**Original Article**

**Epidemic Klebsiella pneumoniae ST258 incidence in ICU patients admitted to a university hospital in Istanbul**

Ozge Unlu¹, Berken Rabun Ersoz², Ayse Istanbullu Tosun³, Mehmet Demirci⁴

¹ Department of Medical Microbiology, School of Medicine, Beykent University, Istanbul, Turkey
² School of Medicine, Beykent University, Istanbul, Turkey
³ Department of Medical Microbiology, International School of Medicine, Medipol University, Istanbul, Turkey
⁴ Department of Medical Microbiology, School of Medicine, Kirklareli University, Kirklareli, Turkey

**Abstract**

Introduction: *Klebsiella pneumoniae* sequence type 258 (ST258) strains are globally distributed multi-drug resistant pathogens and can spread rapidly throughout the world, causing severe healthcare-associated invasive infections with limited antimicrobial treatment options. The aim of this study was to reveal the incidence of *Klebsiella pneumoniae* ST258 strains among the intensive care unit patients in a university hospital in Istanbul.

Methodology: Consecutive nonreplicated 83 *K. pneumoniae* strains were isolated from various clinical samples of intensive care unit patients admitted to a university hospital in Istanbul, between November 2016 to December 2018. Bacterial identifications were performed via VITEK2. Antimicrobial susceptibility tests were conducted with Kirby Bauer’s disc diffusion test except for colistin which was performed with broth microdilution. Real-time PCR method was utilized in order to reveal ST258 positivity among the strains.

Results: Antimicrobial susceptibility results revealed that 56 (67%) *K. pneumoniae* strains were carbapenem-resistant. Real-time PCR results demonstrated that 15 out of 83 (18%) *K. pneumoniae* strain were ST258. According to antimicrobial susceptibility test results of ST258 strains, 8 were found as carbapenem-resistant whereas 7 were found as carbapenem susceptible. 3 out of 8 (37.5%) carbapenem-resistant ST258 strains were found as resistant against all antibiotics tested.

Conclusions: Our study revealed that *K. pneumoniae* ST258 which caused severe infections worldwide so far has also spread to Istanbul. We believe that rapid molecular methods for monitorization of these clones are useful. our results showed that ST258 is not linked to a multi-resistant strain and suggested that it does not contribute to multi-resistance formation alone.

**Key words:** Klebsiella pneumoniae; ST258; antimicrobial resistance; ICU.

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**Introduction**

*Klebsiella pneumoniae* are Gram-negative, encapsulated, nonmotile bacteria that are colonized on human mucosal surfaces [1]. Due to their ability to invade and disseminate to other tissues, it causes several types of life-threatening infections in humans [2,3]. *K. pneumoniae* strains have been known as the most frequent cause of multidrug-resistant gram-negative bacterial infections [4]. Moreover, in the intensive care unit (ICU), in case of any infection with multidrug-resistant bacteria, broad-spectrum antibiotics are needed for treatment and these infections associated with worse outcomes compared to infections due to susceptible strain [5]. The majority of the *K. pneumoniae* strains express carbapenemases that break down most of the beta-lactams [6]. Genes that code for carbapenemases often spread worldwide through clonal expansion and *K. pneumoniae* sequence type 258 (ST258) strains are considered as a “high-risk international clone” [6,7].

*K. pneumoniae* ST258 clones are globally distributed multi-drug resistant pathogens and have capacity to spread rapidly throughout the world, causing severe healthcare-associated invasive infections with limited antimicrobial treatment options [8,9]. These strains may share genetic features that predispose them to pathogenicity or increased transmissibility. Carbapenem resistance is frequent among the strains, thus infections with ST258 strains are treated with regimens containing colistin. However, colistin-resistant strains have been reported from different parts of the world which is one of the major concerns of public health [10,11]. Although there are studies revealing the prevalence of ST258 clones in...
different parts of the world, there is only one case report the presence of ST258 clone in Turkey [12]. However, there is not any study investigating the prevalence of the clone in our country. In light of this information, we aimed to reveal the incidence and antimicrobial susceptibility of *Klebsiella pneumoniae* ST258 strains among the intensive care unit patients in a university hospital in Istanbul for the first time.

**Methodology**

Consecutive nonreplicated 83 *K. pneumoniae* strains were isolated from various clinical samples of intensive care unit patients admitted to a university hospital in Istanbul, between November 2016 to December 2018. Bacterial identifications were conducted with VITEK2 automated system, using VITEK 2 Identification cards, which provide rapid, accurate species-level identification of clinically relevant bacteria (BioMerieux, Marcy L’Etoile, France). Mucoid and lactose positive colony forming, gram negative bacilli are suspected as *K. pneumoniae*, and further identification performed with automated system using VITEK2 Gram-negative bacilli Identification test card (VITEK2 GN ID card). Except for colistin, all other antimicrobial susceptibility tests were performed with Kirby Bauers disc diffusion tests on Mueller-Hinton agar (BioMerieux, Marcy L’Etoile, France). Briefly, a density of 0.5 McFarland bacterial suspension was inoculated in Mueller–Hinton broth and streaked on Mueller-Hinton agar plates, then antibiotic discs were placed on plates and incubated at 37 °C overnight to detect antimicrobial susceptibility tests except colistin. Colistin susceptibility performed by the broth microdilution method and the results were interpreted according to the EUCAST clinical breakpoints [13,14]. The following antibiotic discs were used, amoxicillin-clavulanic acid (20/10 µg, Cat: CT0223B), piperacillin-tazobactam (30/10 µg, Cat: CT1628B), ampicillin (25 µg, Cat: CT0004B), cefotaxime (30 µg, Cat: CT0166B), cefepime (30 µg, Cat: CT0771B), ceftazidime (30 µg, Cat: CT0417B), ceftriaxone (30 µg, Cat: CT0412B), fosfomycin (50 µg, Cat: CT0183B), amikacin (30 µg, Cat: CT0107B), gentamicin (10 µg, Cat: CT0024B), imipenem (10 µg, Cat: CT0455B), meropenem (10 µg, Cat: CT0774B), ciprofloxacin (5 µg, Cat: CT0425B), levofloxacin (5 µg, Cat: CT1587B), trimethoprim-sulfamethoxazole (1.25/23.75 µg, Cat: CT0052B) and tigecycline (15 µg, Cat: CT1841B) (Oxoid, Basingstoke, UK).

All *K. pneumoniae* strains were collected and stored at -80 °C using specific Microbank cryovials containing 20% glycerol (Pro-Lab, Texas, USA). A cryovial bead was inoculated 10 mL Tryptone Soya Broth (TSB, Cat: CM1016B Oxoid Basingstoke, UK) and incubated at 37 °C overnight. One hundred µL inoculum from TSB, streaked on eosin methylene blue (EMB) agar incubated at 37 °C overnight to obtain a single colony for DNA extraction. Real-time PCR method was performed in order to reveal ST258 positivity among the strains. In order to extract bacterial DNA, in a single colony of each strain’s fresh overnight culture on eosin methylene blue (EMB) agar was suspended in 50 µL of ultrapure water. The suspension was heated at 95 °C for 10 minutes and centrifuged at 14,000 rpm for 10 minutes. Thirty microliters of the supernatant were used as a DNA template for real-time PCR [15]. All DNA was stored at -80 °C until processing. Previously designed primers targeting the pilv-l region in the following sequences 5′-TTGGAGCTGATCCTTGCTCT and 5′-TCGATCCATGCTGATGATGT were used to detect ST258 clones among the strains [8]. Real-time PCR amplification and melting curve analysis were performed using a LightCycler 480 II system with software version 1.5 (Roche Diagnostics, Mannheim, Germany). The real-time PCR mixture was prepared using the LightCycler 480 SYBR Green I Mastermix kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer’s instructions. Cycling conditions for the ST258 assays were: initial denaturation for 5min at 95 °C and 40 cycles of 5 seconds at 95 °C and 10 seconds at 58 °C [8]. The fluorescence signal was measured at the end of each annealing step. Following amplification, a melting curve was generated by heating the PCR product to 95 °C with a ramp rate of 0.05 °C/s. Statistical analysis was performed with chi-square test on SPSS vers. 20 software (IBM, USA).

**Results**

Antimicrobial susceptibility results revealed that 56 out of 83 (67%) *K. pneumoniae* strains were carbapenem-resistant. It has been observed that all strains were resistant to ampicillin (100%). Also, 67 out of 83 (81%) *K. pneumoniae* strains are resistant to the third generation cephalosporins, which are usually used to treat *Enterobacteriaceae* infections. In addition to this, resistance rates against trimethoprim-sulfamethoxazole, tigecycline and colistin, which are therapeutic options used instead of beta-lactam antibiotics, were found as 63.86%, 26.67% and 20.0%, respectively. Also, 3 out of 8 (37.5%) carbapenem-resistant ST258 strains were found as resistant against all antibiotics tested. According to susceptibility results, colistin was found to be the best therapeutic choice for
all patients which was followed by tigecycline, amikacin and gentamicin. Antimicrobial susceptibility results of all strains were given in Table 1.

Real-time PCR results demonstrated that 15 out of 83 (18%) K. pneumoniae strain were ST258. According to antimicrobial susceptibility test results of ST258 strains, 8 were found as carbapenem-resistant whereas 7 were found as carbapenem susceptible. There was no statistically significant difference between ST258 incidence and carbapenem resistance ($p > 0.05$). Moreover, in three out of eight (37.5%) ST258 positive Carbapenem-resistant K. pneumoniae (CR-Kp) strains were colistin-resistant whereas there was not encountered any colistin-resistant strains among ST258 Carbapenem sensitive K. pneumoniae (CS-Kp). In other words, in three out of 15 (20%) ST258 strains carbapenem and colistin resistance co-existed. Table 2 and Figure 1 demonstrates the antimicrobial susceptibility results of ST258 positive stains.

### Table 1. Distribution of antimicrobial susceptibility result of all the strains. Resistant: n (%).

| Antimicrobial | CR-Kp (n = 56) | CS-Kp (n = 27) | Total (n = 83) |
|---------------|---------------|---------------|---------------|
| Colistin      | 13 (23.21)    | 2 (7.41)      | 15 (18.07)    |
| Tigecycline   | 20 (35.71)    | 3 (11.11)     | 23 (27.71)    |
| Ampicillin    | 56 (100)      | 27 (100)      | 83 (100)      |
| Gentamicin    | 39 (69.64)    | 4 (14.81)     | 43 (51.81)    |
| Amoxicillin-Clavulanic Acid | 56 (100) | 16 (59.26) | 72 (86.75) |
| Piperacillin-Tazobactam | 55 (98.21) | 12 (44.44) | 67 (80.72) |
| Ceftriaxone   | 55 (98.21)    | 14 (51.85)    | 69 (83.13)    |
| Cefotaxime    | 55 (98.21)    | 14 (51.85)    | 69 (83.13)    |
| Cefazidime    | 54 (96.43)    | 10 (37.04)    | 64 (77.11)    |
| Amikacin      | 41 (73.21)    | 2 (7.41)      | 43 (51.81)    |
| Ciprofloxacin | 49 (87.50)    | 8 (29.63)     | 57 (67.47)    |
| Levofloxacin  | 49 (87.50)    | 8 (29.63)     | 57 (67.47)    |
| Trimethoprim-Sulfamethoxazole | 41 (73.21) | 12 (44.44) | 53 (63.86) |
| Imipenem      | 56 (100)      | 0 (0)         | 56 (67.47)    |
| Meropenem     | 56 (100)      | 0 (0)         | 56 (67.47)    |
| Cefepime      | 54 (96.43)    | 6 (22.22)     | 60 (72.29)    |

CR-Kp: Carbapenem resistant K. pneumoniae; CS-Kp: Carbapenem susceptible K. Pneumoniae.

### Table 2. Antimicrobial susceptibility result of the ST258 clone positive strains. Resistant: n(%).

| Antimicrobial | CR-Kp (n = 8) | CS-Kp (n = 7) | Total (n = 15) |
|---------------|---------------|---------------|---------------|
| Colistin      | 3 (37.50)     | 0 (0)         | 3 (20)        |
| Tigecycline   | 4 (50)        | 0 (0)         | 4 (26.67)     |
| Ampicillin    | 8 (100)       | 7 (100)       | 15 (100)      |
| Gentamicin    | 7 (87.50)     | 0 (0)         | 7 (46.67)     |
| Amoxicillin-Clavulanic Acid | 8 (100) | 1 (14.28) | 9 (60) |
| Piperacillin-Tazobactam | 8 (100) | 4 (57.14) | 12 (80) |
| Ceftriaxone   | 8 (100)       | 3 (42.85)     | 11 (73.34)    |
| Cefotaxime    | 8 (100)       | 3 (42.85)     | 11 (73.34)    |
| Cefazidime    | 8 (100)       | 2 (28.57)     | 10 (66.67)    |
| Amikacin      | 8 (100)       | 0 (0)         | 8 (53.34)     |
| Ciprofloxacin | 8 (100)       | 0 (0)         | 8 (53.34)     |
| Levofoxacin   | 8 (100)       | 0 (0)         | 8 (53.34)     |
| Trimethoprim-Sulfamethoxazole | 8 (100) | 0 (0) | 8 (53.34) |
| Imipenem      | 8 (100)       | 0 (0)         | 8 (53.34)     |
| Meropenem     | 8 (100)       | 0 (0)         | 8 (53.34)     |
| Cefepime      | 8 (100)       | 0 (0)         | 8 (53.34)     |

CR-Kp: Carbapenem resistant K. pneumoniae; CS-Kp: Carbapenem susceptible K. Pneumoniae.

Figure 1. Antimicrobial resistance rate of the ST258 clones in CR-Kp and CS-Kp.
analyzed, it was noticed that the first treatment that some patients received contradicted with the in-vitro antimicrobial susceptibility results of the clone (Table 3). Six patients infected with ST258 received piperacillin and tazobactam combination therapy, however, in-vitro antimicrobial susceptibility tests revealed that only two strains were susceptible to the antibiotic in interest.

**Discussion**

Antimicrobial resistance is an important worldwide problem in the treatment of diseases caused by resistant bacteria. *Klebsiella pneumoniae* is defined as a member of the ESKAPE group microorganisms which comes from their ability to “escape” from the effects of antimicrobial drugs [16]. Therefore, *K. pneumoniae* is known to be an important cause of morbidity and mortality among hospital-acquired and long-term care-related infections [17].

Over the last few decades, there has been a concerning increase in the resistance of *K. pneumoniae* strains to a wide range of antibiotics. Expression of extended spectrum beta-lactamases (ESBLs) and carbapenemases are two major types of antimicrobial resistance mechanisms mainly observed in these strains [1,2]. The prevalence of multiple antibiotic-resistant strains such as CR-Kp has increased in recent years and CR-Kp has become a major public health problem worldwide, as carbapenems are the first-line therapy for infections caused by *K. pneumoniae*, especially ESBL producers [16,18]. Although not all the CR-Kp strains belonging to ST258 clone, previous studies revealed that there is a strong relationship between carbapenem resistance and ST258 clone. In addition to carbapenem

**Table 3. Patients information isolated ST258 Klebsiella pneumoniae strains.**

| Sample         | Age  | Gender | Phenotypically carbapenem susceptibility | Disease                              | Medication                  | Survive                  |
|----------------|------|--------|------------------------------------------|---------------------------------------|-----------------------------|--------------------------|
| P1             | 31   | M      | CR                                       | Rheumatic Valvular Heart Disease      | Tygecycline                 | Remission or clinical improvement |
| P2             | 45   | M      | CR                                       | Lung Cancer, Pneumoniae               | Levofloxacin                | Remission or clinical improvement |
| P3             | 57   | M      | CR                                       | Alcoholic Cirrhosis                   | Meropenem                   | Death*                   |
| P4             | 38   | M      | CR                                       | Heart Failure                         | Colistin+Meropenem          | Remission or clinical improvement |
| P5             | 78   | M      | CS                                       | Heart Failure                         | Piperacillin-Tazobactam     | Death*                   |
| P6             | 57   | M      | CR                                       | Coronary Heart Disease                | Not Used                    | Death*                   |
| P7             | 79   | F      | CR                                       | Chronic Renal Failure + Ileus         | Colistin                    | Remission or clinical improvement |
| P8             | 38   | F      | CR                                       | Parathyroid Ca + Pulmonary Emboli     | Piperacillin-Tazobactam     | Remission or clinical improvement |
| P9             | 53   | M      | CS                                       | Intracranial Hemorrhagry             | Imipenem                    | Remission or clinical improvement |
| P10            | 79   | F      | CR                                       | Chronic Renal Failure + Ileus         | Colistin                    | Remission or clinical improvement |
| P11            | 94   | F      | CS                                       | Chronic Lymphocytic Leukemia          | Piperacillin-Tazobactam     | Remission or clinical improvement |
| P12            | 41   | M      | CS                                       | Intracranial Hemorrhagry             | Piperacillin-Tazobactam     | Death*                   |
| P13            | 76   | M      | CS                                       | Prostat Ca                            | Cefazidime                  | Death*                   |
| P14            | 94   | F      | CS                                       | Chronic Lymphocytic Leukemia          | Piperacillin-Tazobactam     | Remission or clinical improvement |
| P15            | 34   | F      | CS                                       | Colon Ca                              | Piperacillin-Tazobactam     | Death*                   |

*Death (not attributable to infection).
resistance, ST258 positive *K. pneumoniae* strains are known as resistant to all β-lactam antibiotics, also typically have plasmid-derived genes that encode aminoglycoside modifying enzymes and chromosomal mutations that confer fluoroquinolone-resistance, that make them multi-drug resistant strains [16-19]. Furthermore, treating CR-Kp infections is a major clinical challenge, due in part to limited antibiotic options. In spite of being a major health concern worldwide, the data related to the molecular epidemiology and molecular characteristics of these strains are insufficient in our country. When studies investigating the prevalence of ST258 clones among carbapenem-resistant *K. pneumoniae* (CR-Kp) strains were examined, Ocampo et al. reported 37.8% (73 out of 193 strains) ST258 positivity in their study conducted in Colombia in 2016 [19]. Also, Bonura et al. reported 40% (37 out of 94 strains) ST258 positivity among CR-Kp strains in Italy in 2015 [20]. In 2017, Satlin et al. reported that 77 out of 92 (84%) CR-Kp strains were ST258 [21]. In our study, for the first time in our country, we detected 8 (14.2%) strains belonging ST258 clone, among 56 CR-Kp strains.

Mavroidi et al. studied with *K. pneumoniae* strains isolated from intensive care units in Greece in 2016 and reported that 18 of 19 colistin-resistant CR-Kp strains were ST258 positive [22]. Moreover, Bogdanovich et al. reported that all five colistin-resistant CR-Kp strains were ST258 positive in their study conducted in 2011 [11]. Lomonaco et al. did not detect ST258 clone in any of the 10 multi-drug resistant *K. pneumoniae* strains and reported that these strains were belonging to different sequence types in the study conducted in Pakistan in 2018 [23]. In our study, we found colistin resistance rate as 37.5% (three out of eight) ST258 positive CR-Kp strains, which was not as high as it was found in Greece [22]. However, there was no statistically significant difference between ST258 incidence and carbapenem resistance, also ST258 was not linked to a multi-resistant strain.

In addition to this, there is only one case report on the ST258 clone, however, there is not any study revealing the prevalence of ST258 clone in our country [12]. Becker et al. reported ST258 positivity as 19.5% (66 out of 337), also reported ST258 positivity among CR-Kp strains as 28.9% (31 out of 107) in Germany in 2018. According to the results of their study, 35 out of 337 (10.3%) strains were carbapenem sensitive *K. pneumoniae* (CS-Kp) ST258 clone, whereas 31 out of 337 (9.1%) strains were CR-Kp ST258 clone [24].

Diago-Navarro et al. studied with 40 CR-Kp and 8 CS-Kp strains in 2014, detected ST258 clone in 80% (32 of 40 strains) of CR-Kp strains and 33% (3 of 8 strains) of CS-Kp strains [25]. Villa et al. reported that firstly they detected ST258 positive CR-Kp in intraabdominal abscess of a kidney transplant patient but during treatment with tigecycline they isolated ST258 positive CS-Kp in 2013. After examination of these two strains with the next generation sequence system, they reported that plasmid loss and carbapenem susceptibility could be seen after treatment with non-carbapenem antibiotics [26]. Diago-Navarro et al. reported 19% ST258 positivity among 300 *K. pneumoniae* strains in 2016 [27]. Moreover, they reported that 13% of ST258 positive strains were carbapenem susceptible due to the loss of carbapenemase gene which was attributed to ICU residence time and antibiotic use of ST258 positive patients, similarly to the study of Villa et al. [25-27]. The primers used in our study for detecting ST258 clones were targeting the pilv-1 region [8]. Adler et al. [8] reported ST258 positivity in 9 CS-Kp strains when they used these primers in 2014 [8]. Similarly, the studies of Becker et al. [24], Diago-Navarro et al. [25], Diago-Navarro et al. [27] and Adler et al. [26], showed that ST258 clones were detected in carbapenem sensitive *K. pneumoniae* strains as well as CR-Kp. This may be a result of the loss of carbapenemase plasmids due to different antibiotic therapy regimens applied to the patients. However, although the previous studies reported the loss of carbapenemase plasmid, it is seen that majority of the studies on the ST258 clone have been tried to associate the clone only with carbapenem-resistant strains and CS-Kp strains were ignored [19-21]. In the study of Kontopidou et al. [28], conducted in Greece in 2014, it has been reported that they used β-lactam inhibitors for the first 10 days as the first treatment option in ICU patients infected with CR-Kp which were strongly associated with bacteremia [28]. Clancy et al. [29]. reported that piperacillin-tazobactam was the first therapeutic option given to transplant recipients in the first 30 days after the detection of CR-Kp-induced bacteremia [29]. Moreover, in 2016, Ocampo et al. [19] reported that piperacillin-tazobactam was the treatment for patients with CR-Kp, which was similar to other studies in 2016 [19]. Similarly, in our study, CR-Kp and CS-Kp strains were mostly associated with bacteremias and it was found that β-lactam/β-lactamase inhibitor combinations such as piperacillin-tazobactam were frequently given during the treatment of patients. However, it was noticed that the treatment was started empirically, without obtaining in-vitro susceptibility results. Patient survival rates can be increased by rapid detection and
control of infection source and infectious agent-specific combination therapy, whereas inappropriate empirical treatments result in poor clinical outcomes in patients [30].

Conclusions
This molecular epidemiological study is the first study conducted in Turkey in order to reveal the prevalence of ST258 clone, which was reported in only one case study in 2014 [12], among K. pneumoniae strains isolated from intensive care unit patients from a university hospital in Istanbul. In addition to the presence of ST258 clone in CR-Kp strains, ST258 clone positivity was also found in CS-Kp strains. Our results showed that ST258 is not linked to a multi-resistant strain and suggested that it does not contribute to multi-resistance formation alone. We believe that in order to be successful with empirical treatment in intensive care unit patients who are infected with MDR strains, the epidemiological data on K. pneumoniae strains with different clones that can rapidly disseminate multi-drug resistance should be developed with new multicenter and extensive studies.

References
1. Paczoska MK, Mecsas J (2016) Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol Mol Biol Rev 80: 629–661.
2. Bengoechea JA, Sa Pessoa J (2019) Klebsiella pneumoniae infection biology: living to counteract host defences. FEMS Microbiol Rev 43: 123–144.
3. Martin RM, Bachman MA (2018) Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Front Cell Infect Microbiol 8: 4.
4. Hendrik TC, Voor In’t Holt AF, Vos MC (2015) Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing Klebsiella spp.: a systematic review and meta-analyses. PLoS One 10: e0140754.
5. Artelt T, Kaase M, Bley I, Eiffert H, Mellmann A, Küster H, Lange M, Scheithauer S (2018) Transmission risk on a neonatal intensive care unit: Escherichia coli versus Klebsiella pneumoniae. Can J Infect Dis Med Microbiol 2018: 1525072.
6. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH (2016) Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol 7: 895.
7. van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8: 460–469.
8. Adler A, Hkhaba E, Chmelinský I, Giakkoupi P, Vatopoulos A, Mathers AJ, Yeh AJ, Sifri CD, De Angelis G, Tacconelli E, Villegas MV, Quinn J, Carmeli Y (2014) Development and validation of a multiplex PCR assay for identification of the epidemic ST-258/512 KPC-producing Klebsiella pneumoniae clone. Diagn Microbiol Infect Dis 78: 12–15.
9. Adler A, Hussein O, Ben-David D, Masarwa S, Navon-Venezia S, Schwaber MJ, Carmeli Y; Post-Acute-Care Hospital Carbapenem-Resistant Enterobacteriaceae Working Group (2015) Persistence of Klebsiella pneumoniae ST258 as the predominant clone of carbapenem-producing Enterobacteriaceae in post-acute-care hospitals in Israel, 2008-13. J Antimicrob Chemother 70: 89–92.
10. Gomez SA, Pasteran FG, Faccione D, Tijet N, Rapoport M, Lucero C, Lastovetska O, Albornoz E, Galas M; KPC Group, Melano RG, Corso A, Petroni A (2011) Clonal dissemination of Klebsiella pneumoniae ST258 harbouring KPC-2 in Argentina. Clin Microbiol Infect 17: 1520–1524.
11. Bogdanovich T, Adams-Has Duch JM, Tian GB, Nguyen MH, Kwak EJ, Muto CA, Doi Y (2011) Colistin-resistant, Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae belonging to the international epidemic clone ST258. Clin Infect Dis 53: 373–376.
12. Labarca J, Poirel L, Ozdamar M, Turkoglu S, Hakko E, Nordmann P (2014) KPC-producing Klebsiella pneumoniae, finally targeting Turkey. New Microbes New Infect 2: 50–51.
13. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2021) Clinical breakpoints - breakpoints and guidance. January 2021. Available: https://www.eucast.org/clinical_breakpoints/. Accessed: 6 January 2021.
14. Jayol A, Nordmann P, André C, Poirel L, Dubois V (2018) Evaluation of three broth microdilution systems to determine colistin susceptibility of Gram-negative bacilli. J Antimicrob Chemother 73: 1272–1278.
15. Unlu O, Aktas Z, Tugrul HM (2018) Analysis of virulence factors and antimicrobial resistance in Salmonella using molecular techniques and identification of clonal relationships among the strains. Microb Drug Resist 6: 19.
16. Castillo LA, Birnberg-Weiss F, Rodriguez-Rodrigues N, Martire-Greco D, Bigi F, Landoni VI, Gomez SA, Fernandez GC (2019) Klebsiella pneumoniae ST258 negatively regulates the oxidative burst in human neutrophils. Front Immunol 10: 929.
17. Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN (2014) Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella pneumoniae. Proc Natl Acad Sci USA 111: 4988–4993.
18. Meng X, Yang J, Duan J, Liu S, Huang X, Wen X, Huang X, Fu C, Li J, Dou Q, Liu Y, Wang J, Yan Q, Zou M, Liu W, Peng Z, Chen L, Li C (2019) Assessing molecular epidemiology of carbapenem-resistant Klebsiella pneumoniae (CR-KP) with MLST and MALDI-TOF in central China. Sci Rep 9: 2271.
19. Ocampo AM, Chen L, Cienfuegos AV, Roncancio G, Chavda KD, Kreiswirth BN, Jimenez JN (2015) A two-year surveillance in five colombian tertiary care hospitals reveals high frequency of non-CG258 clones of carbapenem-resistant Klebsiella pneumoniae with distinct clinical characteristics. Antimicrobial Agents Chemother 60: 332–342.
20. Bonura C, Giuffrè M, Aleo A, Fasciana T, Di Bernardo F, Stampone T, Giammanco A; MDR-GN Working Group, Palma DM, Mammina C (2015) An update of the evolving epidemic of blaKPC carrying Klebsiella pneumoniae in Sicily, Italy, 2014: emergence of multiple non-ST258 clones. PLoS One 10: e0132936.
21. Satlin MJ, Chen L, Patel G, Gomez-Simmonds A, Weston G, Kim AC, Seo SK, Rosenthal ME, Sperber SJ, Jenkins SG, Hamula CL, Uhlemann AC, Levi MH, Fries BC, Tang YW, Juretschko S, Rojman AD, Hong T, Mathema B, Jacobs MR, Walsh TJ, Bonomo RA, Kreiswirth BN (2017) Multicenter
clinical and molecular epidemiological analysis of bacteremia due to carbapenem-resistant enterobacteriaceae (CRE) in the CRE epicenter of the United States. Antimicrob Agents Chemother 61: e02349-16.
22. Mavroidi A, Katsiari M, Likousi S, Palla E, Roussou Z, Nikolaou C, Maguina A, Platsouka ED (2016) Characterization of ST258 colistin-resistant, blaKPC-producing Klebsiella pneumoniae in a greek hospital. Microbial Drug Resistance 22: 392–398.
23. Lomonaco S, Crawford MA, Lascols C, Timme RE, Anderson K, Hodge DR, Fisher DJ, Pillai SP, Morse SA, Khan E, Hughes MA, Allard MW, Sharma SK (2018) Resistome of carbapenem- and colistin-resistant Klebsiella pneumoniae clinical isolates. PLoS One 13: e0198526.
24. Becker L, Kaase M, Pfiefer Y, Fuchs S, Reuss A, von Laer A, Sin MA, Korte-Berwanger M, Gatermann S, Werner G (2018) Genome-based analysis of carbapenemase-producing Klebsiella pneumoniae isolates from german hospital patients, 2008-2014. Antimicrob Resist Infect Control 7: 62.
25. Diago-Navarro E, Hanington KI, Khan A, Adnan M, Yoon HA, Spitzer H, Fries B (2016) An analysis of carbapenem-sensitive and carbapenem-resistant Klebsiella pneumoniae clinical isolates obtained in Stony Brook University Hospital, Open Forum Infectious Diseases 3 Suppl 1: 332.
28. Kontopidou F, Giamarello H, Katerelos P, Maragos A, Kioumis I, Trikka-Graphakos E, Valakis C, Maltezou HC; Group for the Study of KPC-producing Klebsiella pneumoniae infections in intensive care units (2014) Infections caused by carbapenem-resistant Klebsiella pneumoniae among patients in intensive care units in Greece: a multi-centre study on clinical outcome and therapeutic options. Clin Microbiol Infect 20: O117-23.
29. Clancy CJ, Chen L, Shields RK, Zhao Y, Cheng S, Chavda KD, Hao B, Hong JH, Doi Y, Kwak EJ, Silveira FP, Abdel-Massih R, Bogdanovich T, Humar A, Perlin DS, Kreiswirth BN, Hong Nguyen M (2013) Epidemiology and molecular characterization of bacteremia due to carbapenem-resistant Klebsiella pneumoniae in transplant recipients. Am J Transplant 13: 2619–2633.
30. Izadpanah M, Khalili H (2015) Antibiotic regimens for treatment of infections due to multidrug-resistant Gram-negative pathogens: an evidence-based literature review. J Res Pharm Pract 4: 105–114.

**Corresponding author**
Mehmet Demirci, PhD, Assoc. Prof.
Kirklareli University, School of Medicine, Department of Medical Microbiology, Kayalı Kampüsü, Koçcaz yolu, Kirklareli, 39000, Istanbul, Turkey
Phone: +905337106295
Fax: +902882129679
Email: demircimehmet@hotmail.com

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