Emergence of carbapenemase-producing and colistin resistant *Klebsiella pneumoniae* ST101 high-risk clone in Turkey

GÜLŞEN HAZIROLAN1* and ALPER KARAGÖZ2

1 Department of Medical Microbiology, Faculty of Medicine, Hacettepe University, 06100, Ankara, Turkey
2 Department of Microbiology, Faculty of Molecular Biology and Genetics, Usak University, 64200, Usak, Turkey

Received: August 11, 2020 ● Accepted: September 1, 2020
Published online: November 9, 2020

ABSTRACT

Carbapenemase-producing and colistin resistant *Klebsiella pneumoniae* has become a worldwide healthcare problem. This study describes molecular characterization of carbapenemase-producing and colistin resistant clinical *K. pneumoniae* isolates.

A total of 93 non-replicate carbapenem and colistin resistant *K. pneumoniae* were recovered from clinical specimens in a university hospital during 2017–2019. Detection of *bla*OXA-48, *bla*KPC, *bla*NDM-1, *bla*IMP, *bla*VIM-1 and *mcr-1*, -2, -3, -4, -5, -6, -7, and -8 genes was performed by PCR. The bacterial isolates were assigned to clonal lineages by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

All isolates harbored *bla*OXA-48 and only two isolates harbored *bla*OXA-48 and *bla*NDM-1 genes together. In colistin resistant *K. pneumoniae*, mcr-1 was detected in two (2.1%) isolates. Ninety three isolates of *K. pneumoniae* were categorized into three clusters and five pulsotypes. MLST revealed two different sequence types, ST101 (89/93) and ST147 (4/93).

In our study ST101 was found to be a significantly dominant clone carrying *bla*OXA-48 and among our strains a low frequency of mcr-1 gene was determined. The emergence of colistin resistance was observed in *K. pneumoniae* ST101 isolates. ST101 may become a global threat in the dissemination of carbapenem and colistin resistance.

KEYWORDS

*Klebsiella pneumoniae*, carbapenemase, colistin resistance, mcr-1, *bla*OXA-48, PFGE, MLST, ST101, ST147

INTRODUCTION

*Klebsiella pneumoniae* is an opportunistic pathogen which can cause different types of healthcare-associated infections. Enhanced use of carbapenems in clinical practice, promoted emergence of carbapenem-resistant *K. pneumoniae* (CRKP) worldwide in recent years [1]. CRKP has mainly been link to *K. pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and metallo-β-lactamases (MBLs), such as NDM, IMP, and VIM type carbapenemases. While these plasmid-encoded carbapenemases have been increasingly reported worldwide, their prevalence varies geographically [2]. The first identified OXA-48 producer was a *K. pneumoniae* strain isolated in Turkey in 2003 [3]. Since then, OXA-48 producers have been extensively reported from Turkey as a source of nosocomial outbreaks [4–8]. Worldwide distribution of OXA-48 now includes countries in Europe, in the southern and eastern part of the Mediterranean Sea, and Africa [3–8].

Treatment of infected patients with CRKP is always problematic due to their multidrug resistance phenotype, and several therapeutic options have been considered. Colistin is one of these therapeutic options. However, colistin resistance had been observed in CRKP isolates,
and rapid dissemination of colistin resistant isolates has been recently reported [9]. Colistin resistance mechanisms are presumed to be linked to chromosomal mutation untransferable via horizontal gene transfer [10]. Recently, several plasmid-mediated colistin resistance genes, named mcr, encoding pEtN transferases, have also been reported in K. pneumoniae [11].

Dissemination of CRKP is mainly caused by the spread of a few successful clones. Major representatives of these high-risk clonal lineages include sequence type (ST) 11, ST15, ST307, ST17, ST37, ST101, and ST147 strains [1]. ST258 strains are major players in the worldwide spread of KPC-type carbapenemases, and are responsible for 68% of the CRKP outbreaks [12]. ST101 strains harbor different clinically-relevant resistance determinants, such as carbapenemases of the KPC, OXA-48, VIM, and NDM types [1].

In this study, carbapenemase producing and colistin resistant clinical K. pneumoniae isolates were characterized to evaluate genetic differences and relationships, and prevalence of carbapenem resistance determinants, as well as to determine plasmid-mediated colistin resistance mechanism.

MATERIAL AND METHODS

Bacterial isolates and susceptibility testing

We retrospectively analyzed ninety-three carbapenem and colistin resistant K. pneumoniae isolates consecutively isolated from patients who were hospitalized at the Hacettepe University Hospitals between October 2017 and December 2019. The Hacettepe University Hospitals are tertiary care hospitals of 1,040 beds that provides specialized attention to a population size of ∼5.504 million inhabitants in the capital of Turkey. In the period between October 2017 and December 2019, altogether 8624 K. pneumoniae isolates were obtained from routine microbiological cultures of clinical samples. In total, 93 carbapenem and colistin resistant K. pneumoniae isolates were obtained. These isolates were detected from blood (n = 34), urine (n = 26), abscess (n = 13), tracheal aspirate (n = 11), peritoneal fluid (n = 4), cerebrospinal fluid (n = 2), synovial fluid (n = 1), pleural fluid (n = 1), and pericardial fluid (n = 1). Isolates were identified with conventional tests (Gram staining, catalase and oxidase tests), and matrix assisted lazer desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Germany). All isolates were identified according to the criteria mentioned by Tenover et al. [23].

PFGE

Pulsed-field gel electrophoresis (PFGE) was performed as per a method described in a previous study [22]. A thin slice of plug was digested overnight at 37 °C with 50 U of the XbaI restriction enzyme according to the manufacturer’s instructions. The restriction fragments were separated through PFGE in 1% agarose gel (Bio-Rad, USA) with 0.5x TBE buffer (45 mM Tris, 45 mM boric acid, and 1.0 mM EDTA [pH 8.0]) for 22 h at 200 V and 14 °C, with ramp times of 2 s-40 s using the CHEF Mapper apparatus (Bio-Rad, USA). The gels were stained in ethidium bromide (1 mg/mL), were viewed under an ultra-violet trans-illumination. Digital images were stored electronically. PFGE banding patterns were analysed with the BioNumerics Software (Applied Maths, Belgium) using the dice coefficient and unweighted pair group method with arithmetic averages algorithm. PFGE patterns were compared and analysed according to the criteria mentioned by Tenover et al. [23].

MLST

Multilocus sequence typing (MLST) was performed on K. pneumoniae isolates according to the protocol described on the K. pneumoniae MLST website (https://biggsdb.pasteur.fr/klebsiella/klebsiella.html). Seven conserved housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) were used [24]. MLST results were typed according to the updated international K. pneumoniae MLST database at the Pasteur Institute in Paris, France.

RESULTS

Bacterial isolates and susceptibility testing

In the period between October 2017 and December 2019, a total of 8,624 clinical isolates of K. pneumoniae were isolated from patients admitted to Hacettepe University Hospitals. Among those, 2,259 isolates (26.2%) were non-susceptible to (Thermo Fisher, UK). Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints [13]. Escherichia coli ATCC 25922 and E. coli NCTC 13846 (mcr-1 positive) was used for quality control. The isolates were stored in tryptic soy broth containing 10% (v/v) glycerol at -80 °C until use.

Molecular analysis of carbapenem and colistin resistance

Genomic DNA was isolated using the QIAasympohony DSP Virus/Pathogen kit in the QIAasympohony system according to the manufacturer’s instructions (Qiagen, USA). OXA-48, KPC, NDM-1, IMP, and VIM-1 carbapenemases were identified by PCR amplification and sequencing as described previously [14]. The colistin resistant isolates were screened by simplex PCRs for the presence of mcr-1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7, and mcr-8 genes [15–21] (Supplementary Table S1).
at least one carbapenem by gradient test and were tested for colistin resistance. A total of 93 isolates (4.1%) out of this subset showed a colistin resistant phenotype by Sensititre with minimum inhibitory concentrations (MIC) that ranged between 4 and 128 $\mu$g/mL. Carbapenem and colistin MIC ranges, MIC50 and MIC90 profiles of the isolates are summarized in Table 1. Overall carbapenem and colistin resistance rates are shown in Supplementary Table S2.

Molecular analysis of carbapenem and colistin resistance

The PCR results indicated that \textit{bla\textsubscript{OXA-48}} gene was detected in all \textit{K. pneumoniae} isolates (100%), and 2.1% (n = 2) of the isolates co-produced \textit{bla\textsubscript{OXA-48}} and \textit{bla\textsubscript{NDM-1}}. Other tested carbapenemase genes, such as \textit{bla\textsubscript{KPC}}, \textit{bla\textsubscript{IMP}}, and \textit{bla\textsubscript{VIM-1}} could not detected in any of the isolates. Detection of \textit{mcr-1} genes using PCR technique revealed that two (2.1%) isolates were positive for \textit{mcr-1}. No \textit{mcr-2}, -3, -4, -5, -6, -7, and -8 were detected among all tested isolates.

PFGE

The characteristics of the molecular epidemiology of the 93 carbapenem and colistin resistant \textit{K. pneumoniae} isolates are displayed in Fig. 1. All the 93 carbapenem and colistin resistant \textit{K. pneumoniae} isolates were grouped into three cluster and five pulsotypes. Cluster three is the largest cluster that possesses 71 isolates. Fourteen isolates belonged to cluster one and eight isolates belong to cluster two. PFGE discriminatory power was of 96%, as calculated by Simpson’s Index of Diversity [25].

MLST

Ninety-three carbapenem and colistin resistant \textit{K. pneumoniae} isolates were analysed by MLST, and two ST types were detected. ST101 (95.6%, 89/93) was the dominant ST type followed by ST147 (4.4%, 4/93). Among four isolates of

### Table 1. MIC ($\mu$g/mL) profiles of carbapenem and colistin resistant \textit{K. pneumoniae} isolates

|           | 2017 (n = 18) | 2018 (n = 38) | 2019 (n = 37) |
|-----------|--------------|--------------|--------------|
| **Colistin** |              |              |              |
| MIC range  | 4–64         | 4–128        | 4–64         |
| \textit{MIC\textsubscript{50}} | 4            | 4            | 4            |
| \textit{MIC\textsubscript{90}} | 16           | 32           | 64           |
| **Meropenem** |              |              |              |
| MIC range  | 16–128       | 16–128       | 16–256       |
| \textit{MIC\textsubscript{50}} | 16           | 16           | 16           |
| \textit{MIC\textsubscript{90}} | 32           | 64           | 128          |
| **Imipenem** |              |              |              |
| MIC range  | 16–64        | 16–128       | 8–64         |
| \textit{MIC\textsubscript{50}} | 16           | 16           | 8            |
| \textit{MIC\textsubscript{90}} | 32           | 64           | 32           |
| **Ertapenem** |              |              |              |
| MIC range  | 2–64         | 2–128        | 2–64         |
| \textit{MIC\textsubscript{50}} | 2            | 16           | 16           |
| \textit{MIC\textsubscript{90}} | 32           | 32           | 32           |

![Fig. 1. Dendrogram based on pulsed-field gel electrophoresis pattern analysis (PFGE) of 93 colistin and carbapenem resistant \textit{K. pneumoniae} isolates from different wards and their ST determined via multilocus sequence typing (MLST).](image)

ST147, two isolates carried \textit{bla\textsubscript{OXA-48}} and \textit{bla\textsubscript{NDM-1}} genes, and the other two ST147 clones coproduce \textit{bla\textsubscript{OXA-48}} and \textit{mcr-1} genes (Fig. 1).

DISCUSSION

Multidrug resistant pathogens have become a global problem recently [12]. \textit{K. pneumoniae} is an important nosocomial multidrug resistant pathogen that can cause high morbidity and mortality [26]. After widespread dissemination of carbapenemase producing \textit{K. pneumoniae} isolates; colistin resistance has emerged in \textit{K. pneumoniae} isolates and caused problems in treatment modalities [9, 26, 27]. CRKP isolates that produce carbapenemases such as the OXA-48, KPC, VIM-1, NDM-1, and IMP, have been reported worldwide [1, 3, 5–7, 9, 10, 26]. KPCs are the most clinically common enzymes, and have been detected in North America (especially the United States), South America (Colombia, Argentina), Europe (Greece, Italy, Poland), Asia (China), and the Middle East (Israel) [28–30]. Turkey is a country with a specific epidemiology, where OXA-48 carbapenemase has been extensively identified. However,
first KPC-2-positive *K. pneumoniae* have been identified in Turkey, in 2014 [31]. Since then, sporadic KPC-producing *K. pneumoniae* was reported [32, 33]. In the present study, we didn’t detect *bla*KPC gene among tested carbapenem and colistin resistant *K. pneumoniae* isolates.

MBLs identified in *K. pneumoniae* mainly reported from Japan (IMP), Taiwan (IMP), Indian subcontinent (NDM), Balkan states (NDM), and Greece (VIM) [33]. Recent findings suggest that the Balkan states and the Middle East may act as secondary reservoirs of NDM-1 producers. In the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) study, Turkey was classified as a stage 3 country on a scale of 1–5 (1: no reported case; 5: endemic situation) for the existence of NDM carbapenemases. Among CRKP, NDM-1 carbapenemases detected between 6.3% and 52.9% in our country [34, 35]. Moreover, the coproduction of both OXA-48 and NDM-1 carbapenemases has been frequently reported. We detected two (2.1%) NDM-1-producing isolates, which have already been found to harbour OXA-48 carbapenemases. Interestingly, NDM-1 carbapenemase detected in a very low rate compared to our country results. Our hospital setting is not an endemic region for *bla*NDM-1 positive *K. pneumoniae*. The carbapenemase genes *bla*IMP and *bla*VIM were reported in a low but notable incidence in Turkey like other countries [27, 35–38]. In our study, *bla*IMP and *bla*VIM genes were not detected in any of the isolates.

OXA-48-producing *K. pneumoniae* was first reported from Turkey and it is endemic for our country [30, 32, 36, 37]. The emergence of the OXA-48 enzyme is mediated by the rapid spread of a broad host-range conjugative plasmid harboring the *bla*OXA-48 gene. Plasmid harboring *bla*OXA-48 with the Tn1999.2 transposon detected from a *K. pneumoniae* isolate in Turkey [39]. OXA-48-producing *K. pneumoniae* is also endemic in certain North African and European countries (Morocco, Tunisia, Spain, Belgium) [40]. OXA-48-producing *K. pneumoniae* remain relatively uncommon in the United States and Canada [41]. As it was expected, we detected *bla*OXA-48 gene in all tested CRKP isolates. However, two of these isolates were positive for both *bla*NDM-1 and *bla*OXA-48 genes.

Multilocus sequence typing is an excellent method in evolutionary studies for exploring the common ancestral lineages of bacterial isolates [20]. Various ST types (ST11, ST14, ST101, ST147, and ST258) and resistance mechanisms can be related to carbapenem and colistin resistance in *K. pneumoniae* [41]. A single *K. pneumoniae* clone ST258 was identified extensively worldwide, indicating that it may have contributed to the spread of the *bla*KPC genes [26]. On the other hand, KPC-producing *K. pneumoniae* remains rare for our country [31, 32]. Among all tested isolates, we didn’t detect KPC-producing *K. pneumoniae* and also its emerging high-risk clone ST258. We found that the epidemic Klebsiella pneumoniae isolate in our hospital was in ST101 type. ST101 was previously accepted as a high-risk epidemic clone, and it was reported that the ST101 clone was associated with various β-lactamases, including NDM-1, OXA-48, and CTX-M-15 [38]. Nevertheless, CRKP assigned to ST101 are carbapenem resistant frequently because of the production OXA-48. David et al., analysed the genome sequences of *K. pneumoniae* strains, isolated from patients in 244 hospitals in 32 countries during the European Survey of Carbapenemase-Producing Enterobacteriaceae. CRKP are concentrated in four clonal lineages, ST11, ST15, ST101, ST258/512, and authors identified OXA-48–producing ST101 clones in Romania, Spain, and Turkey [39]. Also, the emergence of colistin resistance has been observed in CRKP isolates, and colistin resistance was shown to be associated with the ST101 clone. Detection of carbapenem and colistin resistant *K. pneumoniae* ST101 was reported from Italy and Serbia [42, 43]. A large multicentre cohort study, describe the molecular characteristics of clinical colistin and carbapenem resistant *K. pneumoniae* isolates. Researchers observed a significant association between ST101, OXA-48, and colistin resistance [44]. Our study reports the clonal expansion of emerging ST101 clone associated with OXA-48 producing and colistin resistance in our hospital settings.

*K. pneumoniae* ST147 is an emerging high-risk clone that was first identified in Greece and has been associated with VIM and KPC carbapenemases in that country [45]. This global ST has also been associated with NDM and OXA-181 carbapenemases in various countries, including Switzerland, Iraq, Canada, United Kingdom, India, and Italy [26, 46]. In the current study, two isolates of ST147 were detected which co-harbored *bla*NDM-1 and *bla*OXA-48 genes.

Carbapenem-resistance among *K. pneumoniae* isolates makes colistin the last therapeutic option for the treatment. With the rise in consumption of colistin, cases of colistin resistant CRKP isolates are reported globally [1, 9, 11]. Chromosomal mutations in *phoP/phoQ, pmrA/pmrB, mgbB* and plasmid-borne mobile colistin resistance genes (*mcr-1* to *mcr-9*) positivity have an important role in increasing colistin resistance in *K. pneumoniae* [47, 10, 11]. The highest colistin resistance rate was reported in Asia (especially Korea and Singapore), followed by Europe (especially Greece) and America, where colistin resistance rates are continually increasing [48]. Nowadays, all known *mcr* genes have been detected in various *K. pneumoniae* isolates, whereas a small number of studies have shown the presence of *mcr* genes in clinical *K. pneumoniae* isolates [49]. Several studies suggested that chromosomal mutations rather than *mcr* genes positivity might have an important role in colistin resistance [47, 44, 50]. Different STs such as ST274, ST461, ST15, ST16, ST416, ST1890, ST37, ST1942, ST101, ST147, ST258, ST152, and ST15 were detected in carbapenem and colistin resistant *K. pneumoniae* [42, 50, 51]. We detected two ST types, ST101 (95.6%) and ST147 (4.4%) in carbapenem and colistin resistant *K. pneumoniae*. In Turkey, a few study investigated *mcr-1* to -3 genes among carbapenem and colistin resistant *K. pneumoniae* isolates and only Arabaci et al., determined *mcr-1* positive KRPC (5.2%) [44, 51–53]. In this study, we investigated *mcr-1* to -8 genes in colistin and carbapenem resistant *K. pneumoniae* and reported the molecular characteristics of *mcr-1* positive CRKP. To our knowledge, this is the first report of *mcr-1* positive *K. pneumoniae* isolates that produce both NDM-1 and OXA-48 while also belonging to the one of the emerging clones ST147 from Turkey.
CONCLUSION

We identified two different STs, namely, ST101 and ST147 among the carbapenem and colistin resistant K. pneumoniae isolates identified during 2017 and 2019. ST101 is an epidemic ST and has been associated with OXA-48. Our results show that this ST also has the ability to develop colistin resistance. Early detection and surveillance can prevent the spread of carbapenem and colistin resistant K. pneumoniae isolates.

Ethical committee approval: Not required.

Conflict of interest: The authors declare no competing interests.

ACKNOWLEDGEMENTS

None.

SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at https://doi.org/10.1556/030.2020.01275.

REFERENCES

1. Palmieri M, D’Andrea MM, Pellegrin AC, Mirande C, Béfic S, Cirkovic I, et al. Genomic epidemiology of Carbapenem- and Colistin-resistant Klebsiella pneumoniae isolates from Serbia: predominance of ST101 strains carrying a novel OXA-48 plasmid. Front Microbiol 2020;11:294.

2. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of Carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol 2016;7:895.

3. Poirel L, Héritier C, Toliin V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 2004;48:15–22.

4. Carrrè A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, et al. Spread of OXA-48–encoding plasmid in Turkey and beyond. Antimicrob Agents Chemother 2010;54:1369–73.

5. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. Outbreak of OXA-48–positive carbapenem-resistant Klebsiella pneumoniae isolates in France. Antimicrob Agents Chemother 2011;55:2420–3.

6. Moquet O, Bouchiat C, Kinana A, Seck A, Arouna O, Bercion R, et al. Class D OXA-48 carbapenemase in multidrug-resistant enterobacteria, Senegal. Emerg Infect Dis 2011;17:143–4.

7. Benouda A, Touzani O, Khairallah MT, Araj GF, Matar GM. First detection of oxacillinase-mediated resistance to carbapenems in Klebsiella pneumoniae from Morocco. Ann Trop Med Parasitol 2010;104:327–30.

8. Poirel L, Ros A, Carrér A, Fortineau N, Carricajo A, Berthelot P, et al. Cross-border transmission of OXA-48–producing Enterobacter cloacae from Morocco to France. J Antimicrob Chemother 2011;66:1181–2.

9. Di Tella D, Tamburro M, Guerrizio G, Fanelli I, Sannarco ML, Ripabelli G. Molecular epidemiological insights into colistin-resistant and Carbapenemases-producing clinical Klebsiella pneumoniae isolates. Infect Drug Resist 2019;12:3783–95.

10. Olaitan AO, Morand S, Rolain J-M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 2014;5:643.

11. Sun J, Zhang H, Liu YH, Feng Y. Towards understanding MCR-like colistin resistance trends. Microbiol 2018;26:794–808.

12. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 2017;41:252–75.

13. EUCAST Clinical Breakpoints http://www.eucast.org/clinical_breakpoints.

14. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010;65:490–5.

15. Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, Yan SG, et al. mcr-1 identified in Avian Pathogenic Escherichia coli (APEC). PLoS One 2017;12:e0172997.

16. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro Surveill 2016;21.

17. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. Novel plasmid-mediated colistin resistance gene mcr-3 in Escherichia coli. mBio 2017;8:e01166–17.

18. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppini A, et al. Novel plasmid-mediated colistin resistance mcr-4 gene in Salmonella and Escherichia coli, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 2017;22.

19. Borowiak M, Fischer J, Hammerl JA, Hendriksen SR, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. Enterica serovar Paratyphi B. J Antimicrob Chemother 2017;72:3317–24.

20. Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmid-mediated colistin resistance gene mcr-7.1 in Klebsiella pneumoniae. J Antimicrob Chemother 2018;73:1791–95.

21. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg Microbes Infect 2018;7:122.

22. D’Agata EM, Gerrits MM, Tang YW, Samore M, Kusters JG. Comparison of pulsed-field gel electrophoresis and amplified fragment-length polymorphism for epidemiological investigations of common nosocomial pathogens. Infect Control Hosp Epidemiol 2001;22:530–40.

23. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2333–9.

24. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol 2005;43:4178–82.
25. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson’s index of diversity. J Clin Microbiol 1988;26:2465–66.

26. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 2015;59:5873–84.

27. Menekşe Ş, Çağ Y, İşlık ME, Şahin S, Hacıseyitoglu D, Can F, et al. The effect of colistin resistance and other predictors on fatality among patients with bloodstream infections due to Klebsiella pneumoniae in an OXA-48 dominant region. Int J Infect Dis 2019;86:208–11.

28. Walther-Rasmussen J, Hoiby N. Class A carbapenemases. J Antimicrob Chemother 2007;60:470–82.

29. Deshpande LM, Rhomberg PR, Sader HS, Jones RN. Emergence of serine carbapenemases (KPC and SME) among clinical strains of Enterobacteriaceae isolated in the United States Medical Centers: report from the MYSTIC Program (1999–2005). Diag Microbiol Infect Dis 2006;56:367–72.

30. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228–36.

31. Labarca J, Poirel L, Ozdamar M, Turkoglu S, Hakko E, Nordmann P. KPC-producing Klebsiella pneumoniae, finally targeting Turkey. Labarca New Microbes New Infect 2014;2:50–1.

32. Tekeli A, Dolapci I, Evren E, Uğuzman E, Karahan ZC. Characterization of Klebsiella pneumoniae coproducing KPC and NDM-1 Carbapenemases from Turkey. Microb Drug Resist 2020;26:1218–25.

33. Sagiroglu P, Hasdemir U, Altnkanat 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;48:885–90.

34. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Klebsiella pneumoniae, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 2015;59:5873–84.

35. Tekintaş Y, Çilli F, Eraç B, Yaşar M, Aysiemir SS, Hosgör Limoncu M. Comparison of phenotypic methods and polymerase chain reaction for the detection of carbapenemase production in clinical Klebsiella pneumoniae isolates. Mikrobiyol Bul 2017;51:269–76.

36. Çakar A, Akyoun Y, Gür D, Karatuna O, Öğünç D, Özhak Baysan B, et al. Investigation of carbapenemases in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae strains isolated in 2014 in Turkey. Mikrobiyol Bul 2016;1:21–33.

37. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. European network on carbapenem-resistant Klebsiella pneumoniae. Clin Microbiol Infect 2012;18:413–31.

38. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 2012;67:1597–606.

39. Düzgün AO, Saral A. Next-generation sequencing of plasmid carrying bla(OXA-48) in Klebsiella pneumoniae from Turkey. Acta Microbiol Immunol Hung 2019;66:261–72.

40. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, et al. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nat Microbiol 2019;4:1919–29.

41. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing enterobacteriaceae. Emerg Infect Dis 2011;17:1791–8.

42. Novovic K, Trudic A, Brikic S, Vasiljevic Z, Kojić M, Medić D, et al. Molecular epidemiology of colistin resistant, carbapenemase-producing Klebsiella pneumoniae in Serbia from 2013 to 2016. Antimicrob Agents Chemother 2017;61:e02550–16.

43. Del Franco M, Paone L, Novati R, Giacomazzi CG, Bagattini M, Galotto C, et al. Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Valle d’Aosta region, Italy, shows the emergence of KPC-2 producing Klebsiella pneumoniae clonal complex 101 (ST101 and ST1789). BMC Microbiol 2015;15:260.

44. Can F, Menekse S, Ispir P, Atac N, Albayrak O, Demir T, et al. Impact of the ST101 clone on fatality among patients with colistin-resistant Klebsiella pneumoniae infection. J Antimicrob Chemother 2018;73:1235–41.

45. Giakkoupi P, Papagiannitsis CC, Miragou V, Pappa O, Polemis M, Tryfinopoulou K, et al. An update evolving epidemic of blaKPC-2-carrying Klebsiella pneumoniae in Greece (2009–10). J Antimicrob Chemother 2011;66:1510–13.

46. Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JD. Surveillance and molecular epidemiology of Klebsiella pneumoniae isolates that produce carbapenemases: first report of OXA-48-like enzyme-sin North America. Antimicrob Agents Chemother 2016;57:130–6.

47. Jayol A, Poirel L, Brink A, Villegas MV, Yılmaz M, Nordmann P. Resistance to colistin associated with a single amino acid change in protein PmrB among Klebsiella pneumoniae isolates of worldwide origin. Antimicrob Agents Chemother 2014;58:4762–6.

48. Bialvaei AZ, Samadi Kafli H. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin 2015;31:707–21.

49. Wang Y, Liu F, Hu Y, Zhang G, Zhu B, Gao GF. Detection of mobile colistin resistance gene mcr-9 in carbapenem-resistant Klebsiella pneumoniae strains of human origin in Europe. J Infect 2020;80:578–606.

50. Longo LGA, de Sousa VS, Krachyte GB, Justo-da-Silva LH, Rocha JA, Superti SV, et al. Colistin resistance emerges in pandrug-resistant Klebsiella pneumoniae epidemic clones in Rio de Janeiro, Brazil. Int J Antimicrob Agents 2019;54:579–86.

51. Guducuoğlu H, Gursoy NC, Yakupogullari Y, Parlak M, Karaslan G, Sunnetcioglu M, et al. Hospital outbreak of a colistin-resistant, carbapenemase-producing Klebsiella pneumoniae strain. J Infect 2018;76:966–72.

52. Borsa BA, Demirci M, Gungordu-Dalar Z, Karabiyik G, Aygun G, Kucukbasmaci O. Molecular mechanisms of colistin resistance among Klebsiella pneumoniae strains. Clin Lab 2019;65:1123–30.

53. Arabacı C, Dal T, Başyırtı T, Genişel N, Durmac R. Investigation of carbapenemase and mcr-1 genes in carbapenem-resistant Klebsiella pneumoniae isolates. J Infect Dev Ctries 2019;30:504–9.