Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Investigation of three different methods for detection of ESBL production and antibiotic resistance percentage of ESBL producing Gram negative bacteria

Gram negatif bakterilerde GSBL üretiminin üç farklı yöntemle araştırılması ve antibiyotik direnç oranları

Mustafa GÜZEL[1], Yasemin GENÇ[2], Altan AKSOY[2], Penka MONCHEVA[1], Petya HRISTOVA[1]

ABSTRACT

Objective: In this study, it was aimed to evaluate the efficacy of chromogenic agar for rapid and accurate identification of bacteria producing extended spectrum beta-lactamase (ESBL) and to investigate the antibiotic resistance rates of 105 bacteria that were determined to produce ESBL.

Methods: ESBL production was investigated using combined disc method, E-test and chromogenic agar. Additionally, susceptibility patterns of these strains to 21 antibiotics were studied according to the criteria of CLSI (Clinical and Laboratory Standards Institute). Zone diameters categorized as susceptible were evaluated, and strains showing intermediate susceptibility were considered as resistant. Fisher's chi-square test was used in statistical analysis.

Results: A hundred and five strains (81 *Escherichia coli*, 24 *Klebsiella* spp.) were found to produce ESBL by combined disc method, while 96 strains were found to produce ESBL by E-test and 99 strains by chromogenic agar. The sensitivity and positive predictive value of chromogenic agar for ESBL production was 94.8% and 91.9%, respectively. All strains were found to be resistant to cefuroxime, cefazolin and cefotaxime, the antibiotics that ESBL producing strains are considered to be resistant to. Among beta-lactam/beta-lactamase inhibitor combinations, the highest resistance was

ÖZET

Amaç: Çalışmada genişlemiş spektrumlu beta-laktamaz (GSBL) üreten bakterilerin doğru ve hızlı tanımlanması için kromojenik agarın etkinliğini test etmek ve GSBL ürettiği tespit edilen 105 bakteride antibiyotik direnç oranlarının tespit edilmesi amaçlanmıştır.

Yöntemler: Kombine disk yöntemi, E test yöntemi ve kromojenik agar ile GSBL üretimi tespit edilmiştir. Ayrıca bu şuşların 21 antibiyotiğe karşı duyarlılıkları CLSI (Clinical Laboratory Standards Institute) kriterlerine göre çalışılmıştır. Duyarlı kabul edilen zon çapları değerlendirilmiş, orta duyarlı suşlar dirençli kabul edilmiştir. İstatistiksel değerlendirme Fisher's ki-kare testiyle yapılmıştır.

Bulgular: Kombine disk yöntemi ile 105 (81 tanesi *Escherichia coli* ve 24 tanesi *Klebsiella* spp.), E test ile 96 ve kromojenik agar ile 99 suşun GSBL ürettiği tespit edilmiştir. Kromojenik agar yöntemi ile GSBL tespitinin duyarlılığı %94,8. GSBL üreten suşların dirençli kabul edildiği sefuroksim, sefazolin ve sefotaksim'e tüm suşların dirençli olduğu görülmüştür. Beta-laktam/beta-laktamaz inhibitörü kombinasyonları içerisinde ampisilin-sulbaktam direncinin (%75,2) yüksek olduğu bu grupta en az dirençli antibiyotiklerin piperasilin-tazobaktam (%31,4) ve sefoperazon -sulbaktam (%32,4) olduğu tespit edilmiştir. Karbapenemlere (imipenem,

[1] Univercity of Sofia "st. Kliment Ohridski" Faculy of Biology, Department of Mikrobiology, SOFIA, BULGARIA
[2] Ankara Numune Research and Education Hospital, Medical Mikrobiology, ANKARA, TURKEY

İletişim / Corresponding Author : Mustafa GÜZEL
Univercity of Sofia "st. Kliment Ohridski" Faculy of Biology, Department of Mikrobiology, SOFIA, BULGARIA
Tel : +90 532 494 32 50 E-posta / E-mail : dr.mustafaguzel@hotmail.com

Geliş Tarihi / Received : 20.03.2015
Kabul Tarihi / Accepted : 05.06.2015

DOI ID : 10.5505/TurkHijyen.2015.33239

Güzel M, Genç Y, Aksoy A, Moncheva P, Hristova P. Comparison of three different methods for detection of ESBL production and antibiotic resistance percentage of ESBL producing Gram negative bacteria. Turk Hij Den Biyol Derg, 2015; 72(2): 131-8.

against ampicillin-sulbactam (75.2%), and the lowest resistance was against piperacillin-tazobactam (31.4%) and cefoperazone-sulbactam (32.4%). A total of 8 strains (7.6%) were found to be resistant to carbapenems (imipenem, meropenem, and ertapenem) and the lowest resistance rate was observed to this group of antibiotics. *Klebsiella* spp. strains were found to be more resistant to beta-lactam-beta-lactamase inhibitors, aminoglycosides, trimethoprim-sulfamethoxazole and chloramphenicol than that of *E. coli*, but *E. coli* strains were found to be more resistant to quinolones ($p<0.05$).

Conclusion: It was observed that the use of chromogenic agar has no advantage in detecting ESBL enzymes, the lowest resistance rate in ESBL producing strains was to carbapenems, and the species of ESBL producing bacteria was important in determining the resistance rates and selection of the appropriate antibiotic for treatment.

Key Words: Extended spectrum beta- lactamase, chromogenic agar, antimicrobial susceptibility

meropenem ve ertapenem) dirençli toplam 8 (%7,6) suş tespit edilmiş olup en düşük direnç oranı karbapenem grubu antibiyotiklerde saptanmıştır. Beta-laktam/beta-laktamaz inhibitörü kombinasyonları, aminoglikozidler, trimetoprim - sülfametoksazol ve kloramfenikole *Klebsiella* spp. suşlarının *E. coli*'den daha dirençli olduğu fakat kinolonlara *E. coli* suşlarının daha dirençli olduğu tespit edilmiştir ($p< 0.05$).

Sonuç: GSBL tespitinde kromojenik agar kullanımının bir avantaj sağlamadığı, GSBL üreten suşlarda en düşük direnç oranına sahip antibiyotiklerin karbapenemler olduğu, GSBL üreten bakterilerin türlerinin direnç oranlarını belirlemede ve tedavide kullanılacak antibiyotiğin seçiminde önemli olduğu görülmüştür.

Anahtar Kelimeler: Genişlemiş spektrumlu beta-laktamaz, kromojenik agar, antimikrobiyal duyarlılık

INTRODUCTION

The frequency of infections caused by bacteria that are resistant to beta-lactam antibiotics is gradually increasing. These bacterial agents causing community-acquired or hospital-acquired infections have been determined to produce large amounts of extended-spectrum beta-lactamase (ESBL) enzymes. *E. coli* belonging to the *Enterobacteriaceae* family and *Klebsiella* spp. are the bacteria causing community and hospital-acquired infections. The most important mechanism of resistance in these bacteria is ESBL production. ESBL is generally transported by plasmids and it hydrolyzes penicillin along with oxyimino cephalosporins and aztreonam. However, these enzymes do not have any effect on cephamycin group cephalosporins (cefoxitin and cefotetan). Additionally, plasmids encoding these enzymes carry genetic material against many antibiotics other than beta-lactams. Therefore, concurrent aminoglycoside, quinolone, chloramphenicol and trimethoprim-sulfamethoxazole resistance may be present in ESBL-producing bacteria (1,2).

Today, carbapenem derivatives are among the most commonly preferred antibiotics for the treatment of infections with gram-negative bacteria that produce ESBL. Carbapenems have been found effective against plasmid-mediated ESBL enzymes or enzymes other than ESBL as well as chromosome mediated beta-lactamases (1).

Mortality of infections caused by ESBL-positive bacteria has been increased, length of hospital stay has been prolonged, treatment costs have been increased, and clinical and microbiologic response

have been decreased in recent times. Therefore, rapid and accurate identification of ESBL producing bacteria is quite important for the selection of appropriate antibiotic treatment (1).

Hence, in our study, we aimed to compare the efficiency of the tests used in ESBL detection and determine the antibiotic resistance percentage of 105 ESBL-producing isolates.

MATERIAL and METHODS

The study included 105 isolates (81 *E. coli* and 24 *Klebsiella* spp.) obtained from different patients and clinical specimens (urine and wound) between 2013 and 2014 in Ankara Numune Research and Education Hospital. These isolates were identified using MALDI TOFF mass spectrometry (BD, Sparks, USA) and determined to be ESBL positive by combined disc method.

Combined disc method

Bacterial suspension adjusted to McFarland 0.5 was cultivated in Mueller Hinton agar (Oxoid) medium, then cefotaxime (30µg) and cefotaxime/clavulanic acid (30µg/10µg) and ceftazidime (30µg) and ceftazidime/clavulanic acid (30µg/10µg) discs (BD BBL) were placed. After 24 hours of incubation, a difference of ≥5 mm in the zone diameter was interpreted in favor of ESBL production (3). Additionally, antibiotic susceptibility of these isolates against 21 antibiotics were tested according to the CLSI (Clinical and Laboratory Standards Institute, 2014) criteria (3). Zone diameters those were defined as susceptible were evaluated, and isolates with intermediate susceptibility were accepted to be resistant.

E. coli ATCC 25922 was used as the control strain. One hundred and five strains that were determined to be ESBL positive were stored at -80 C°.

Phenotypic confirmation using the E-test

Isolates were inoculated to 5% sheep blood agar. A solution consisting of the isolates in 0.5% saline was prepared, the turbidity of the solution was adjusted to 0.5 McFarland, then the isolates were inoculated into Mueller Hinton agar medium. E-test strips containing cefotaxime and cefotaxime/clavulanic acid (0.25-16/0.016-1µg/mL, Biomerieux) were placed in the medium and the plates were evaluated after 24 hours of incubation at 37°C. The isolates having a MIC value for cefotaxime/clavulanic acid of ≥8 times higher than that for cefotaxime were accepted to be ESBL positive (3).

Detection of ESBL production using chromogenic agar

The samples were inoculated to chromogenic agar (Biomerieux) medium and were evaluated after incubation for 24 hours at 37°C. According to the manufacturers' recommendations, *E. coli* colonies showing green color and *Klebsiella* spp. colonies with red color were accepted to be ESBL positive.

Statistical analysis

Statistical analysis was performed by using SPSS 11.0 statistics program, and Fisher's Chi-square test. In statistical evaluation, a p value of < 0.05 was considered to be significant.

The antibiotic resistance percentage of the isolates that were determined to produce ESBL by each of the three methods were compared, and no statistically significant difference was found ($p>0.05$).

It was observed that resistance percentage were high for ampicillin, cephalosporins, aztreonam, trimethoprim – sulfamethoxazole and quinolones.

Table 1. The distribution of the resistance percentage of various antimicrobials those were determined by disk diffusion, according to three different methods of ESBL detection (combined disc method, E-test, Chromogenic agar).

Antibiotics	Combined disk synergy test (n=105)	E test (n=96)	Chromogenic agar (n=99)
Ampicillin	105 (100)	96 (100)	99 (100)
Ampicillin-Sulbactam	79 (75.2)	70 (72.9)	75 (75.7)
Amoxicillin-Clavulanic acid	**66 (62.8)**	**57 (59.4)**	**62 (62.6)**
Ticarcillin-Clavulanic acid	58 (55.2)	51 (53.1)	54 (54.5)
Piperacillin-Tazobactam	33 (31.4)	30 (31.2)	31 (31.3)
Cefoperazone- Sulbactam	**34 (32.4)**	**29 (30.2)**	**32 (32.3)**
Cefazolin	105 (100)	96 (100)	99 (100)
Cefuroxime	105 (100)	96 (100)	99 (100)
Cefotaxime	**105 (100)**	**96 (100)**	**99 (100)**
Ceftazidime	73 (69.5)	69 (71.9)	69 (69.7)
Cefepime	62 (59.1)	60 (62.5)	58 (58.9)
Gentamicin	40 (38.1)	37 (38.5)	37 (37.4)
Amikacin	9 (8.6)	9 (9.4)	7 (7.1)
Ciprofloxacin	59 (56.2)	56 (58.3)	54 (54.5)
Levofloxacin	53 (50.5)	50 (52.1)	49 (49.5)
Imipenem	4 (3.8)	2 (2.1)	3 (3.1)
Meropenem	7 (6.7)	4 (4.2)	6 (6.1)
Ertapenem	**8 (7.6)**	**5 (5.2)**	**7 (7.1)**
Trimethoprim-sulfamethoxazole	63 (60)	59 (61.4)	60 (60.6)
Aztreonam	65 (61.9)	63(65.6)	63 (63.6)
Chloramphenicol	**11 (10.5)**	**10 (10.4)**	**11 (11.1)**

Data are presented as number (%).

A total of 8 (7.6%) isolates were found to be resistant to carbapenems (imipenem, meropenem and ertapenem). The lowest resistance percentage was recorded to be against carbapenem antibiotics. The most susceptible antibiotic in this group was imipenem.

Among beta-lactam/beta-lactamase inhibitor combinations, antibiotics with the lowest resistance

Table 2. Antibiotic resistance percentage of extended spectrum beta-lactamase positive isolates by E-test (n=96)

Antibiotics	Resistance percentage n (%)		p
	E. coli (n=74)	*Klebsiella* spp. (n=22)	
Ampicillin	74 (100)	22 (100)	-
Ampicillin-Sulbactam	52 (70.3)	18 (81.8)	< 0.05
Amoxicillin-Clavulanic acid	**39 (52.7)**	**18 (81.8)**	**< 0.05**
Ticarcillin-Clavulanic acid	35 (47.3)	16 (72.7)	< 0.05
Piperacillin-Tazobactam	19 (25.7)	11 (50)	< 0.05
Cefoperazone- Sulbactam	**19 (25.7)**	**10 (45.5)**	**< 0.05**
Cefazolin	74 (100)	22 (100)	-
Cefuroxime	74 (100)	22 (100)	-
Cefotaxime	**74 (100)**	**22 (100)**	**-**
Ceftazidime	50 (67.6)	19 (86.4)	< 0.05
Cefepime	43 (58.1)	17 (77.3)	< 0.05
Gentamicin	26 (35.1)	11 (50)	< 0.05
Amikacin	5 (6.7)	4 (18.2)	< 0.05
Ciprofloxacin	45 (60.8)	11 (50)	< 0.05
Levofloxacin	42 (56.7)	8 (36.4)	< 0.05
Imipenem	2 (2.7)	0	-
Meropenem	3 (4.1)	1 (4.5)	-
Ertapenem	3 (4.1)	2 (9.1)	-
Trimetoprim - sulfamethoxazole	43 (58.1)	16 (72.7)	< 0.05
Aztreonam	44 (59.5)	19 (86.4)	< 0.05
Chloramphenicol	**5 (6.7)**	**5 (22.7)**	**< 0.05**

percentage were piperacillin-tazobactam and cefoperazone-sulbactam.

One hundred and five, 96 and 99 isolates were found to produce ESBL by combined disc method, E-test and chromogenic agar, respectively. Of the 105 strains, which were found ESBL positive with combined disc method, 96 were found positive by E test and 99 were found positive using chromogenic

agar. When the E test method was taken as a reference, of the strains producing ESBL using chromogenic agar, 8 were found to be false positive and 5 were found to be false negative. Accordingly, sensitivity of the chromogenic agar was 94.8%.

As expected, cephalosporin resistance was high in ESBL producing *E. coli* and *Klebsiella* spp. strains. According to the CLSI data, ESBL producing bacteria are considered resistant against cefuroxime, cefazolin and cefotaxime. All of the isolates in this study were found to be resistant against these antibiotics.

When compared to *E. coli* strains, *Klebsiella* spp. were more resistant to beta-lactam/beta-lactamase inhibitor combinations, aminoglycosides, trimethoprim-sulfamethoxazole and chloramphenicol, however *E. coli* strains were more resistant to quinolones than *Klebsiella* spp. ($p<0.05$).

DISCUSSION

In the CLSI 2010 guidelines a change was made in the susceptibility zone diameters of cephalosporins and it was indicated that ESBL producing isolates were already resistant against these antibiotics (3). In the study of Hombach et al. (4) that was performed according to 2013 CLSI data on 150 *Enterobacteriaceae* isolates producing ESBL, cefuroxime and cefotaxime resistance was found to be 91.3%, ceftazidime resistance was found to be 46% and cefepime resistance was found to be 15.3%. Although the resistance percentage against cefuroxime and cefotaxime found in the present study were similar, resistance percentage against ceftazidime (69%) and cefepime (59%) were found to be higher. Cefepime, a fourth generation cephalosporin, is especially effective to SHV ESBLs. However, cefepime shows an inoculum effect, and is inactivated due to increased beta-lactamase production. In addition, the increase in cefepime use was demonstrated to cause outbreaks of infections due to ESBL-producing organisms. Because of these reasons, it has been emphasized that cefepime should not be primarily used in the treatment of infections caused by ESBL producing microorganisms. If it will be used, it is recommended to be used in high doses, and if possible, along with the other antibiotics that would be effective (quinolones or aminoglycosides) (2).

Among beta-lactam/beta-lactamase inhibitor combinations, resistance to ampicillin-sulbactam was high, and the most susceptible antibiotics were piperacillin-tazobactam and cefoperazone-sulbactam in our study. Various clinical studies have shown that beta-lactam/beta-lactamase inhibitor combinations are effective in the treatment of infections caused by ESBL producing bacteria. Seniha et al. in 2011 (5), found that resistance percentage of piperacillin-tazobactam and cefoperazone-sulbactam were 75.8% and 59.1% in *Klebsiella* spp., and 25.8% and 21% in *E. coli* isolates. The corresponding rates in our study were 50% and 45.5% in *Klebsiella* spp., and 25.7% in *E. coli* isolates (for both antibiotics). Likewise, resistance percentage of *Klebsiella* spp. was higher than that of *E. coli* isolates. Certain microorganisms may produce more than one beta-lactamases concomitantly or may express the same beta-lactamase at high amounts, which can lead to the development of resistance also against beta-lactamase inhibitors (2).

Although resistance percentage of *Klebsiella* spp. is generally higher than that of *E. coli* isolates, quinolone resistance was found to be higher in *E. coli* isolates. This may be attributed

to the frequent prescription of quinolones for urinary tract infections mostly caused by *E. coli* isolates.

Currently, carbapenem derivatives are the most commonly preferred antibiotics in the treatment of ESBL producing gram-negative bacterial infections. In the SENTRY study involving 42 centers in the United States of America (USA) (6) carbapenem resistance in K. pneumoniae isolates was found to be 6.1%, and in the study of Özgen et al (7) carbapenem resistance was found in 23 (11%) of 210 *Enterobacteriaceae* isolates producing ESBL. The lower resistance percentage found in the present study [8 (7.6%)] may be explained by the fact that Özgen et al. used blood samples of patients who received intense antibiotic treatment in their study.

In the study of Réglier-Poupet et al. (8), combined disc method was compared with chromogenic agar in terms of detection of ESBL. Chromogenic agar was shown to be effective, with a sensitivity and positive predictive value of 88% and 38.7%, respectively. The higher sensitivity (94.8%) observed in our study may be explained by the fact that those researchers evaluated the combined disc method as the reference method and that chromosomal cephalosporinase produced by Enterobacter spp. strains included in the study led to false positivity. It was concluded that the use of chromogenic agar in ESBL detection was not effective due to the facts that chromogenic agar had no advantage of rapid diagnosis and had a low positive predictive value.

In conclusion, it has been demonstrated that the use of chromogenic agar does not have any advantage for detection of ESBL, carbapenems are the antibiotics with the lowest resistance percentage in ESBL producing bacteria, and identification of beta-lactamase producing bacterial species are important in terms of determination of resistance percentage and selection of the antibiotics that will be used in the treatment.

REFERENCES

1. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev, 2005; 18(4): 657-86.
2. Sercan U, Hakan L, Dilek A. Önemli ve sorunlu gram negatif bakteri infeksiyonları. Bilimsel Tıp Yayınevi, 2004; 85-94.
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 24th Informational Supplement. Document M100-S24, 2014. CLSI, Wayne, Pa.
4. Michael H, Brice Mouttet and Guido VB. Consequences of revised CLSI and EUCAST guidelines for antibiotic susceptibility patterns of ESBL- and AmpC b-lactamase-producing clinical Enterobacteriaceae isolates. J Antimicrob Chemother, 2013; 68: 2092–2098.
5. Seniha SA, Asuman I, Simin C, Ayse NO, Naz C, Seyfi CO, Sebahat A. Gram-negative bacilli causing infections in an intensive care unit of a tertiary care hospital in Istanbul, Turkey J Infect Dev Ctries, 2014; 8(5): 597-604.

6. Kaiser DM, Castanheira M, Jones RN, Tenover F, Lynfi R. Trends in Klebsiella pneumoniae carbapenemase-positive K. pneumoniae in US hospitals: report from the 2007-2009 SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis, 2013; 76(3): 356-60.

7. Özgen KE, Hatice AU, Alper E, Barış B, Burçin Ş, Gülşen H. İnvazif enfeksiyonlara neden olan GSBL pozitif Enterobacteriaceae İzolatlarında Karbapenem Direnci. Mikrobiyol Bul, 2014; 48(1): 59-69.

8. Réglier-Poupet H, Naas T, Carrer A, Cady A, Adam JM, Fortineau N, Poyart C, Nordmann P. Performance of chromID ESBL, a chromogenic medium for detection of Enterobacteriaceae producing extended-spectrum beta-lactamases. J Med Microbiol, 2008; 57(3): 310-5.