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

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RESEARCH ARTICLE

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 linked 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,

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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 habitants 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 laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Germany). All isolates were identified with a score ≥ 2.0 ; accurate identification to the species level by MALDI-TOF MS. Antibiotic susceptibility profiles of isolates were determined by BD PhoenixTM automated susceptibility testing system (Becton Dickinson and Company BD, USA). Isolates non-susceptible to at least one carbapenem (ertapenem, meropenem, and imipenem) were also tested for carbapenem resistance by gradient test (bioMérieux, France) according to manufacturer's instructions. CRKP was defined when the isolate was resistant to ertapenem, meropenem or imipenem by gradient test. Colistin MICs were determined for 93 CRKP isolates using the SensititreTM plate

(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 QIAasymphony DSP Virus/Pathogen kit in the QIAasymphony 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).

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://bigsd.bpasteur.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

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

	2017 (<i>n</i> = 18)	2018 (<i>n</i> = 38)	2019 (<i>n</i> = 37)
Colistin			
MIC range	4–64	4–128	4–64
MIC ₅₀	4	4	4
MIC ₉₀	16	32	64
Meropenem			
MIC range	16–128	16–128	16–256
MIC ₅₀	16	16	16
MIC ₉₀	32	64	128
Imipenem			
MIC range	16–64	16–128	8–64
MIC ₅₀	16	16	8
MIC ₉₀	32	64	32
Ertapenem			
MIC range	2–64	2–128	2–64
MIC ₅₀	2	16	16
MIC ₉₀	32	32	32

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 $\mu\text{g/m}$. 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 *bla*_{OXA-48} gene was detected in all *K. pneumoniae* isolates (100%), and 2.1% (*n* = 2) of the isolates co-produced *bla*_{OXA-48}, and *bla*_{NDM-1}. Other tested carbapenemase genes, such as *bla*_{KPC}, *bla*_{IMP}, and *bla*_{VIM-1} could not be detected in any of the isolates. Detection of *mcr* 1–8 genes using PCR technique revealed that two (2.1%) isolates were positive for *mcr*-1. No *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 *K. pneumoniae* isolates are displayed in Fig. 1. All the 93 carbapenem and colistin resistant *K. pneumoniae* isolates were grouped into three clusters 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 96%, as calculated by Simpson's Index of Diversity [25].

MLST

Ninety-three carbapenem and colistin resistant *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

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

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

DISCUSSION

Multidrug resistant pathogens have become a global problem recently [12]. *K. pneumoniae* is an important nosocomial multidrug resistant pathogen that can cause high morbidity and mortality [26]. After widespread dissemination of carbapenemase producing *K. pneumoniae* isolates; colistin resistance has emerged in *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*, *mcrB* 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 Arabacı et al., determined *mcr-1* positive KRCP (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 a 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.

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Conflict of interest: The authors declare no competing interests.

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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, Pelegrin AC, Mirande C, Brkic 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, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004;48:15–22.
4. Carrër 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, Carrër 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, Sammarco ML, Ripabelli G. Molecular epidemiological insights into colistin-resistant and Carbapenemase-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, Ruhel 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, Luppi 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;22:550–4.
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;33:2233–9.
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, Işık ME, Şahin S, Haciseyitoğlu 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). *Diagn 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 İ, Evren E, Oguzman E, Karahan ZC. Characterization of *Klebsiella pneumoniae* coproducing KPC and NDM-1 Carbapenemases from Turkey. *Microb Drug Resist* 2020;2:118–25.
33. Sağiroğlu P, Hasdemir U, Altınkanat Gelmez G, Aksu B, Karatuna O, Söyletir G. Performance of “RESIST-3 O.K.N. K-SeT” immuno-chromatographic assay for the detection of OXA-48 like, KPC, and NDM carbapenemases in *Klebsiella pneumoniae* in Turkey. *Braz J Mikrobiol* 2018;4:885–90.
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, Eriç B, Yaşar M, Aydemir ŞŞ, Hoşgör 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, Akyön Y, Gür D, Karatuna O, Ögünç 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 carbapenemases. *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, Brkic S, Vasiljević 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, Menekşe S, İspir 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, Miriagou 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 in North America. *Antimicrob Agents Chemother* 2016;57:130–6.
47. Jayol A, Poirel L, Brink A, Villegas MV, Yilmaz 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;58:4762–8.
48. Bialvaei AZ, Samadi Kafil 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, Kraychete 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. Guducuoglu H, Gursoy NC, Yakupogullari Y, Parlak M, Karasin G, Sunnetcioglu M, et al. Hospital outbreak of a colistin-resistant, NDM-1- and OXA-48-producing *Klebsiella pneumoniae*: high mortality from pandrug resistance. *Microb Drug Resist* 2018;24:966–72.
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;65:1125–30.
53. Arabacı Ç, Dal T, Başyigit T, Genişel N, Durmaz R. Investigation of carbapenemase and mcr-1 genes in carbapenem-resistant *Klebsiella pneumoniae* isolates. *J Infect Dev Ctries* 2019;30:504–9.