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Investigation of *bla*$_{KPC}$ Gene by PCR in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates in a Tertiary Care Hospital in Turkey

Türkiye'deki Üçüncü Basamak Bir Hastanede Klinik Örneklerden İzole Edilen Karbapenem Dirençli *Escherichia coli* ve *Klebsiella pneumoniae* İzolatlarında *bla*$_{KPC}$ Geninin PCR ile Araştırılması

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Abstract

Introduction: One of the most common mechanisms in the development of carbapenem resistance is acquiring carbapenemases. A new and different carbapenemase, *Klebsiella pneumoniae* carbapenemase (KPC), has recently emerged in Turkey. *Klebsiella pneumoniae* carbapenemases are encoded by the *bla*$_{KPC}$ gene and can be transferred between different species. Reports showing the presence of *bla*$_{KPC}$ gene in isolates from Turkey are limited. The current study aimed to investigate the presence of *bla*$_{KPC}$ gene in carbapenem resistant *K. pneumoniae* and *Escherichia coli* isolates in a tertiary care hospital in Turkey.

Materials and Methods: Patient samples from various clinical units in Adana Numune Training and Research Hospital between November 2013 and October 2014 were sent to our laboratory at the same hospital. *K. pneumoniae* and *E. coli* strains isolated from different clinical samples were identified using both conventional methods and the VITEK® 2.0 automated identification system (bioMérieux, France). Imipenem-cilastatin, meropenem, and ertapenem susceptibility was analyzed using a VITEK® 2.0 antibiotic susceptibility testing system (bioMérieux, France). The presence of blaKPC gene was investigated by polymerase chain reaction.

Results: A total of 49 resistant *K. pneumoniae* and 33 E. coli isolates were included in the study. Most were isolated from urine specimens. Among the carbapenems we tested, highest resistance rates were to ertapenem (98.0% in *K. pneumoniae* and 84.8% in *E. coli* isolates). One *K. pneumoniae* isolate was found positive for the blaKPC gene.

Conclusion: According to our literature survey, this study is among the first reports from Turkey presenting the isolation of KPC-producing *K. pneumoniae*. This finding indicates the need to monitor carbapenem resistance arising from *bla*$_{KPC}$, which may spread horizontally. Moreover, surveillance of antibiotic sensitivity rates and observation of regional differences will be useful guidelines in determining infection control and antimicrobial use management policies.

Keywords: Carbapenemase, *bla*$_{KPC}$ gene, molecular epidemiology, urinary tract infection, modified Hodge test

Öz

Giriş: Karbapenem direncinin gelişiminde en önemli mekanizmalarından biri karbapenemazlardır. Son zamanlarda yeni ve farklı bir karbapenemaz olan *Klebsiella pneumoniae* karbapenemaz (KPC) Türkiye'de de görülmeye başlanmıştır. *Klebsiella pneumoniae* karbapenemazlar *bla*$_{KPC}$ geni tarafından kodlanır ve farklı türler arasında aktarılabilir. Türkiye'de *bla*$_{KPC}$ geni bulunan izolat bildirimleri oldukça azdır. Bu nedenle çalışmamızda

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Türkiye'deki bir üçüncü basamak hastanede karbapenem dirençli *Klebsiella pneumoniae* ve *Escherichia coli* kökenlerinde bla_{KPC} geninin varlığının araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmamızda Kasım 2013-Kasım 2014 tarihleri arasında Adana Numune Eğitim ve Araştırma Hastanesi'nin çeşitli klinik birimlerinden Tıbbi Mikrobiyoloji Laboratuvarı'na gönderilen hasta örnekleri alındı. Farklı klinik örneklerden izole edilen *K. pneumoniae* ve *E. coli* kökenleri konvansiyonel yöntemlere ek olarak VITEK® 2.0 (bioMérieux, Fransa) otomatize sistemi ile tanımlandı ve imipenem-silastatin, meropenem ve ertapenem duyarlılıkları VITEK® 2.0 otomatize antibiyotik duyarlılık sistemi (bioMérieux, Fransa) ile çalışıldı. Ayrıca, bla_{KPC} geni varlığı polimeraz zincir reaksiyonu yöntemi ile araştırıldı.

Bulgular: Karbapenem dirençli 49 adet *K. pneumoniae* ve 33 adet *E. coli* suşu çalışmaya dahil edildi. Test ettiğimiz karbapenem grubu antibiyotikler içinde en yüksek direnç oranı *K. pneumoniae*'da %98,0 ve *E. coli*'de %84,8 ile ertapeneme karşı tespit edildi. Polimeraz zincir reaksiyonu yöntemi ile bir *K. pneumoniae* izolatında bla_{KPC} geni varlığı belirlendi.

Sonuç: Literatür taramalarımızda ulaşabildiğimiz kadarıyla, çalışmamız Türkiye'deki KPC üreten *K. pneumoniae* izolasyonunu sunan ilk yayınlardan biridir. Bu bulgu, bla_{KPC} ile horizontal olarak yayılabilen karbapenem direncinin araştırılması gerektiğini göstermiştir. Enfeksiyon kontrolü ve antimikrobiyal kullanım politikalarının belirlenmesinde, antimikrobiyal duyarlılık oranlarının sürekli olarak izlenmesi ve bölgesel farklılıkların gözlemlenmesi oldukça önemli yol göstericilerdir.

Anahtar Kelimeler: Karbapenemaz, bla_{KPC} geni, moleküler epidemiyoloji, üriner sistem enfeksiyonu, modifiye Hodge testi

Introduction

Carbapenems are highly effective antibiotics in the treatment of *Enterobacteriaceae* infections. However, carbapenem-resistant *Enterobacteriaceae* (CRE) strains have been reported all over the world in recent years[1]. Carbapenem-resistant *Enterobacteriaceae* infections comprise public health threat due to the limited treatment options[2].

Different mechanisms are known in the development of carbapenem resistance.

These include (i) decreased permeability of the outer membrane, (ii) beta-lactamase (carbapenemase) production, (iii) decreased affinity of penicillin-binding proteins, (iv) high-level production of cephalosporinase, and (v) loss or alteration of porins[3]. One of the most common mechanisms of carbapenem resistance is carbapenemases such as VIM, IMP, NDM, and OXA. Another carbapenemase, *Klebsiella pneumoniae* carbapenemase (KPC), has recently been detected in Turkey[2,4,5]. *Klebsiella pneumoniae* carbapenemase is encoded by the bla_{KPC} gene and can be transferred between different species; its universal spread is mainly attributable to the Tn3-type transposon (Tn4401)[5]. The presence of carbapenemase in *Enterobacteriaceae* was first reported in 1993[6]. *Klebsiella pneumoniae* carbapenemase was first reported in the Eastern United States in 1996 and caused epidemics, mainly in New York. Subsequently, the gene was also reported in Europe and South Asia[6-8].

The current study aimed to investigate the presence of bla_{KPC} gene in carbapenem-resistant *K. pneumoniae* and *E. coli* strains isolated from different clinical units between November 2013 and November 2014 in the microbiology laboratory of Adana Numune Training and Research Hospital.

Materials and Methods

Bacterial Isolates

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by a Local Ethics Committee (Adana Numune Training and Research Hospital protocol number: 2014/7). The study was conducted using patient samples sent to our laboratory from different clinical units of the hospital between November 2013 and November 2014. Each sample was used to inoculate three types of agar plates: 5% sheep blood agar (GBL, Turkey); Eosin methylene blue agar (GBL, Turkey), and chocolate agar (GBL, Turkey). The plates were incubated in aerobic conditions for 24-48 hours at 37 °C and growth was evaluated. Catalase-positive, oxidase-negative, non-fermentative bacteria with Gram-negative bacillary appearance on stained microscopic examination were identified by classical methods. In addition, isolates were identified at the species level in accordance with the manufacturer's recommendations with VITEK® 2.0 automated bacterial identification and antibiotic susceptibility system (bioMérieux, France). Isolates identified as *K. pneumoniae* or *E. coli* were aliquoted and stocked at -20 °C.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility patterns of isolates identified as *K. pneumoniae* and *E. coli* to imipenem-cilastatin (IMP), ertapenem (ETP), and meropenem (MEM) were assessed with the VITEK® 2.0 automated system (bioMérieux, France). The results were categorized as sensitive or resistant according to Clinical and Laboratory Standards Institute (CLSI) criteria[9]. Intermediate strains were regarded as resistant because they could produce carbapenemases[4]. *K. pneumoniae* ATCC BAA-1705 was employed as a KPC-positive control strain and *K. pneumoniae* ATCC BAA-1706 was used as a KPC-negative control strain. Any detected carbapenem resistance was reported to the clinical units of origin for implementation of necessary contact isolation procedures.

Investigation of Extended-spectrum Beta-lactamase (ESBL) Presence

Investigation of extended-spectrum beta-lactamase positivity was confirmed by either double-disc synergy test method or combined disc method[9]. AmpC beta-lactamase detection in the isolates was performed according to CLSI recommendations. ATCC 25922 *E. coli* and ATCC 700603 *K. pneumoniae* isolates were used as controls in all tests[10].

Modified Hodge Test (MHT) and Boronic Acid Inhibition Test

Modified Hodge test was used to detect carbapenemases phenotypically. E. coli ATCC 25922, *K. pneumoniae* ATCC BAA-1705 (positive control), *K. pneumoniae* ATCC BAA-1706 (negative control), and ETP disc (10 µg) were used. After 24 hours of incubation at 35 °C on a Mueller-Hinton agar (MHA) medium, a clover leaf appearance was considered positive[10].

In the boronic acid inhibition test, an inhibition zone at least 5 mm larger in diameter around the ETP/boronic acid (10 µg/400 µg) disc compared to the ETP-only disc (10 µg) after 24 h incubation on MHA medium was accepted as positive[11].

Isolates were stored at -20 °C in tryptic soy broth medium (Fluka, 22092, USA) containing 15% glycerol (Merck, Germany) until analysis[12].

DNA Isolation and Polymerase Chain Reaction (PCR)

The isolates were passaged onto 5% sheep blood agar from stock medium. DNA extraction was performed using GF-1 nucleic acid extraction kit (Vivantis, Malaysia) according to manufacturer's recommendations. Thus, the template DNA samples to be used in the PCR process were obtained. Polymerase chain reaction was performed employing bla_{KPC} forward (5'-ATGTCACTGTATCGCCGTCT-3') and reverse (5'-TTTTCAGAGCCTTACTGCCC-3') primers (IDT, USA) in order to detect all known bla_{KPC} types ($bla_{KPC-1-7}$)[13]. For electrophoresis, 1% agarose gel was prepared and ethidium bromide was added to obtain a final concentration of 0.5 µg/ml. In order to evaluate product size, 5 µl of a 100 base pair (bp) DNA ladder (New England BioLabs, UK) was added to the first and last wells. *K. pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 were used as KPC positive and negative controls, respectively.

Bands formed during electrophoresis were observed on a UV transilluminator and images were obtained with the VersaDoc™ Imaging System (Bio-Rad, USA).

Results

During the study period, the microbiology laboratory isolated 3802 *K. pneumoniae* and 2576 *E. coli* strains from patient samples in various wards. In total, 152 of these isolates were determined to be resistant to carbapenems. Forty-nine carbapenem-resistant *K. pneumoniae* and 33 *E. coli* isolates were included in the study after excluding subsequent isolates of the same strain and antibiotic susceptibility pattern from a patient. The most common type of specimen included in the study was urine samples, followed by blood and catheter samples. Distribution of isolates in terms of sample type is shown in Table 1, and distribution of clinical units from which the samples were sent is shown in Table 2.

As can be seen in Table 3, the highest susceptibility rate of the tested carbapenems was detected against IMP in *E. coli* isolates, with 78.8%. The lowest sensitivity was found against ETP in *K. pneumoniae* isolates, with 2%.

The MHT, which is a phenotypic screening test for carbapenemase, was positive in 61.5% of *K. pneumoniae* isolates and 45.5% of *E. coli* isolates. Boronic acid inhibition test, which is a phenotypic screening test for class A carbapenemases, was positive in 14.3% of *K. pneumoniae* isolates. Data regarding extended-spectrum beta-lactamase, boronic acid inhibition test, and MHT positivity rates are shown in Table 3.

Amplification with the generic bla_{KPC} primers yielded a band of 882 bp in one of the isolates (Figure 1). The positive band belonged to a *K. pneumoniae* isolate from the urine specimen of a 77-year-old Turkish man who presented to the urology department for nephrolithiasis and benign prostatic hyperplasia. He had no history of travel abroad in the recent past. This isolate was determined to be ESBL-positive, resistant to ceftriaxone, cefotaxime, ceftazidime, and all three carbapenems tested; susceptible to gentamicin, amikacin, cefepime, and tigecycline.

Discussion

Carbapenem resistance in *Enterobacteriaceae* was first reported in 1997 in the United States and in 2001 in Turkey[14]. Subsequently, CRE epidemics were reported at different times in various regions[14]. Carbapenem-resistant *Enterobacteriaceae* can be encountered in almost every specimen type[15]. In our

Table 1. Distribution of carbapenem-resistant isolates according to types of clinical specimens

	Specimen type % (n)						
	Urine	Blood	Catheter	Sputum	Wound	Peritoneal fluid	Total
K. pneumoniae	57.1 (28)	18.4 (9)	8.2 (4)	8.2 (4)	6.1 (3)	2.0 (1)	100 (49)
E. coli	78.8 (26)	6.1 (2)	3.0 (1)	3.0 (1)	6.1 (2)	3.0 (1)	100 (33)
Total	65.9 (54)	13.4 (11)	6.1 (5)	6.1 (5)	6.1 (5)	2.4 (2)	100 (82)

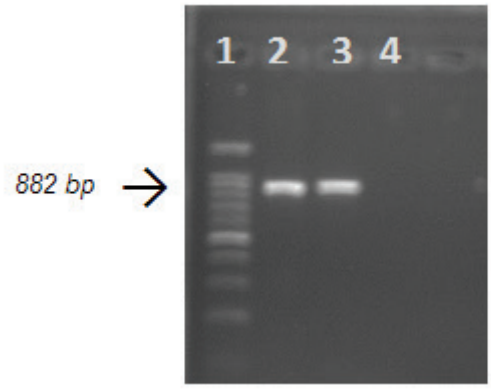

Figure 1. Gel image of isolate with bla_{KPC-2} gene band (Lane 1: 100 bp DNA marker; Lane 2: isolate of *K. pneumoniae* carrying bla_{KPC} gene; Lane 3: KPC-positive control strain [*K. pneumoniae* ATCC BAA-1705]; Lane 4: KPC-negative control strain [*K. pneumoniae* ATCC BAA-1706])

Table 2. Distribution of carbapenem-resistant isolates according to clinical units

	Clinical units % (n)			
	Outpatient	Inpatient	ICU	Total
K. pneumoniae	18.4 (9)	36.7 (18)	44.9 (22)	100 (49)
E. coli	42.4 (14)	48.5 (16)	9.1 (3)	100 (33)
Total	28.0 (23)	41.5 (34)	30.5 (25)	100 (82)

ICU: Intensive care unit

study, carbapenem-resistant *E. coli* and *K. pneumoniae* isolates were detected mostly in urine specimens. Similar to our study, Tseng et al.[8] studied 163 carbapenem-resistant *K. pneumoniae* strains in Taiwan and found that they were most commonly in urine samples 36.2%. Balkan et al.[15] examined bloodstream infections caused by CREs and showed that 5.5% of these infections originated from the urinary tract. Us et al.[7] examined 26 carbapenem-resistant *K. pneumoniae* strains, 50% of which originated from blood and 23% from urine.

Gram-negative bacteria, which cause serious hospital infections, are responsible for a significant proportion of infections in intensive care units (ICUs)[14]. Members of the *Enterobacteriaceae* family are among the most common pathogens in ICUs[16]. *K. pneumoniae* is reported to be the fourth most common cause of pneumonia and the fifth most common cause of bacteremia in patients receiving treatment in ICUs, while, *E. coli* is the most common cause of urinary tract infections[17,18]. Severe infections in the ICU are usually treated with carbapenem group antibiotics[7]. When the distribution of CRE isolates in our study were analyzed, *K. pneumoniae* was found to be mostly isolated from patients treated in ICU (44.9%) and *E. coli* was mostly isolated from the other inpatients (48.5%). Similarly, in a study conducted in Malatya, Kuzucu et al.[19] reported that 70% of *E. coli* strains and 71.7% of *Klebsiella* strains were isolated from inpatient wards. In a study conducted by Us et al.[7] in Ankara,

Table 3. Antibiotic susceptibility rates of carbapenem-resistant isolates and rates of extended-spectrum beta-lactamase, boronic acid inhibition test, modified Hodge test, and AmpC positivity

	Types of antibiotics % (n)						ESBL		AmpC		MHT		Boronic acid inhibition test	
	Imipenem		Meropenem		Ertapenem									
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
K. pneumoniae (n=49)	32.7 (16)	67.3 (33)	49.0 (24)	51.0 (25)	2.0 (1)	98.0 (48)	34.7 (17)	65.3 (32)	14.3 (7)	85.7 (42)	61.2 (30)	38.8 (19)	55.1 (27)	44.9 (22)
E. coli (n=33)	51.5 (17)	48.5 (16)	78.8 (26)	21.2 (7)	15.2 (5)	84.8 (28)	51.5 (17)	48.5 (16)	3.0 (1)	97.0 (32)	45.5 (15)	54.5 (18)	39.4 (13)	60.6 (20)

ESBL: Extended-spectrum beta-lactamase, MHT: Modified Hodge test

54% of carbapenem-resistant *K. pneumoniae* strains were detected in the ICU.

Isolates belonging to the *Enterobacteriaceae* family may be resistant to antibiotics such as carbapenems that are used as last-resort treatment for serious infections. Carbapenem-resistance may further limit treatment options and lead to treatment failure[20]. Differential susceptibility to carbapenems may occur due to relative penetration rates through minor porins, differential susceptibility to efflux, or relative susceptibility to slow hydrolysis by AmpC enzymes or ESBLs[21,22]. Higher than data from other countries but similar to those of other Turkish studies, the highest rate of resistance to the carbapenems that we tested in our study was to ETP (98.0% in *K. pneumoniae*, 84.8% in *E. coli*). In 2008, Güzel Tunçcan et al.[23] conducted a study in Ankara and found reported no carbapenem resistance in 58 *E. coli* and 37 *Klebsiella* spp. strains isolated from various clinical specimens. However, in 2011, Kuzucu et al.[19] reported 0.7% IMP and MEM resistance and 1.4% ETP resistance in ESBL-positive *E. coli* and *Klebsiella* spp. strains in Malatya. In 2015 in the USA, Pecora et al.[24] detected carbapenem resistance in 1.5% of 41 *K. pneumoniae* isolates from various clinical specimens. In Brazil, Biberg et al.[25] detected carbapenem resistance in 12.2% of 360 *Klebsiella* spp. isolates.

There are many mechanisms of carbapenem resistance among *Enterobacteriaceae*. One of these, carbapenemase production, has become a major public health problem worldwide[1]. The KPC type carbapenemase is one of these critical mechanisms[2]. The presence of the bla_{KPC} gene was first detected in the USA in 1996[8]. Studies have shown that the bla_{KPC} gene is gradually spreading among *K. pneumoniae* strains[11]. In France, China, Colombia, and Scotland, sporadic cases of KPC-producing strains were observed, while prolonged outbreaks were reported in Israel and Greece in 2004 and 2007, respectively[26].

Reports of *K. pneumoniae* isolates carrying the bla_{KPC} gene have been very limited in Turkey. A strain showing KPC gene positivity was first reported by Labarca et al.[27] in 2014 in a case report of an 80-year-old Romanian patient treated in an ICU in Kocaeli. In the same year, Poirel et al.[28] identified two *K. pneumoniae* isolates producing KPC-2 carbapenemase in İstanbul. In 2016, as part of the European Survey of Carbapenemase Producing Enterobacteriaceae (EuSCAPE) project, 155 carbapenemase suspected *K. pneumoniae* (n=134, 86.5%) and *E. coli* (n=21, 13.5%) isolates submitted from 18 centers in various regions of Turkey were investigated, but none was positive for KPC[20]. Recently, Sağıroğlu et al.[29] reported the presence of KPC in *K. pneumoniae* strains isolated from two patients. In 2016, Kuskucu et al.[30] reported two KPC-2-producing *E. coli* isolates in Turkey. In this study, we investigated the presence of bla_{KPC} gene in carbapenem-resistant *E. coli* and *K. pneumoniae* isolates and detected bla_{KPC} gene in one of the *K. pneumoniae* isolates. According to our literature survey, this report is one of the few reports reporting the presence of KPC-producing *K. pneumoniae* isolates in Turkey.

This study was carried out in a state hospital and has some limitations due to financial and technical issues resulting in lack of sequencing the positive gene, and screening other carbapenemase genes in the laboratory. The bla_{KPC}-positive strain and the other isolates used in the study could not survive because of technical problems in the hospital.

Conclusion

In conclusion, continuous monitoring of sensitivity rates and observation of regional differences will provide guidance in determining infection control and antimicrobial usage policies. In addition, surveillance of resistance genes with the potential for horizontal spreading, like in this study, will likely contribute to the prevention of carbapenemase-induced resistance.

Ethics

Ethics Committee Approval: The study was approved by the Adana Numune Training and Research Hospital of Local Ethics Committee (protocol number: 2014/7).

Informed Consent: It was not received.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: G.Ö., E.Ö., H.K., B.M.S., F.B., Design: G.Ö., E.Ö., H.K., B.M.S., C.K.B., Data Collection or Processing: G.Ö., E.Ö., S.K.S., H.A., C.K.B., Analysis or Interpretation: G.Ö., E.Ö., Literature Search: E.Ö., F.B., C.K.B., Writing: G.Ö., E.Ö., C.K.B.

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References

1. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V; European Network on Carbapenemases. Identification and screening of carbapenemase-producing *Enterobacteriaceae*. Clin Microbiol Infect. 2012;18:432-8.
2. Kanerva M, Skogberg K, Ryynänen K, Pahkamäki A, Jalava J, Ollgren J, Tarkka E, Lyytikäinen O. Coincidental detection of the first outbreak of carbapenemase-producing *Klebsiella pneumoniae* colonisation in a primary care hospital, Finland, 2013. Euro Surveill. 2015;20:pii=21172.
3. Yiğit H, Queenan AM, Anderson GJ, Domenech-Sanchez AD, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2001;45:1151-61.

4. Bae IK, Kang HK, Jang IH, Lee W, Kim K, Kim JO, Jeong SH, Lee K. Detection of Carbapenemases in Clinical *Enterobacteriaceae* Isolates Using the VITEK AST-N202 Card. Infect Chemother. 2015;47:167-74.
5. Bina M, Pournajaf A, Mirkalantari S, Talebi M, Irajian G. Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* isolated from the clinical samples by the phenotypic and genotypic methods. Iran J Pathol. 2015;10:199-205.
6. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase producing *Enterobacteriaceae*. Emerging Infectious Diseases. 2011;17:1791-8.
7. Us E, Tekeli A, Arıkan Akan O, Dolapci I, Şahin F, Karahan ZC. Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* strains isolated between 2004-2007 in Ankara University Hospital, Turkey. Mikrobiyol Bul. 2010;44:1-10.
8. Tseng IL, Liu YM, Wang SJ, Yeh HY, Hsieh CL, Lu HL, Tseng YC, Mu JJ. Emergence of Carbapenemase Producing *Klebsiella pneumoniae* and spread of KPC-2 and KPC-17 in Taiwan: A Nationwide Study from 2011 to 2013. PLoS One. 2015;10:e0138471.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Seventeenth Informational Supplement. Wayne, PA. M100-S17;2007.
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Nineteenth Informational Supplement. Wayne, PA. M100-S17;2009.
11. Baykal A, Coplu N, Simsek H, Esen B, Gur D. Investigation of the presence of extended spectrum beta-lactamase, KPC- type carbapenemase and plasmid-mediated AmpC beta-lactamase in *E. coli* and *K. pneumoniae* strains Isolated from Blood. Mikrobiyol Bul. 2012;46:159-69.
12. Robson RL, Essengue S, Reed NA, Horvat RT. Optochin resistance in *Streptococcus pneumoniae* induced by frozen storage in glycerol. Diagn Microbiol Infect Dis. 2007;58:185-90.
13. Schechner V, Straus-Robinson K, Schwartz D, Pfeffer I, Tarabeia J, Moskovich R, Chmelnitsky I, Schwaber MJ, Carmeli Y, Navon-Venezia S. Evaluation of PCR-based testing for surveillance of kpc-producing carbapenem-resistant members of the *Enterobacteriaceae* family. J Clin Microbiol. 2009;47:3261-5.
14. Çiftci İH, Karakeçe E, Aşık G, Demiray T, Er H. An Investigation on the Presence of OXA-48 and KPC in Carbapenem Resistant *Klebsiella pneumoniae* Strains. ANKEM Derg. 2013;27:49-54.
15. Balkan II, Aygun G, Aydin S, Mutcali SI, Kara Z, Kuskucu M, Midilli K, Şemen V, Aras S, Yemişen M, Mete B, Özaras R, Saltoğlu N, Tabak F, Öztürk R. Blood stream infections due to OXA-48-like carbapenemase-producing *Enterobacteriaceae*: treatment and survival. Int J Infect Dis. 2014;26:51-6.
16. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) Study. EPIC International Advisory Committee. JAMA. 1995;274:639-44.
17. Samra Z, Ofir O, Lishtzinsky Y, Madar-Shapiro L, Bishara J. Outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing KPC-3 in a tertiary medical centre in Israel. Int J Antimicrob Agents. 2007;30:525-9.
18. Yoon BI, Kim HS, Kim SD, Cho KJ, Kim SW, Ha US, Cho YH5, Sohn DW. Changes in bacterial species and antibiotic sensitivity in intensive care unit: acquired urinary tract infection during 10 years interval (2001-2011). Urol J. 2014;11:1478-84.
19. Kuzucu C, Yetkin F, Gorgec S, Ersoy Y. Investigation of the susceptibilities of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* spp. strains to ertapenem and other carbapenems. Mikrobiyol Bul. 2011;45:28-35.
20. Cakar A, Akyon Y, GUr D, Karatuna O, Ogunc D, Ozhak Baysan B, Çöplü N, Çağatay M, Kılıç A, Baysallar M, Bakıcı Z, Çelik C, Gülay Z, Aydemir Ş, Tünger A, Kılıç H, Erçal BD, Aşçı Toraman Z, Zer Y, Büyüktaş A, Ay S, Aktaş Z, Kayacan Ç, Bayramoğlu G, Aydın F, Dündar D, Hasdemir U, Ayaş R, Yanık K, Günaydın M, Güdücüoğlu H, Parlak M. Investigation of carbapenemases in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in 2014 in Turkey. Mikrobiyol Bul. 2016;50:21-33.
21. Woodford N, Dallow JW, Hill RL, Palepou MF, Pike R, Ward ME, Warner M, Livermore DM. Ertapenem resistance among *Klebsiella* and Enterobacter submitted in the UK to a reference laboratory. Int J Antimicrob Agents. 2007;29:456-9.
22. Kuskucu MA, Karakullukcu A, Ailiken M, Otlu B, Mete B, Aygun G. Investigation of carbapenem resistance and the first identification of *Klebsiella pneumoniae* carbapenemase (KPC) enzyme among *Escherichia coli* isolates in Turkey: A prospective study. Travel Med Infect Dis. 2016;14:572-6.
23. Güzel Tunçcan Ö, Tozlu Keten D, Dizbay M, Hızel K. Antimicrobial Susceptibility of Ertapenem Comparing with other Antibiotics against Nosocomial *Escherichia coli* and *Klebsiella* Strains. ANKEM Derg. 2008;22:188-92.
24. Pecora ND, Li N, Allard M, Li C, Albano E, Delaney M, Dubois A, Onderdonk AB, Bry L. Genomically ınformed surveillance for carbapenem-resistant *Enterobacteriaceae* in a health care system. MBio. 2015;6:e01030.
25. Biberg CA, Rodrigues AC, do Carmo SF, Chaves CE, Gales AC, Chang MR. KPC-2-producing *Klebsiella pneumoniae* in a hospital in the Midwest region of Brazil. Braz J Microbiol. 2015;46:501-4.
26. Maltezou HC, Giakkoupi P, Maragos A, Bolikas M. Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). J Infect. 2009;58:213-9.
27. Labarca J, Poirel L, Ozdamar M, Turkoglu S, Hakko E, Nordmann P. KPC-producing *Klebsiella pneumoniae*, finally targeting Turkey. New Microbe New Infect. 2014;2:50-1.
28. Poirel L, Yilmaz M, Istanbullu A, Arslan F, Mert A, Bernabeu S, Nordmann P. Spread of NDM-1-producing *Enterobacteriaceae* in a neonatal intensive care unit in Istanbul, Turkey. Antimicrob Agents Chemother. 2014;58:2929-33.
29. Sağıroğlu P, Hasdemir U, Altınkanat Gelmez G, Aksu B, Karatuna O, Söyletir G. Performance of "RESIST-3 O.K.N. K-SeT" immunochromatographic assay for the detection of OXA-48 like, KPC, and NDM carbapenemases in *Klebsiella pneumoniae* in Turkey. Braz J Microbiol. 2018;49:885-90.
30. Kuskucu MA, Karakullukcu A, Ailiken M, Otlu B, Mete B, Aygun G. Investigation of carbapenem resistance and the first identification of *Klebsiella pneumoniae* carbapenemase (KPC) enzyme among *Escherichia coli* isolates in Turkey: A prospective study. Travel Med Infect Dis. 2016;14:572-6.