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Original research-Orijinal araştırma

A retrospective investigation of extended spectrum beta lactamase production in gram negative bacteria strains isolated from inpatients of an university hospital.

Bir üniversite hastanesi hastalarından izole edilen gram negatif basillerde geniş spektrumlu beta-laktamaz üretiminin retrospektif olarak araştırılması

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Abstract

Aim. We aimed to investigate retrospectively the extended spectrum beta lactamase (ESBL) production status of Gr (-) bacillus that were isolated from the cultures of several clinical specimens from the inpatients of our hospital in the years 2005 and 2006. **Methods.** Gram negative bacillus such as Escherichia coli (E.coli), Klebsiella pneumonia (K. pneumonia), Klebsiella oxytoca (K. oxytoca) were isolated from the cultures of the specimens of blood, urine, wound swab, sputum, trans tracheal aspirate material (TTA), vaginal swab, abscess, cerebrospinal fluid, peritoneal paracentesis fluid and catheter tip of 925 patients in the year 2005 and 1184 patients in the year 2006. Becton Dickinson Phoenix 100 model full automatic systems were used to identify bacteria and their sensitivity/resistance profile and to investigate whether these isolated gr(-) bacteria produce ESBL or not. **Results.** 2109 Gram negative bacterial strains (E.coli, K. pneumonia, K. oxytoca) were investigated for ESBL in two-year period (2005-2006). ESBL was positive in 591 of them. 273 out of 925 gram negative strains (30%) showed ESBL positivity in 2005 while 318 out of 1184 gram negative strains (27%) showed ESBL positivity in 2006. There was not a significant difference between the years 2005 and 2006 in terms of ESBL positivity ($p>0.05$). Two hundred and eleven (30%) strains of 709 E.coli, 44 (27%) strains of 164 K.pneumoniae and 18 (35%) strains of 52 K.oxytoca were found to be ESBL positive in the year 2005. Two hundred thirty seven (29%) strains of 831 E.coli, 54 (21%) strains of 253 K.pneumoniae and 27 (27%) strains of 100 K.oxytoca were found to be ESBL positive in the year 2006. There were no significant differences in the ratios of E.coli, K.pneumoniae and K.oxytoca between the years 2005 and 2006 in terms of ESBL positivity. **Conclusion.** Although there were no significant differences in the ratios of ESBL positivity between the 2005 and 2006 years, ESBL positivity still remains to be an important problem for our hospital when these high ratios are considered.

Keywords: Gram negative bacteria, extended spectrum beta lactamase, nosocominal infection

Özet

Amaç. Hastanemizdeki 2005 ve 2006 yıllarındaki çeşitli klinik numunelerin kültürlerinden izole edilen gram negatif basillerin genişlemiş spektrumlu beta laktamaz (GSBL) üretim durumunu retrospektif olarak araştırmayı amaçladık. **Yöntem.** 2005 yılında 925 ve 2006 yılında 1184 hastanın kan, idrar, yara yeri sürüntüsü, balgam transtrakeal aspirasyon materyali, vajinal sürüntü, abse, serebrospinal sıvı, periton ve parasentez sıvıları ve kateter ucu gibi klinik numunelerinin kültürlerinden Escherichia coli (E.coli), Klebsiella pneumonia (K. pneumonia) ve Klebsiella oxytoca (K. oxytoca) gibi gram negatif bakteriler izole edildi. Bakterinin tanınması, sensitivite/direnç profilinin belirlenmesi ve bu izole edilen gram negatif bakterilerin ESBL üretip üretmediğini tespit etmek için Becton Dickinson Phoenix 100 model full automatic system kullanıldı. **Bulgular.** İki yıllık periyot (2005-2006) içinde 2109 gram negatif bakteri türünde (E.coli, K. pneumoniae, K. oxytoca) GSBL çalışılmıştır. Bunların 591'inde GSBL pozitif bulunmuştur. 2005'de 925 gram negatif bakteri türünden 273'ü (%30) , 2006'da 1184 gram negatif bakteri türünden 318'i (%27) GSBL pozitif idi. 2005 ve 2006 yılları arasında GSBL pozitiflik oranları arasında anlamlı bir fark bulunmamıştır ($p>0,05$). 2005 yılında 709 E.coli suşundan 211'inde (%30), 164 K. pneumoniae suşundan 44'ünde (%27) ve 52 K. oxytoca suşundan 18'inde

(%35) GSBL pozitif bulunmuştur. 2006 yılında 831 E. coli suşundan 237'si (%29), 253 K. pneumoniae suşundan 54'ü (%21) ve 100 K. oxytoca suşundan 27'si (%27) GSBL pozitif bulunmuştur. GSBL pozitifliği açısından; 2005 ve 2006 yılları arasında E.coli, K. pneumoniae ve K. oxytoca oranlarında anlamlı bir fark bulunamamıştır (p>0,05). **Sonuç.** 2005 ve 2006 yıllarında GSBL pozitiflik oranları açısından yıllara göre istatistiksel olarak anlamlı bir değişiklik olmamasına karşın, elde edilen oranlara bakılacak olursa GSBL pozitifliğinin halen hastanemiz için önemli bir problem olduğu görülmektedir.

Anahtar sözcükler: Gram negatif bakteriler, genişlemiş spektrumlu beta laktamaz, hastane enfeksiyonu

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Introduction

Hospital infections are one of the most important problems due to the difficulties of treatment, high mortality and morbidity [1]. There can be many differences about hospital infection agents and resistance profiles between countries, hospitals and also between different divisions of the same hospital. Therefore each hospital has to establish its self flora and resistance profile. This is important for both controlling the hospital infections and choosing empirical antibiotics. In recent years, the problem of increasing resistance to antibiotics has become a threat for the whole world. Beta lactamase production that hydrolyses beta lactam antibiotics is an important resistance mechanism of many bacteria. Approximately 150 of 350 beta lactamases are wide spectrum beta lactamases and can be transferred between bacteria due to plasmidic specialities [2, 3]. Extended spectrum beta lactamases (ESBL) are enzymes that gain resistance to aztreonam and oxyimino-beta lactams such as cefotaxime, ceftazidime, ceftriaxone, and their genetic code is on plasmids [4, 5]. These enzymes were first derived from klebsiella pneumonia in Europe in 1983 and after that they were shown in the other members of Enterobacteriaceae [6, 7]. It has been shown by hybridization experiments that ESBLs appear as a result of point mutations in TEM-1, TEM-2 and SHV-1 beta lactamase genes [5]. ESBLs hydrolyze third generation cephalosporins and aztreonam, and make the treatment with beta lactam antibiotics difficult. ESBLs can be hydrolyzed by beta lactamase inhibitors and enzymes are not effective against carbapenems (imipenem, meropenem), cephamycins (cefoxitin, moxalactam) and temocillin [6-10]. ESBL producing bacteria can be determined by the resistance to cephotaxim, ceftriaxone, ceftasidime, and/or aztreonam with routine sensitivity experiments. Sometimes however the resistance to these antibiotics couldn't be identified by using sensitivity experiments done with ESBL producing isolates and this situation causes treatment failure [7, 8]. Different methods like double disk synergy test (DDST), E-test and three dimensional tests can be used for the identification of ESBL producing isolates [11, 12]. The most common method to determine ESBL production is DDST. It is suitable for daily use and most research has been done on it. Also a synergy between beta-lactam and beta-lactamase inhibitors is searched by this test [13, 14]. Several factors increase infection risk with ESBL producing bacteria such as long term hospitalization, staying in ICU, urinary and venous catheters and the use of wide spectrum beta lactam antibiotics [15]. The incidence of ESBL producing bacteria as a hospital infection agent has become increased in the recent years and due to their multiple drug resistance their treatment is also difficult [16, 17]. It is reported that showing this enzyme production is mandatory because ESBL producing isolates can be found as sensitive at routine antibiogram although they are resistant to penicillins, cephalosporins and aztreonam and that problems can be faced during treatment with these antibiotics. [10].

In this study, the production of ESBL was retrospectively investigated in the Gram negative bacteria strains isolated from different clinical specimens in the Microbiology laboratory, Cumhuriyet University Research and Practice Hospital in inpatients between the years 2005-2006.

Materials and methods

In this retrospective study, ESBL production of Gr (-) bacillus that were isolated from the cultures of several clinical specimens taken from the inpatients of different departments of Cumhuriyet University Research and Practice Hospital between January 2005 and December 2006 and sent to Microbiology Laboratory were investigated.

We investigated whether ESBL production was available in gram negative bacillus such as Escherichia coli (E.coli), Klebsiella pneumonia (K. pneumonia), Klebsiella oxytoca (K. oxytoca) isolated from the cultures of the specimens of blood, urine, wound swab, sputum, trans tracheal aspirate material (TTA), vaginal swab, pus, abscess, cerebrospinal fluid, peritoneal paracentesis fluid, catheter etc of 925 patients in the year 2005 and 1184 patients in the year 2006 in order to determine a change in 2 years by scanning the results of patient records. Preparations were made from all clinical materials taken by sterile ecuvion or injector from inpatients of different departments of hospital in suitable conditions and cultured to the plates including 5% sheep blooded brain heart infusion agar (Merck KGaA, Germany), chocolate agar (Merck KGaA, Germany) and eosin methylene blue agar (Merck KGaA, Germany).The preperations were analyzed by a microscope in terms of gr (+) or gr (-) bacteria presence. Cultured plates were evaluated after one night incubation at 35^{o}C. Plates that had no reproduction were evaluated after one more 24 hours of incubation. Microorganisms reproduced in mediums were taken to the tubes which includes ID broth (Phoenix ID Broth 4.5 mL Becton Dickinson and Company, Ireland) to identification according to application proposals of kits and systems. Densities were adjusted to McFarland 0.5, then 0.25 mL was transferred from ID broth tubes to AST broth tubes (Phoenix AST broth 8 mL Becton Dickinson and Company, Ireland) for sensitivity/resistance tests and distributed to panels. These panels were used at the Becton Dickinson Phoenix 100 model full automatic systems to identify bacteria and their sensitivity/resistance profile and to investigate whether these isolated gr(-) bacteria produce ESBL or not.

The data of our study were evaluated by Chi-square and Fisher's Chi-square tests using SPSS (ver 13.0) program $p<0.05$ was considered as significant.

Results

ESBL (E.coli, K.pneumonia, K.oxytoca) production was investigated in a total of 2109 clinical specimens that had been sent to the laboratory in the years 2005 and 2006. 519 of them were positive. 273 (30%) specimens out of 925 from the year 2005 were ESBL positive while 652 (70%) of them were ESBL negative. 318 (27%) specimens out of 1184 in the year 2006 year were ESBL positive while 866 (73%) were ESBL negative (Table 1). There was no significant difference by means of ESBL positivity between the two years ($p>0.05$). Two hundred eleven (30%) E.coli strains out of 709 were ESBL positive while 498 (70%) were negative at the year 2005. 237 (29%) E.coli strains out of 831 were ESBL positive while 594 (71%) were negative at the year 2006 (Table 1). No significant difference was found regarding ESBL positivity in E.coli strains between the two years ($p>0.05$). There was no significant difference in terms of the ESBL positivity of E.coli strains isolated from different clinical specimens when their distribution according to years was investigated ($p>0.05$) (Table 2). Fortyfour (27%) K.pneumonia strains out of 164 were ESBL positive while 120 (73%) were negative at 2005. However, 54 (21%) K.pneumonia strains of 253 were ESBL positive while 199 (79%) were negative at the year 2006 (Table 1). No significant difference was found in the ESBL positivity of K.pneumonia between the two years ($p>0.05$).

Table 1. Yearly distribution of ESBL findings in some gram (-) bacillus

Bacteria	Year								
	2005				2006				
	ESBL (+)		ESBL (-)		ESBL (+)		ESBL (-)		Result
	n	(%)	n	(%)	n	(%)	n	(%)	
E.coli	211	30	498	70	237	29	594	71	p>0.05
K. pneumoniae	44	27	120	73	54	21	199	79	p>0.05
K. oxytoca	18	35	34	65	27	27	73	73	p>0.05
Total	273	30	652	70	318	27	866	73	p>0.05

Table 2. Yearly ESBL positivity of *E.coli* strains isolated from different clinical specimens.

Clinical specimens	Year								
	2005				2006				
	ESBL (+)		ESBL (-)		ESBL (+)		ESBL (-)		Result
	n	(%)	n	(%)	n	(%)	n	(%)	
Blood	8	20	33	80	6	15	35	85	p>0.05
Urine	100	26	293	74	132	25	395	75	p>0.05
Wound swab	65	50	65	50	55	43	74	57	p>0.05
Sputum	10	27	27	73	21	46	25	54	p>0.05
TTA	8	38	13	62	5	63	3	37	p>0.05
Vaginal swab	1	7	14	93	3	10	26	90	p>0.05
Other	19	26	53	74	15	29	36	71	p>0.05
Total	211	30	498	70	237	29	594	71	p>0.05

TTA : Trans tracheal aspirate

There was a significant difference at wound swab specimens about ESBL positivity of K.pneumonia isolated from several clinical specimens between the two years (p<0.05). ESBL positivity rates of the year 2006 were reduced when compared with the rates of 2005 at wound swab specimens. There was no significant difference regarding the other clinical specimens (p>0.05) (Table 3).

Table 3. Yearly ESBL positivity of *K. pneumoniae* strains isolated from different clinical specimens.

Clinical specimens	Year								
	2005				2006				
	ESBL(+)		ESBL(-)		ESBL (+)		ESBL (-)		Result
	n	(%)	n	(%)	n	(%)	n	(%)	
Blood	3	8	34	92	3	10	26	90	p>0.05
Urine	18	29	44	71	37	27	99	73	p>0.05
Wound swab	11	61	7	39	5*	24	16	76	P<0.05
Sputum	2	11	16	89	3	8	35	92	p>0.05
TTA	3	20	12	80	0	0	5	100	p>0.05
Vaginal swab	0	-	0	-	1	33	2	67	p>0.05
Other	7	50	7	50	5	24	16	76	p>0.05
Total	44	27	120	73	54	21	199	79	p>0.05

* significant decrease when compared with the year 2005

18 (35%) K.oxytoca strains out of 52 were ESBL positive while 34 (65%) were negative at 2005. Besides, 27(27%) K.oxytoca strains out of 100 were ESBL positive while 73 (73%) were negative at the year 2006 (Table 1). No significant difference was found about ESBL positivity regarding K.oxytoca strains between the two years (p>0.05).There was no significant difference about ESBL positivity of K.oxytoca isolated from several clinical specimens distributed according to years (p>0.05) (Table 4).

Table 4. Yearly ESBL positivity of *K. oxytoca* strains isolated from different clinical specimens.

Clinical specimens	Year								
	2005				2006				
	ESBL (+)		ESBL (-)		ESBL (+)		ESBL (-)		Result
	n	(%)	n	(%)	n	(%)	n	(%)	
Blood	0	0	1	100	1	20	4	80	p>0.05
Urine	10	37	17	63	18	28	47	72	p>0.05
Wound swab	2	18	9	82	4	31	9	69	P>0.05
Sputum	2	33	4	67	2	20	8	80	p>0.05
TTA	1	33	2	67	2	50	2	50	p>0.05
Other	3	75	1	25	0	0	3	100	p>0.05
Total	18	35	34	65	27	27	73	73	p>0.05

Discussion

ESBLs found in Gr (-) bacillus is enzymes that are responsible for the resistance to wide spectrum cephalosporins and aztreonam [18]. The infections due to ESBL releasing Enterobacteriaceae seem to be increased in our country like the entire world. As well as ESBLs are mostly found at Klebsiella and E.coli, it is increasing among the other pathogens and opportunist bacteria [19]. As most of the antibiotics can remain ineffective against ESBL positive bacteria, the ESBL production status of the pathogens must be known during treatment with antibiotics [11].

In our study, we retrospectively investigated whether E.coli, K. pneumonia and K. oxytoca, isolated from several departments was producing ESBLs. Higher rates of ESBL were reported in the studies of low socio-economic level and low health service quality countries. The high ESBL rate of these countries increases morbidity, mortality and health costs [20]. However, the rate of ESBL producing bacteria in high socio-economic level countries like USA, Japan and in most of Europe is 1% and there is not a problem yet in these countries [20]. In our country there are different results from different centers about ESBL positivity. But the common feature is that ESBL rates of K.pneumonia are higher than the rates of E.coli. While, Bülüç et al. [21] found the ESBL rates 48% and 14% for K.pneumonia and E.coli respectively, Tünger et al. [22] found 41.7% and 13.4% for nasocomial strains. On the other hand, Kiremitçi et al. [23] found these rates as 42% and 23.3% and Gönüllü et al. [24] declared a rate of 52% and 40%. In the studies from other countries ESBL producing rates were higher for K.pneumonia than E.coli too. Datta et al. [25] reported even more different results (12.5% and 16.1%). In our study, while 211 (30%) E.coli strains out of 709 were ESBL positive, 44 (27%) K.pneumonia strains out of 164 were ESBL positive and 18 (35%) K.oxytoca strains out of 52 were ESBL positive at the year 2005. 237 (29%) E.coli strains out of 831 were ESBL positive, 54 (21%) K.pneumonia strains out of 253 were ESBL positive and 27(27%) K.oxytoca strains out of 100 were ESBL positive at the year 2006.

Aktaş et al. [26] found ESBL positivity to be 19.2% in 52 E.coli and 58.3% in 12 K.pneumonia strains at their study by DDST method. Şahin et al. [27] found ESBL positivity in 19.4% of 108 E.coli strains and 15.9% of 44 Klebsiella spp strains with the same method. Similarly, Akçam et al. [28] reported ESBL positivity in 6 (7.2%) of 83 E.coli strains and in 14 (35%) of 40 Klebsiella strains. Several studies of other countries showed ESBL positivity between 13.0%-86.6% at Klebsiella strains and between 11.0%-63.6% at E.coli strains [29-31]. It is suggested that the different ESBL rates of several studies are related to the changes in enzyme production frequency of bacteria in different conditions [15]. There can be many differences about hospital infection agents and resistance profiles between countries, hospitals and also between different divisions of the same hospital. Therefore each hospital has to establish its self flora and resistance profile. This is important in both controlling hospital infections and choosing empirical antibiotics.

It is known that the ESBL production of K.pneumonia strains isolated from patients who had a medical history of treatment in intensive care units, a recent operation, catheter or

invasive procedure applications, long hospital stay, use of extended spectrum beta-lactame antibiotics are found to be in higher ratio than that of the strains isolated from society [32, 33].
In a study about this issue, Delialioglu et al. [34] investigated E.coli and Klebsiella isolated from inpatient and outpatients. They determined ESBL production at 94 (18.3%) of 514 E.coli strains, 52 (29.7%) of 175 K.pneumonia strains and 1 (4.2%) of 24 K.oxytoca strains. They found ESBL production of E.coli and K.pneumonia isolated from inpatients to be significantly higher. Celen et al. [35] investigated ESBL production in 182 gram negative bacteria which they accepted as hospital infectious agents. The bacteria were isolated from Dicle University hospital for two years (2003-2004). They had found the ESBL production rate of gram negative bacteria in their same hospital to be 31% in 2001 and the difference was not significant between the 2001 and 2003-2004 ratios. Similarly our results of 2005 and 2006 were 30% and 27% respectively and there was not a statistically significant difference between these two years.

ESBL positivity has been shown by using several tests such as disc diffusion, liquid dilution, agar dilution, E-test, half automatic and automatic systems [36, 37]. We used automated Phoenix system (Phoenix, Becton Dickinson and Company, Ireland). As automated systems give results in short time duration, antibiotics can also be started in a short time, therapy can be completed in a short time and the duration of hospitalization is decreased. Besides, systems have data management systems so that the data can be transferred automatically to laboratory computing systems and it prevents errors and provides easy collection of statistical data regarding the antibiotic susceptibility [36]. Although there was no statistically significant difference in the changes of ESBL positivity in our hospital between the years 2005 and 2006, our ESBL positivity rates show that ESBL positivity is still a real problem for our hospital. This study emphasizes the importance of rational antibiotic use in decreasing antibiotic resistance ratios which usually is an overlooked/uncontrolled issue. Also we think that our GSBL ratios can be used for comparison in more detailed future studies.

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