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DETERMINATION OF EXTENDED-SPECTRUM β-LACTAMASE (ESBL) PRODUCTION BY DOUBLE DISK SYNERGY METHOD AND E TEST IN KLEBSIELLA STRAINS*

Murat GÜNAYDIN[1], Cafer EROĞLU[2], Yavuz UYAR[1], Meryem ÇETİN[1], Mustafa SÜNBÜL[2], Hakan LEBLEBİCİOĞLU[2]

SUMMARY

Extended-spectrum β-lactamase (ESBL) production was determined by both DDSM and E test in 58 (75.3%) *Klebsiella pneumoniae* and 19 (24.7%) *Klebsiella oxytoca* strains isolated from hospitalized patients. Ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP) and aztreonam (ATM) disks were placed 25 mm (center to center) from the amoxicillin-clavulanic acid (AMC) disk in DDSM. A ratio of ceftazidime MIC (Minimum Inhibitory Concentration)/ceftazidime+clavulanic acid MIC (TZ/TZL) equal to or greater than 8 indicates the presence of ESBL enzymes in E test. ESBL positivity in *Klebsiella* spp. was detected by E test and DDSM to be 55.8% and 46.8%, respectively. ESBL positivity by ATM (97.2%) and FEP (88.9%) disks was statistically higher than CRO (72.2%) and CAZ (58.3%) disks ($p<0.05$). Although, ESBL was detected in a greater number of strains with the E test than DDSM, this was not statistically significant ($p>0.05$).

The results showed that both E test and DDSM were practically useful for detection of ESBLs in *Klebsiella* spp.

ÖZET

Klebsiella suşlarında çift disk sinerji yöntemi ve E test ile genişlemiş spektrumlu β-laktamaz (GSBL) varlığının araştırılması.

Hastanede yatan hastalardan izole edilen 58 (% 75.3) *Klebsiella pneumoniae* ve 19 (% 24.7) *Klebsiella oxytoca* suşunda genişlemiş spektrumlu β-laktamaz oluşturma çift disk sinerji yöntemi (ÇDSY) ve E test ile araştırılmıştır. ÇDSY'da seftazidim (CAZ), seftriakson (CRO), sefepim (FEP) ve aztreonam (ATM) diskleri, amoksisilin-klavulanik asit (AMC) diskine merkezleri arasındaki uzaklık 25 mm olacak şekilde yerleştirilmiştir. E test ile seftazidim/seftazidim-klavulanik asit (TZ/TZL) MİK (Minimum İnhibitör Konsantrasyon) oranı 8 ve üzeri değerler GSBL pozitifliği olarak değerlendirilmiştir.

Klebsiella suşlarında GSBL pozitifliği E test ve ÇDSY ile sırasıyla % 55.8 ve % 46.8 oranında bulunmuştur. ATM (% 97.2) ve FEP (% 88.9) disklerinde GSBL pozitifliği, CRO (% 72.2) ve CAZ (% 58.3) disklerine göre istatistiksel olarak daha yüksek bulunmuştur ($p<0.05$). GSBL varlığı, E test ile daha fazla sayıda suşta saptanmasına rağmen ÇDSY ile arasında istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0.05$).

Her iki testin de *Klebsiella* suşlarında GSBL varlığını araştırmak için kullanılabilece-

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Ondokuz Mayıs University, Medical Faculty, 1- Dept. of Microbiology and Clinical Microbiology, 2- Dept. of Infectious Diseases and Clinical Microbiology, Samsun.

ği sonucuna varılmıştır.

INTRODUCTION

As important agents of hospital infection, *Klebsiella* strains are responsible for 8-15% of nosocomial infections. Most of these strains are multiple-drug-resistant (9,10). The most important mechanism in resistance to β-lactam antibiotics is the production of the β-lactamases (4). Extended spectrum beta-lactamases (ESBL) positive strains are resistant to cefotaxime and aztreonam besides penicillins and first generation cephalosporins (3,4). ESBL-producing strains can not be detected by routine disk diffusion method (1,2,3,11). ESBL (in strains with hospital origin, especially) easily can be identified by antibiograms having special disk location or E test method.

In this study, the ESBL activity has been investigated in *Klebsiella* strains by both E test and double disk synergy method (DDSM).79

MATERIALS AND METHODS

Klebsiella spp. (n=77) were isolated from intensive care unit of Ondokuz Mayıs University Medical Faculty during 1999. The organisms were identified by API 32 E semi-automatic system (Bio-Merieux). Strains were as follow: *K.pneumoniae* 58 (73.3%), *K.oxytoca* 19 (24.7%). These strains were isolated from the cultures of urine (38), blood (21), exuda (15), sputum (2), and cerebrospinal fluid (1).

Detection of ESBL activity in *Klebsiella* with DDSM: Broth cultures of strains were adjusted to 0.5 McFarland standard and spread over Mueller-Hinton agar (Difco) plates. 20/10 μg amoxicillin/clavulanic acid (AMC) disks were placed in the center of plates and 30 μg ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), cefoxitin (FOX), aztreonam (ATM) and 10 μg imipenem (IPM) disks (Oxoid) were placed at a distance of 25 mm from center to center (13,14). After 16-18 hours incubation at 37°C, enhancement of inhibition zones of the oximino beta-lactam antibiotics towards to AMC disk or presence of inhibition zone in the middle disks were interpreted as indicative of presence of ESBL (13,14,17).

Detection of ESBL activity in *Klebsiella* strains with E test: Cultures at the McFarland 0.5 turbidity, were spread over Mueller-Hinton agar (Difco). Approximately 10-15 minutes later, E test strips (AB Biodisk) including ceftazidime (TZ) and ceftazidime+clavulanic acid (TZL) were applied to plates. After 16-18 hours incubation at 35°C, minimum inhibitory concentrations (MIC) were evaluated, and recorded. According to the test procedure, the 8 or higher MIC ratio of TZ/TZL was interpreted as ESBL positivity (1,18).

The Kappa statistics was used for agreement between two methods. The agreement was explained as Cohen Kappa. Moreover, defining of ESBL by two method was compared with chi-square (X^2) test.

RESULTS

The ESBL-production was found in 36 (46.8%) and in 43 (55.8%) *Klebsiella* strains by DDSM and E test respectively ($p>0.05$). 29 strains gave positive and 27 strains gave ne-

gative results by both methods for ESBL production. Seven negative strains by E test were found to be positive by DDSM and 14 negative strains by DDSM were found to be positive by E test. According to the Kappa test agreement the value of Cohen Kappa (κ) was found as 45% between E test and DDSM. Results of both two tests were compared with regard to agreement. The E test and DDSM agreement was found 81% (κ=0.81) and 64% (κ=0.64), respectively, when compared with total result of two tests.

Out of 36 ESBL positive strains by DDSM, enlargement of inhibition zones were detected in 35, 32, 26 and 28 strains around ATM, FEP, CRO and CAZ disks, respectively (Table 1). Thus, ATM and FEP were found to be significantly more useful than CAZ and CRO in detection of ESBL production ($p<0.05$).

Table 1. ESBL detection in DDSM by individual beta-lactam antibiotics.

Antibiotic	n	%
Aztreonam (ATM)	35	97.2
Cefepime (FEP)	32	88.9
Ceftriaxone (CRO)	26	72.2
Ceftazidime (CAZ)	21	58.3

The ESBL was detected in 18 (50%) of 36 strains by all of four disks in DDSM. In two strains only ATM disk gave positive results. ESBL production was detected by two or three disk combinations in remaining strains.

DISCUSSION

Resistance due to inducible chromosomal or extended spectrum TEM and SHV beta-lactamases in the nosocomial strains generally can not be identified by routine antibiogram tests. To show the resistance, a special setting of antibiotic disks is necessary (2,11,14,18).

It was established that outbreaks from ESBL producing *Klebsiella* spp. have been associated with the use of 3rd generation cephalosporins (ceftazidime, especially) (22). Decreasing of 3rd generation cephalosporin usage reduces the resistant strains. Some reports suggest that, resistance to CAZ certainly is important for determining of ESBL-producer strains (18).

In our hospital, the ESBL production has been detected in 66 (51.2%) of 129 *E.coli* and 107 (83.6%) of 128 *K.pneumoniae* strains; and in the same strains ESBL production has been detected in 55.5% of *K.pneumoniae,* in 15.5% of *E.coli* by E test by Leblebicioğlu et al. (16). In table 2 some studies related with ESBL production were given.

Tallis et al. (21) from Israel showed ESBL production in 69% of 68 nosocomial *K.pneumoniae* strains by E test. ESBL production was detected as 13% in *Klebsiella* spp. isolated from 4 patients with disk diffusion method in China (12). In *Klebsiella* strains ESBL production was found to be 49%, 31%, 24%, 17%, 16%, 9%, 1% in Portugal, Belgium, France, Italy, Holland, Germany, and Spain, respectively (19). In our country, this rate is between 44-47%.

Table 2. ESBL-positivity in Klebsiella strains..

Reference	Bacteria (n)	DDSM*		E test	
		n	%	n	%
Çokça and Tekeli (6)	K.pneumoniae (36)	16	44	-	-
Kaygusuz et al (15)	K.pneumoniae (127)	89	70	-	-
	K.oxytoca (25)	15	60	-	-
Gülay et al (11)	K.pneumoniae (44)	39	44	-	-
Akata (2)	K.pneumoniae (27)	12	44	-	-
Abacıoğlu et al (1)	K.pneumoniae (24)	15	62.5	12	50
Eskitürk et al (8)	Klebsiella spp (46)	24	52	24	52
Leblebicioğlu et al (16)	K.pneumoniae (128)	71	55.5	-	-
Derbentli et al (7)	K.pneumoniae (35)	14	40	-	-
Kaleli et al (14)	K.pneumoniae (38)	18	47	-	-
Tallis et al (21)	K.pneumoniae (68)	-	-	47	69
Peterson et al (20)	K.pneumoniae (25)	15	60	-	-
Andrzejewska et al (5)	K.pneumoniae (52)	21	40.4	-	-
Ho et al (12)	Klebsiella spp (472)	61	13	-	-
In our study	Klebsiella spp. (77)	36	46.8	43	55.8

*DDSM=Double Disk Synergy Method.

Although we found higher rates of ESBL production by E test compared with DDSM the difference was not significant. In conclusion we suggest that both E test and DDSM should be used together but not separately to get higher rates of ESBL production in *Klebsiella* spp. Especially, the use of DDSM alone is not enough to detect the ESBL production.

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