INVESTIGATION OF THE SUSCEPTIBILITY OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING ESCHERICHIA COLI AND KLEBSIELLA SPP. STRAINS TO COMBINATIONS OF CARBAPENEMS AND BETA-LACTAMASE INHIBITORS

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ABSTRACT

Objective: *Beta-lactamase inhibitor combinations are among to the limited number of agents effective for entended spectrum beta-lactamase (ESBL) strains which cause a considerable health problem worldwide. But the common concern about the ESBLs is the rapid resistance development. We aimed to measure in vitro activity of imipenem, meropenem, and combination of cefoperazone-sulbactam, piperacillin-tazobactam and amoxicillin-clavulanic acid against the ESBL producing Klebsiella and E. coli strains.*

Material and Method: *A total of 300 strain, 230 E. coli, 43 K. pneumoniae and 27 K. oxytoca collected from the inpatients with nosocomial infections admitted to our Institute. ESBL production of the strains were determined by using Double-disk synergy (DDS) test. The susceptibility of the strains against to the studied antibiotics was investigated with broth microdulition method according to the recommendations of NCCLS.*

Results: *No resistance to imipenem and meropenem among E. coli and Klebsiella spp was detected. Susceptibility of E. coli strains to piperacillin-tazobactam, cefoperazone-sulbactam and amoxicillin-clavulanic acid was found as 75.2%, 72.6% and 29.3, respectively. Piperacillin-tazobactam and cephoperazon-sulbactam were determined as equally active as 69.8% to K. pneumoniae strains. Susceptibility of K. oxytoca strains to piperacillin-tazobactam and cephoperazon-sulbactam was found as 55.8% and 40.7%, orderly. The susceptibility of amoxicillin-clavulanic acid among K. pneumoniae and K. oxytoca strains was detected as 23.3% and 18%, respectively.*

Conclusion: *Piperacillin-tazobactam was found relatively more active against ESBL producing E. coli and K. oxytoca strains, than the cefoperazone-sulbactam. Since both antibiotics were found equally sensitive in K. pneumoniae strains they may be used in life-threatening infections when susceptible.*

Key Words: *Beta-lactamase, antimicrobial resistance, Escherichia coli, Klebsiella pneumonia* ***Nobel Med 2013; 9(1): 89-94***

GENİŞLEMİŞ SPEKTRUMLU BETA-LAKTAMAZ ÜRETEN ESCHERİCHİA COLİ VE KLEBSİELLA SPP. SUŞLARINA KARŞI KARBAPENEMLER VE BETA-LAKTAM/BETA-LAKTAMAZ İNHİBİTÖR KOMBİNASYONLARININ DUYARLILIĞININ ARAŞTIRILMASI

ÖZET

Amaç: Dünyada önemli bir sağlık sorununa neden olan genişlemiş spektrumlu beta-laktamaz (GSBL) suşları için etkili sınırlı sayıda seçenek arasında beta-laktamaz inhibitörlü kombinasyonları yer almaktadır. Ancak ESBL'ler hakkında yaygın endişe hızlı direnç gelişimidir. Bu çalışmada, çeşitli polikliniklerden ve yatan hastalardan enfeksiyon etkeni olarak izole edilen GSBL pozitif E. coli ve Klebsiella spp. suşlarında karbapenemler ve sefaperazon-sulbaktam, piperasilin-tazobaktam ve amoksisilin-klavulanat duyarlılıklarının belirlenmesi amaçlanmıştır. Bu çalışmada ESBL üreten Klebsiella ve E. coli suşlarına karşı imipenem, meropenem ve sefoperazon-sulbaktam, piperasilin-tazobaktam ve amoksisilin-klavulanat kombinasyonlarının in vitro aktivitesinin değerlendirilmesi amaçlanmıştır.

Materyal ve Metod: Çalışmaya nozokomiyal infeksiyonu tanısı alan hastalardan alınan örneklerden izole edilen 230 E. coli, 43 K. pneumoniae ve 27 K. oxytoca olmak üzere toplam 300 suş alındı. Suşların GSBL üretimi, çift disk sinerji testi ile saptandı. Suşların çalışılan antibiyotiklere karşı duyarlılıkları National Committee for Clinical Laboratory Standards (NCCLS) önerilerine göre broth mikrodilüsyon yöntemi ile araştırıldı.

Bulgular: E. coli, Klebsiella spp. suşlarında imipenem ve meropeneme direnç saptanmadı. E. coli suşlarında piperasilin-tazobaktam %75,2, sefoperazon-sulbaktam %72,6 ve amoksisilin-klavulanat %29,3 oranında duyarlı saptandı. K. pneumoniae'da piperasilin-tazobaktam ve sefoperazon-sulbaktam duyarlılığı eşit olup %69,8 olarak saptandı. K. oxytoca'da ise piperasilin-tazobaktam duyarlılığı %55,8, sefoperazon-sulbaktam %40,7 olarak bulundu. Amoksisilin-klavulanat duyarlılığı K. pneumoniae ve K. oxytoca'da % 23,3 ve %18 olarak saptandı.

Sonuç: GSBL üreten E. coli ve K. oxytoca'da piperasilin-tazobaktam sefoperazon-sulbaktama göre göreceli olarak daha etkin bulunmuştur. K. pneumoniae'da ise her iki antibiyotiğin etkinliği eşit bulunduğundan hayatı tehdit eden ciddi enfeksiyonlarda duyarlı olduklarında kullanılabilirler.

Anahtar Kelimeler: Beta-laktamaz, antimikrobiyal direnç, Escherichia coli, Klebsiella pneumoniae **Nobel Med 2013; 9(1): 89-94**

INTRODUCTION

Extended spectrum beta-lactamases (ESBLs) were first identified in 1980s shortly after extended-spectrum cephalosporins resistant to beta-lactamases were developed.[1-3] Although they are seen in many members of Enterobacteriaceae, ESBLs are most frequently found in *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *E. coli*.[1,4] Phenotypes of extended-spectrum beta lactamase can vary between countries, cities, and even hospitals.[3]

The fact that ESBLs hydrolyze all penicillins, 1st, 2nd and 3rd generation cephalosporins and aztreonam and that they develop resistance against antibiotic groups other than beta-lactam, like aminoglycosides, quinolones, trimethoprim-sulphamethoxazole limits the treatment alternatives that can be used to treat infections caused by microorganisms carrying ESBL. Carbapenems are still the most effective antibiotics that can be used against bacteria producing ESBL.[5,6] However, it is important to generate alternative treatments to these agents in order to prevent carbapenem resistance that may develop later. Beta-lactam + β-lactamase inhibitor combinations can be used as a treatment alternative, if they are found susceptible in the treatment of infections caused by bacteria carrying ESBL. Consequently, both their tendency to spread rapidly and limited treatment alternatives seriously increase mortality and morbidity in infections caused by microorganisms producing ESBL and bring about unfavorable consequences in treatment costs.[7]

The present study aims to determine the susceptibility of carbapenem and cefoperazone-sulbactam, piperacillin-tazobactam, and amoxicillin-clavulanate in ESBL-positive *E. coli* and Klebsiella spp, subtypes isolated as the infection agent in outpatients and inpatients.

MATERIAL and METHOD

Culture, Bacteria Subtypes and Bacteria Identification

This study included a total of 300 bacteria, of which 230 were *E. coli*, 43 *K. pneumoniae*, and 27 *K. oxytoca* subtype, which were identified by double-disk synergy test to produce ESBL, and which were isolated from polyclinics of Fırat University Medical School Research and Application Hospital and inpatients diagnosed as hospital infection according to CDC (Centers for Disease Control and Prevention) criteria. Samples collected from the patients were →

planted in blood agar (Oxoid UK) and Eosin Methylene Blue (EMB) agar (Oxoid UK) plates; in addition, blood, pleural fluid and CSF samples were incubated in a BACTEC 9050 fully automated blood culture system, besides solid plates. The subtypes of the isolated bacteria were identified in consideration of their biochemical characteristics and using API ID 32 E (Bio-Mérieux/ France) automated identification kits. ESBL-positive 300 subtypes (*E. coli*, *K. pneumoniae* and *K. oxytoca*) were put into microstores (The Ropewalk, Lancshire, England) as the storage plate and kept at -20°C until analyses. All subtypes were checked again before analyses.

Antibiotic Susceptibility Tests

Antibiograms of subtypes were performed in accordance with disk-diffusion method as recommended by NCCLS.[8] The susceptibility of ESBL-positive bacteria to imipenem and meropenem from the carbapenem group, and to cefoperazone-sulbactam, piperacillin-tazobactam and amoxicillin-clavulanic acid from the beta-lactam+lactamase inhibitor combinations was examined according to broth microdilution method as suggested by NCCLS.[8] Meropenem, imipenem, piperacillin-tazobactam, cefoperazone-sulbactam and amoxicillin-clavulanate dust raw materials supplied by the manufacturing firm were used. Sterile 96-well polystyrene microplates were employed in the MIC analyses. Mueller-Hinton Broth (Oxoid/England) media were used as the growth medium. While studying meropenem, imipenem and cefoperazone-sulbactam, *E. coli* ATCC 25922 subtype was used as the control subtype, and while studying amoxicillin-clavulanate and piperacillin-tazobactam, *E. coli* ATCC 35218 subtype was used as the control subtype.

Bacteria suspensions were prepared from the colonies taken from the fresh cultures according to McFarland 0.5 standard so as to have a final concentration of $5x10^5$ cfu/ml. The starting concentrations were diluted as 32 µg/ml for carbapenems, 256/4 µg/ml for piperacillin-tazobactam, 256/256 µg/ml for cefoperazone-sulbactam (CPS), and 128/64 µg/ml for amoxicillin-clavulanate (AMC). Antibiotic dilutions were 32-0.015 µg/ml for imipenem and meropenem, 256/32-0.125/32 µg/ml for piperacillin-tazobactam (PIP-TAZ), 256/256-0.125/0.125 µg/ml for cefoperazone-sulbactam, and 128/64-0.06/0.03 µg/ml for amoxicillin-clavulanate. Bacteria without antibiotic and plates were checked for each subtype. Microplates were covered and incubated at 35°C for 18-24 hours. At the end of this period, whether or not there was growth in the wells in the plates was evaluated by identifying turbidity. The concentration at which turbidity disappeared was recorded as "minimal inhibitor concentration" (MIC) on the serial dilution scale for that particular subtype.

Table 1: Clinical samples isolated from the ESBL-producing subtypes and their numerical distribution

Clinical samples	**N**
Urine	231
Cerebrospinal fluid (CSF)	1
Blood	21
Respiratory samples	
Sputum	5
Endotracheal intubation tube	4
Throat culture	2
Bronchial lavage	2
Wound samples	
Surgical wound	24
Abscess	4
Diabetic foot	3
Drain	2
Catheter	1
Total	300

Table 2: Susceptibility rates of ESBL-positive bacteria to antibiotics

Bacteria	**IPM**	**MEM**	**AMC**	**CPS**	**PIP-TAZ**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
E. coli	230 (100)	230 (100)	68 (29.6)	167 (72.6)	173 (75.2)
K.pneumoniae	43 (100)	43 (100)	10 (23.3)	30 (69.8)	30 (69.8)
K. oxytoca	27 (100)	27 (100)	5 (18.5)	11 (40.7)	15 (55.8)
Total	300 (100)	300 (100)	82 (26)	208 (69.3)	218 (72.6)

IPM: Imipenem, MEM: Meropenem, AMC: Amoxicillin-clavulanat CPS: Cefoperazone-sulbactam, PIP-TAZ: Piperacillin-tazobactam

The MIC average at which growth of half of the bacteria subtypes was prevented was calculated as MIC_{50} and the antibiotic concentration where 90% did not grow was recorded as MIC_{90}.

Statistical Evaluation

For the statistical evaluation of the data, chi-square test and Fischer's exact chi-square test were used in SPSS version 12.01 software. Level of significance was set at $p<0.05$.

RESULTS

Of the 300 cases from whom the bacteria were isolated, 159 (53%) were males and 141 (47%) were females. Mean age was 44.85±21.82 in the cases whose ages ranged between 1 and 87. Distribution and number of the clinical samples from which a total of 300 subtypes was isolated are presented in Table 1.

Of the subtypes, 143 (47.4%) were isolated from the samples sent from the surgery clinics, 111 (36.8%) from the internal medicine clinics, and 46 (15.2%) from the intensive care units. Susceptibility of the ESBL-producing subtypes to the studied antibiotics is presented in Table 2.→

Table 3: Distribution of antibiotic resistance by clinics

CLINIC	IPM	MEM	AMC	CPS	PIP-TAZ
	n (%)	n (%)	n (%)	n (%)	n (%)
Surgery	0 (0)	0 (0)	79 (49.7)	19 (33.3)	15 (46.9)
Internal medicine	0 (0)	0 (0)	54 (34)	27 (47.4)	10 (31.3)
Intensive Care	0 (0)	0 (0)	26 (16.8)	11 (19.3)	7 (21.9)
Total	0 (0)	0 (0)	159 (100)	57 (100)	32 (100)

IPM: Imipenem, MEM: Meropenem, AMC: Amoxicillin-clavulanat CPS: Cefoperazone-sulbactam, PIP-TAZ: Piperacillin-tazobactam

Table 4: MIC_{50}-MIC_{90} values and MIC ranges of the studied antibiotics

		MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MIC range
IPM	E	0.5	0.5	0.25/4
	K	0.5	0.5	<0.03/4
MEM	E	0.12	0.12	0.03/2
	K	0.12	0.12	0.06/4
AMC	E	16	32	0.25/>128
	K	32	128	1/>128
CPS	E	8	64	0.12/>256
	K	16	128	0.12/>256
PIP-TAZ	E	8	64	0.5/>256
	K	16	128	1/>256

E: E. coli, K: Klebsiella spp. IPM: Imipenem, MEM: Meropenem, AMC: Amoxicillin-clavulanat CPS: Cefoperazone-sulbactam, PIP-TAZ: Piperacillin-tazobactam

There was no resistance against imipenem or meropenem in our study. Of the combined antibiotics containing beta-lactamase inhibitor, the most effective one against *E. coli* was found to be piperacillin-tazobactam (75.2%). Of the 32 subtypes resistant to PIP-TAZ, 19 (59%) were found resistant to CPS, and 26 (81%) were found resistant to AMC. Of the 57 subtypes resistant to CPS, 50 (87.7%) were found resistant against AMC, too. Two subtypes that were resistant to CPS and PIP-TAZ were also found resistant to AMC. A total of 15 subtypes, of which 11 were *E. coli*, 3 were *K. pneumoniae*, and 1 was *K. oxytoca*, were found resistant to all three antibiotics. Table 3 shows the distribution of antibiotic resistances by the clinics the subtypes were isolated from. An examination of all subtypes demonstrated that while there was no resistance against imipenem and meropenem, 159 subtypes were resistant to amoxicillin-clavulanate, 57 were resistant to cefoperazone-sulbactam, and 32 to piperacillin-tazobactam. MIC_{50} and MIC_{90} values of the analyzed drugs are presented in Table 4.

MIC_{50} values of piperacillin-tazobactam and cefoperazone-sulbactam were found within susceptibility limits for *E.coli* and Klebsiella spp. MIC_{90} values for both antibiotics were within resistance limits for Klebsiella spp., whereas MIC_{90} value of cefoperazone-sulbactam was found at the resistance limit for *E. coli*. MIC_{50} and MIC_{90} levels of amoxicillin-clavulanate were measured within resistance limits for Klebsiella spp

DISCUSSION

ESBL enzymes are commonly found in particularly Klebsiella types and *E. coli* subtypes isolated from patients who stay in surgery clinics or intensive care units for a long period of time, who undergo an invasive intervention or have an open wound, and whose general condition is poor.[1,4,9] It is necessary to know the prevalence of ESBL-positive bacteria in a hospital, not only to guide the clinician in determining empirical antibiotic treatment alternatives, but also in terms of the spreading of resistant hospital infection agents and measures to be taken against them. Although bacteria producing extended spectrum beta-lactamase seem to be susceptible to in vitro 3rd generation cephalosporins and monobactams, these antibiotics may fail in treatment.[9,10]

Treatment alternatives that can be used to treat infections caused by bacteria having ESBL are fairly limited. Some studied have found that the resistance of ESBL-positive subtypes to antibiotics other than beta-lactams is significantly higher than that of the subtypes which do not produce ESBL.[11] Carbapenems are still the most effective antibiotics that can be used against bacteria producing ESBL.[5,6,12] Clinicians have preferred carbapenems in both empirical and prophylactic antibiotic treatments due to the extreme prevalence of ESBL enzymes in hospitals recently. This has led to unwarranted and intensive use of carbapenems, which in turn has brought about serious hospital infections caused by bacteria subtypes resistant to imipenem or meropenem.[6,13]

Gioia and Livermore who followed the antibiotic susceptibility of ESBL-producing isolates over the years found that in a couple of years piperacillin-tazobactam resistance increased two folds and quinolone resistance increased about 3 to 4 folds, that no resistance developed against meropenem, but high MIC-value (2-4 µg/ml) susceptibility was seen at a rate of 1%.[14] Imipenem and meropenem resistance in ESBL-positive *E. coli* and *K. pneumoniae* was reported to be 7-8% and 8% respectively, in some studies conducted in Turkey and in several international studies.[15-17] In a study of the Asian-Pacific Surveillance Program (SENTRY) covering the period between 1998 and 2002, no resistance was found against imipenem in *E. coli* and *K. pneumoniae*, but when the distribution of MIC values by countries was examined, it was seen that MIC_{50} values ranged between 0.25-1 µg/ml and MIC_{90} between 0.25-2 µg/ml in *K. pneumoniae*, and MIC_{50} values ranged between 0.12-0.25 µg/ml and MIC_{90} values between 0.25-2 µg/ml in *E. coli*.[18] No resistance against imipenem and meropenem was found in our study, either. In the present study, $MIC_{50/90}$ →

values of imipenem for *E. coli* and Klebsiella spp. were 0.5/0.5 µg/ml and those values of meropenem for these two microorganisms were 0.12/0.12 µg/ml; these values are below 4 µg/ml, which is the resistance limit for carbapenems, and demonstrate that they still preserve their high effectiveness against both subtypes concerned. However, the number of subtypes that have high MIC-value (2-4 µg/ml) susceptibility is 7 and 3 for *E. coli* and Klebsiella spp., respectively. This is an indicator of the decrease in imipenem and meropenem effectiveness, and requires dose adjustment in serious infections caused by subtypes that have these MIC values.

One approach used in the treatment of infections caused by ESBL-producing bacteria is to combine an antibiotic not resistant to beta-lactamase with a beta-lactamase inhibitor. Combinations like amoxicillin-clavulanate, cefoperazone-sulbactam, ticarcillin-clavulanate, and piperacillin-tazobactam can be used for this purpose. CPS and PIP-TAZ can be used with success in the treatment of infections caused by subtypes of TEM and SHV origin. Leleu et al. argued that even low doses of piperacillin-tazobactam combination could be effective in the treatment of infections like endocarditis and meningitis, caused by ESBL-producing *K. pneumoniae*.[19] However, Gioia et al. found that the effectiveness of piperacillin-tazobactam combination in *Klebsiella* subtypes producing ESBL was very erratic, and that susceptibility rates varied according to the concentration of the tazobactam component.[20] In another study, treatment failed in more than half of the ESBL infections treated with piperacillin-tazobactam in a period of two years, and it was concluded that routine use of this agent in ESBL infections was not appropriate.[21]

Various studies reported that susceptibility of ESBL-producing subtypes to piperacillin-tazobactam was 12-100% for *E. coli* and 12-96% for *K. pneumoniae*.[16-19] Susceptibility values of 75.6% and 58.9% found by Casellas et al. are similar to the results we obtained in this study.[22] Susceptibility to PIP-TAZ was found 75.2% in *E. coli*, 69.8% in *K. pneumoniae*, and 55.6% in *K. oxytoca* in our study. In addition, we established that the rate of susceptibility of PIP-TAZ in *E. coli* subtypes was higher than that in Klebsiella sybtypes, which is consistent with the above-mentioned studies. Another combination that can be resorted to in ESBL-producing microorganisms, and particularly in subtypes of TEM and SHV origin, is cefoperazone-sulbactam. In the present study we found that CPS susceptibility was 72.6% in *E. coli*, 69.8% in *K. pneumoniae*, and 40.7% in *K. oxytoca*. Although the effectiveness of CPS and PIP-TAZ combinations to *E. coli* and *K. pneumoniae* is similar, it can be speculated that the higher effectiveness of PIP-TAZ to *K. oxytoca* relative to CPS may result from the fact that tazobactam, which is the beta-lactamase inhibitor in piperacillin-tazobactam combination, hydrolyzes ESBL enzymes better than sulbactam. CPS susceptibility in ESBL-producing *E. coli* and *K. pneumoniae* subtypes is found 90% in *E. coli* and 94.1% in *K. pneumoniae* in the literature. In our country CPS susceptibility to these subtypes was reported between 32.7% and 91%.[23,24] CPS susceptibility in our country was found higher than that in the literature due to the irrational policies of antibiotic use, which were carried out in the past.

AMC usually has a low effectiveness in ESBL-producing subtypes. There are only few studies reporting high susceptibility rates. Spanu et al. found 85% AMC susceptibility in ESBL-producing Enterobactericeace and emphasized that combinations with a beta lactamase inhibitor could be a better alternative after carbapenems in the treatment of infections caused by ESBL-producing microorganisms.[11] However, there are also studies where AMC susceptibility in ESBL-producing *E. coli* and *K. pneumoniae* subtypes was found at such low rates as 17.2-24%.[23,24] In our study AMC susceptibility was 29.6% in *E. coli*, 23.3% in *K. pneumoniae*, and 18.5% in *K. oxytoca*, and was lower than CPS and PIP-TAZ in both microorganisms.

In conclusion, piperacillin-tazobactam was found relatively more effective than cefoperazone-sulbactam in *E. coli* and *K. oxytoca*. The effectiveness of both antibiotics was found similar in *K. pneumoniae*. We think that these two antibiotics should be used after susceptibility tests are conduced in particularly life-threatening, serious systemic infections. Amoxicillin-clavulanate seems to be far below the sufficient effectiveness in the treatment of these infections. Imipenem and meropenem are agents that can be used reliably in the treatment of ESBL-producing subtypes.

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