detected, for *E. coli* (n=534) sensitivity 98.1%, specificity 99.5%, PPD 98.1%, NPD 99.5%, for *K. pneumoniae* (n=193) sensitivity 97.7%, specificity 100%, PPD 100%, NPD 98.1% was detected and VITEK 2 automatize system can be used rountinely in laboratories for ESBL detection.[19] Kacmaz et al.[20] reported that in some cases where the reliability of the DSS test is decreased (for example, accompanied by different resistance mechanisms such as the production of carbapenemases such as high-level Amp C beta lactamase, metallo beta lactamase and *K. pneumoniae* beta lactamase, excretion mechanisms and decreased permeability with ESBL) It may mask the presence of ESBL and also that the bacteria which resistant to clavulanic acid cannot be evaluated with the DDS test.

Singh et al.[21] compared the 57 ESBL positive strains with six methods. Between these methods, concordance was found with combined disk test and 100% MIC value. VITEK 2 sensitivity 91.8% specificity 97.24%, PPD 93.3% was found in all strains. The highest sensitivity and specificity have been demonstrated with combined disc (93.44%) and double disc synergy (100%) techniques, respectively. They reported that VITEK-2 has an acceptable capacity to detect ESBL strains compared to traditional phenotypic methods.[21] ChromID ESBL agar (BioMerieux, France) that developed in recent years, is a chromogenic selective broth that developed to identify ESBL positive Enterobacteriaceae strains earlier than other methods used. The sensitivity and specificity of this broth in detecting ESBL-producing microorganisms were reported as 97% and 92.9% respectively, by Alıskan et al.[22] from our country.

In our study when we compared VITEK 2 Automatize System's results with DDS and Combined Disc Methods which are ESBL verification tests, sensitivity 100%, specificity 92.4%, PPD 92.8%, NPD 100% was found in all strains.

CONCLUSION

ESBL-positivity is an important problem of resistance in Gram-negative bacteria, one of the most important risk factors is antibiotic use. Therefore, attention should be paid to the use of appropriate antibiotics in the appropriate indication. In addition, it was found that the VITEK 2 automated system in laboratories was compatible with confirmation tests in detecting ESBL positivity during the decision-making process in the selection of antibiotics that play a role in the treatment of these infections.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval was taken from Clinical Researches Ethics Committee of Dr. Lütfi Kırdar Training and Research Hospital (Date: 29.11.2016, Decision No: 2016/514/96/2).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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