AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

68 (2021) 3, 162–168

DOI:
10.1556/030.2021.01400

ORIGINAL RESEARCH PAPER

Spread of NDM-producing *Klebsiella pneumoniae* in a tertiary Greek hospital

KONSTANTINA KONTOPOULOU[1], GEORGIOS MELETIS[2*], STYLIANI PAPPA[2], SOFIA ZOTOU[1], KATERINA TSIOKA[2], PANAGIOTA DIMITRIADOU[1], ELENI ANTONIADOU[3] and ANNA PAPA[2]

[1] Laboratory of Biopathology, General Hospital of Thessaloniki "G. Gennimatas", Thessaloniki, Greece

[2] Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3] Intensive Care Unit, General Hospital of Thessaloniki "G. Gennimatas", Thessaloniki, Greece

Received: January 17, 2021 • Accepted: February 04, 2021
Published online: March 3, 2021

ABSTRACT

Bacterial carbapenem resistance, especially when mediated by transferable carbapenemases, is of important public health concern. An increased number of metallo-β-lactamase (MBL)-producing *Klebsiella pneumoniae* strains isolated in a tertiary hospital in Thessaloniki, Greece, called for further genetic investigation.

The study included 29 non-repetitive carbapenem resistant *K. pneumoniae* isolates phenotypically characterized as MBL-producers collected in a tertiary hospital in Greece. The isolates were screened for the detection of carbapenemase genes (*K. pneumoniae* carbapenemase (bla_{KPC}), Verona-integron-encoded MBL-1 (bla_{VIM-1}), imipenemase (bla_{IMP}), oxacillinase-48 (bla_{OXA-48}) and New Delhi MBL (bla_{NDM})). The genetic relationship of the isolates was determined by Random Amplified Polymorphic DNA (RAPD) analysis. The whole genome sequences (WGS) from two NDM-positive *K. pneumoniae* isolates were further characterized.

The presence of New Delhi MBL (bla_{NDM}) gene was confirmed in all *K. pneumoniae* isolates, while bla_{KPC} and bla_{VIM-1} genes were co-detected in one and two isolates, respectively. The RAPD analysis showed that the isolates were clustered into two groups. The whole genome sequence analysis of two *K. pneumoniae* isolates revealed that they belonged to the sequence type 11, they carried the bla_{NDM-1} gene, and exhibited differences in the number and type of the plasmids and the resistant genes.

All MBL-producing *K. pneumoniae* isolates of the study harbored a bla_{NDM} gene, while WGS analysis revealed genetic diversity in resistance genes. Continuous surveillance is needed to detect the emergence of new clones in a hospital setting, while application of antimicrobial stewardship is the only way to reduce the spread of multi-resistant bacteria.

KEYWORDS

Klebsiella pneumoniae, NDM, carbapenemases, Greece

INTRODUCTION

Klebsiella pneumoniae is a notorious opportunistic nosocomial pathogen involved in serious and often life-threatening hospital-acquired infections; therefore, the emergence of multi-drug-resistant, and especially carbapenem-resistant strains, is considered as a major public health problem [1, 2]. Carbapenem resistance is mainly mediated by enzymes able to hydrolyze carbapenems and almost all other β-lactams [3]. They are encoded by carbapenemase-encoding genes, mainly bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, and lately, bla_{NDM}, that

*Corresponding author.
E-mail: meletisg@hotmail.com

emerged in the Indian subcontinent [4] and subsequently has been reported in Europe and other continents [5–8].

According to the most recent annual surveillance report from the European Centre for Disease Prevention and Control (ECDC), Greece presents high percentage of carbapenem-resistant isolates among invasive *K. pneumoniae* strains [9] and is considered endemic for KPC and VIM-producing *K. pneumoniae* [10, 11]. Since 2011, when the first detection of NDM-producing strains was reported in Greece [12], several sporadic cases and outbreaks have been reported causing great concern [13–17]. Up to now the NDM *K. pneumoniae* strains in Greece belong to the sequence type (ST) 11. The present study presents the molecular epidemiology and resistance mechanisms of NDM-encoding *K. pneumoniae* isolated in a tertiary hospital in Greece.

MATERIAL AND METHODS

Strains

From August 15, 2019 to February 15, 2020, during the routine phenotypic screening for carbapenemase production among carbapenem-resistant Gram-negative pathogens isolated at a 270-bed tertiary hospital in Greece, an unusual increase of metallo-β-lactamase (MBL)-producing *K. pneumoniae* was observed prompting for further investigation. Twenty-nine non-repetitive *K. pneumoniae* isolates which phenotypically gave an evidence for MBL production were included in the study. Seventeen isolates were recovered from blood, urine and pus samples and twelve were recovered during surveillance (5 from pharyngeal and 7 from rectal swab cultures). Most patients (17/29, 63%) were hospitalized in the intensive care unit (ICU).

Bacterial identification and antimicrobial susceptibility testing were performed by the Vitek2 automated system (bioMérieux, Marcy l'Étoile, France), while the MICs of imipenem and meropenem were further determined by E-test (bioMérieux, Marcy l'Étoile, France); results of antimicrobial testing were interpreted in accordance to Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines.

Detection of β-lactamase genes

Phenotypic testing for the presence of MBL and/or KPC carbapenemases was performed with the combined disk test (CDT) using meropenem disks without and with phenyl boronic acid and/or EDTA [18]. The presence of bla_{KPC}, bla_{IMP}, $bla_{OXA\text{-}48\text{-}like}$, and bla_{NDM} genes was investigated by a multiplex PCR [19], while an additional PCR was applied for the detection of $bla_{VIM\text{-}1}$ gene [20]. The genetic relationship of the isolates was determined by random amplification of polymorphic DNA test (RAPD) [21]. The clustering analysis on RAPD patterns and the dendrogram construction were performed based on the pairwise similarities among the RAPD profiles applying the UPGMA method in the BioNumerics software package (version 5.1, Applied Maths available from: http://www.applied-maths.com/bionumerics).

Whole Genome Sequence analysis

The whole genome sequence (WGS) of two randomly selected NDM-producing *K. pneumoniae* isolates (213A and 248D) was obtained and analyzed by next generation sequencing. Specifically, following DNA extraction, the concentration of double strand (ds) DNA was measured with Qubit using Qubit ds DNA HS assay kit (Q32851, Life Technologies) and NGS was performed using the Ion Torrent S5 PGM Platform (Life Technologies Corporation, Grand Island, NY, USA); shearing, purification, ligation, barcoding, size selection, library amplification and quantitation, emulsion PCR and enrichment were performed according to manufacturer's instructions and the products were loaded on a 316 chip. The Ion PGMHi-Q (200) chemistry (Ion PGM Hi-Q Sequencing kit, A25592) was applied (Thermo Fisher Scientific).

Identification of multi-locus sequence type (MLST), plasmids and antimicrobial resistance genes was performed applying the tools using MLST 2.0, Resfinder 4.1, KmerResistance 2.2, Comprehensive Antibiotic Resistance Database (CARD), and PlasmidFinder 2.1 [22–26].

RESULTS

All isolates were resistant to imipenem, meropenem, ceftazidime, piperacillin/tazobactam, while 37.94 and 44.83% were non-susceptible to amikacin and gentamicin, respectively; aztreonam and colistin resistance was observed in 79.31 and 10.34% of the isolates, respectively. The bla_{NDM} gene was detected in all *K. pneumoniae* isolates, while bla_{KPC} and bla_{VIM} genes were co-detected in one (33AP) and two (3051D and 322D) isolates, respectively (Table 1).

RAPD DNA profiles of the 29 *K. pneumoniae* isolates showed bands ranging from 200-to 3,000 base pairs. Based on RAPD patterns the isolates were divided into two major groups (A and B) which differed by 28.4%. A clear chronological grouping of the isolates was observed, with group A containing isolates collected during August-November 2019 (with 4 subgroups), and group B containing isolates collected during December 2019–February 2020 (with 7 subgroups) (Fig. 1). The in-group difference was <10%.

The two isolates from which the WGS was obtained, presented high minimum inhibitory concentrations to imipenem, meropenem, ceftazidime, piperacillin/tazobactam, and aztreonam; 213A was sensitive to amikacin and gentamicin and colistin resistant, while 248D was resistant to amikacin and gentamicin and colistin sensitive. The main characteristics of their whole genome analysis are seen in Table 2. Both isolates belonged to MLST ST11 and carried the $bla_{NDM\text{-}1}$ gene. In silico analysis revealed that only IncFIA (HI1) plasmid was present in both isolates, with the rest belonging to various incompatibility groups (Table 2).

Table 1. Characteristics and detection of β-lactamase-encoding genes in carbapenem-resistant *Klebsiella pneumoniae* isolates

Isolate	Hospital unit	Isolation date	Source	KPC	NDM	VIM-1	IMP	OXA-48
1972A	Int. Medicine	20/8/2019	blood	neg	**pos**	neg	neg	neg
2270O	Urology	25/8/2019	urine	neg	**pos**	neg	neg	neg
2989O	Urology	10/9/2019	urine	neg	**pos**	neg	neg	neg
2185D	Urology	12/9/2019	pus	neg	**pos**	neg	neg	neg
3202O	Urology	26/9/2019	urine	neg	**pos**	neg	neg	neg
267Y	ICU	22/10/2019	pus	neg	**pos**	neg	neg	neg
2569B	ICU	11/11/2019	blood	neg	**pos**	neg	neg	neg
3879O	Int. Medicine	16/11/2019	urine	neg	**pos**	neg	neg	neg
2964D	ICU	9/12/2019	pharyngeal swab	neg	**pos**	neg	neg	neg
149 MB	ICU	11/12/2019	BAL	neg	**pos**	neg	neg	neg
3051D	ICU	16/12/2019	rectal swab	neg	**pos**	**pos**	neg	neg
3045D	ICU	16/12/2019	rectal swab	neg	**pos**	neg	neg	neg
3053D	ICU	16/12/2019	rectal swab	neg	**pos**	neg	neg	neg
4299O	Urology	18/12/2019	urine	neg	**pos**	neg	neg	neg
331Y	Gen. Surgery	25/12/2019	pus	neg	**pos**	neg	neg	neg
4053O	Int. Medicine	27/12/2019	urine	neg	**pos**	neg	neg	neg
81O	ICU	8/1/2020	urine	neg	**pos**	neg	neg	neg
33AP	Int. Medicine	13/1/2020	pharyngeal swab	**pos**	**pos**	neg	neg	neg
37AP	Int. Medicine	13/1/2020	pharyngeal swab	neg	**pos**	neg	neg	neg
86D	ICU	13/1/2020	rectal swab	neg	**pos**	neg	neg	neg
165O	Int. Medicine	15/1/2020	urine	neg	**pos**	neg	neg	neg
127D	ICU	17/1/2020	rectal swab	neg	**pos**	neg	neg	neg
213A	ICU	27/1/2020	blood	neg	**pos**	neg	neg	neg
248D	ICU	3/2/2020	pharyngeal swab	neg	**pos**	neg	neg	neg
264A	ICU	3/2/2020	blood	neg	**pos**	neg	neg	neg
255D	ICU	3/2/2020	rectal swab	neg	**pos**	neg	neg	neg
314A	ICU	6/2/2020	blood	neg	**pos**	neg	neg	neg
221D	ICU	10/2/2020	pharyngeal swab	neg	**pos**	neg	neg	neg
322D	ICU	10/2/2020	rectal swab	neg	**pos**	**pos**	neg	neg

Int. Medicine: Internal Medicine, Gen. Surgery: General Surgery, ICU: Intensive care unit, BAL: bronchoalveolar lavage, neg: negative, pos: positive.

Regarding genes conferring resistance to antibiotics, most were detected in *K. pneumoniae* 248D (Table 2). Co-occurrence of beta-lactamases was observed. Besides the $bla_{\text{NDM-1}}$ gene, several additional chromosomal and plasmid-mediated beta-lactamase genes were detected, including $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-10}}$, $bla_{\text{SHV-11}}$, $bla_{\text{TEM-1B}}$, and $bla_{\text{VEB-1}}$ genes. Both strains harbored the plasmid-mediated quinolone resistance genes *oqxA* and *oqxB*, while genes conferring resistance to doxycycline, penicillins and rifampicin were detected only in 248D. Both strains harbored the aminoglycoside-modifying enzymes genes *aac(6)-Ib-cr*, and *aph (3)-Ib*, while 248D co-harbored the *aac(6)-Ib, ant(2′)-Ia, ant(3″)-IIe* genes and the 16S rRNA methylase gene *rmtB*. A variety of genes associated with efflux pump and reduced permeability were also detected.

DISCUSSION

Since the early 2000s, Greece is affronting a critical situation regarding the presence of carbapenemase-encoding genes in hospitals, as VIM-1 carbapenemases emerged in 2002, KPC-2 in 2007, NDM-1 in 2011, OXA-48 in 2012 [27] and, since 2010, *K. pneumoniae* strains co-producing two carbapenemases have been also detected [28]. As reported recently, the Greek situation differs greatly from other European countries where OXA-48, is the most frequently encountered carbapenemase [16]. The increased number of metallo-β-lactamase (MBL)-producing *K. pneumoniae* isolates observed in the summer of 2019 in a tertiary hospital in Thessaloniki, Greece, called for further genetic investigation since MBL–producing *K. pneumoniae* are associated with high rates of morbidity and mortality in nosocomial settings [29]. It was shown that all isolates of the study harbored the bla_{NDM} gene, while three NDM-producing isolates carried additional carbapenemase genes (two bla_{VIM} and one bla_{KPC}). A previous 4-year (January 2013–December 2016) survey in 8 Greek hospitals showed that 71% of 481 phenotypically MBL positive carbapenem non-susceptible *K. pneumoniae* isolates carried the $bla_{\text{NDM-1}}$ gene [30]. Since 2016, there was a clear predominance of KPC over metallo-β-lactamases in the country [31, 32] which recently seems to change. This could be at least partially attributed to the implementation of ceftazidime/avibactam [33] that shows optimal results against KPC-producers [34].

Epidemiological data showed that the detection of NDM-producing *K. pneumoniae* was not limited in certain units,

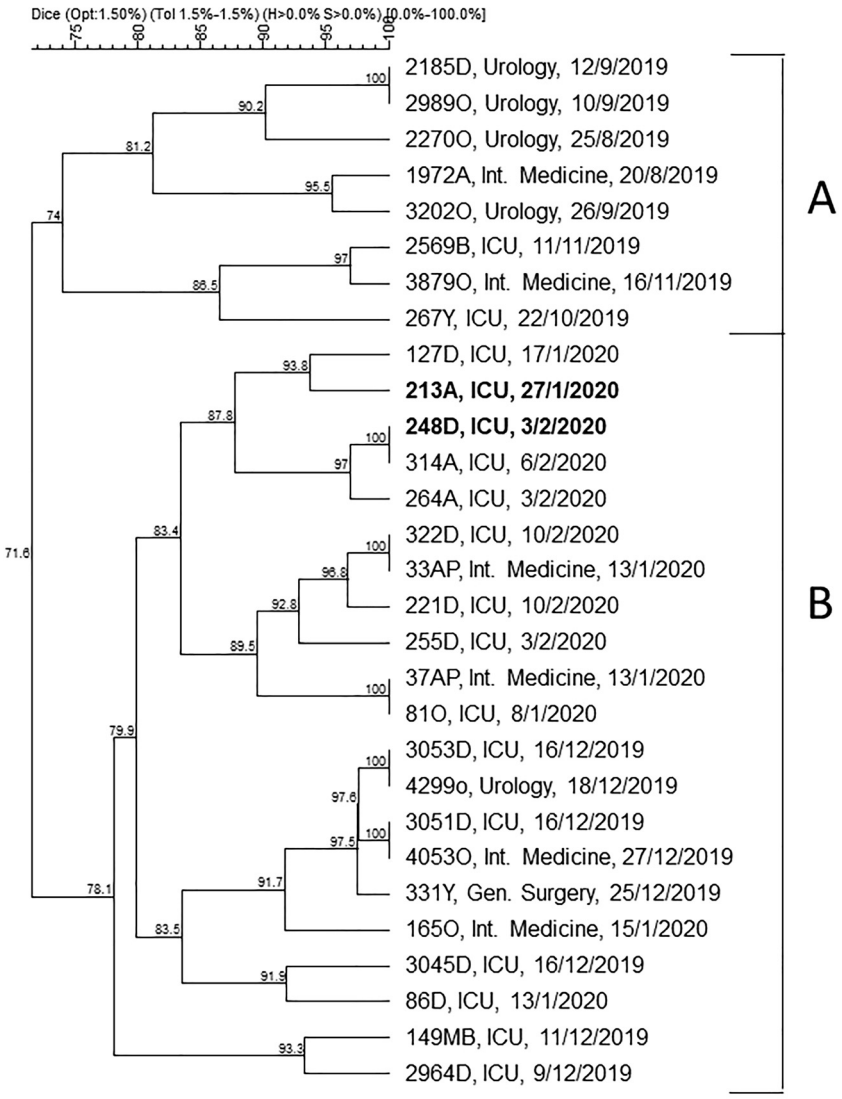

Fig. 1. RAPD profiles of the 29 *K. pneumoniae* isolates of the present study

although most (63%) were isolated from patients hospitalized in ICU. Twelve isolates were taken during colonization control, which shows the value of surveillance in order to detect silent dissemination, since carbapenem-resistant *Enterobacterales* colonization may rapidly result in infection, especially in critically ill patients [35].

A low level of similarity (<28%) was observed among the RAPD patterns of the two time-dependent groups, suggesting the circulation of two different clones. Even within groups, the isolates differed each other (although less than 10%), reflecting the great heterogeneity of the isolates.

The analysis of the whole genome sequences of the two *K. pneumoniae* isolates revealed that they belonged to ST11, which is a common NDM-bearing lineage in several countries worldwide, and it seems that currently is established in Greece. A noteworthy variability in the genetic characteristics was observed between the two isolates, regarding the number and content of plasmids and the number and types of antibiotic resistance genes (Table 2). The IncFIA (HI1) plasmid was present in both isolates, and it is known that plasmids of the IncF group represent one of the most common plasmid types contributing to the spread of antibiotic resistance genes in *Enterobacteriaceae* [36]. In previous studies in Greece the identified plasmids in NDM-producing ST11 *K. pneumoniae* isolates belonged to the IncFII group [12, 30]. In the present study, an IncFII plasmid was detected in one isolate, however, a variety of other plasmids were identified, including IncC and IncR. The content of resistance genes differed between the two isolates, which reflects the difference in antibiotic resistance. A discrepancy was seen in amikacin resistance of 213A isolate since it was found sensitive (MIC = 16 mg/L); however, *aac (6)-Ib-cr* gene which confers resistance to certain aminoglycosides (amikacin, isepamicin, and tobramycin) and fluoroquinolones was detected in the WGS. This can be explained by the fact that AAC (6′)-Ib-cr enzymes are less effective against aminoglycosides compared to other members of the same subclass [37]. As expected, 213A

Table 2. Genetic characteristics of *K. pneumoniae* isolates 213A and 248D

	Isolate	
	213A	248D
Sequence size	5377931	5787820
No of contigs	363	505
GC content (%)	57.4	57.0
Sequence type	11	11
Plasmid replicons	IncR, IncFIA (HI1)	ColRNAI, IncC, IncFIA (HI1), IncFIB(K), IncFII(K)
Genes for efflux pump/reduced permeability	*marA, LptD, oqxA, oqxB, OmpA, ermR, baeR, adeF*	*marA, LptD, oqxA, oqxB, tet(A), tet(D), OmpA, ermR, baeR, adeR*
Resistance to:		
β-lactams	NDM-1, OXA-1, SHV-11	NDM-1, OXA-1, OXA-10, SHV-11, CTX-M-15, TEM-1B, VEB-1
Aminoglycosides	*aac(6′)-Ib-cr, aph(3″)-Ib*	*aac(6′)-Ib, aac(6′)-Ib-cr, aac(3)-IIe, aph(3″)-Ib, aph(3′)-Ia aph(6)-Id, aadA1, ant(2″)-Ia, rmtB*
Quinolones	*aac(6′)-Ib-cr, oqxA, oqxB*	*aac(6′)-Ib-cr, oqxA, oqxB*
Sulphonamides	*sul2*	*sul2*
Trimethoprim	*dfrA14, oqxA, oqxB*	*dfrA14, oqxA, oqxB*
Fosfomycin	*fosA*	*fosA*
Phenicol	*oqxA, oqxB*	*cmlA1, oqxA, oqxB*
Tetracycline (doxycycline)		*tet(A), tet(D), tet(G)*
Penicillins		TEM-1
Rifampicin		ARR-2

isolate was sensitive to gentamicin, in contrast to the 248D isolate which contained a plethora of aminoglycoside-modifying enzymes genes and the 16S rRNA methylase gene *rmt*B (Table 2).

Limitation of the study was the low number of whole genome sequenced isolates, and especially the lack of WGS of group A isolates. However, even the analysis of two isolates of the same group showed that although the NDM-producing *K. pneumoniae* strains belonged to the same sequence type and were isolated in the same unit of the hospital within one-week interval, they differed greatly in their genetic characteristics. As indicated previously, the "one size fits all" approaches to identifying effective antimicrobial regimens against carbapenem-resistant *K. pneumoniae* strains are not effective [38].

In conclusion, all MBL-producing *K. pneumoniae* isolates recovered from a Greek hospital during a 6-month period carried a bla_{NDM} gene, while WGS analysis showed that isolates, even within similar genetic group, exhibit high genetic diversity. Continuous surveillance is necessary to detect the emergence of new bacterial clones in hospital settings, while application of antimicrobial stewardship is the only way to reduce the spread of multi-drug resistant bacteria.

Nucleotide sequence accession numbers

The whole genome sequences of Thessaloniki-248D-2020 and Thessaloniki-213A-2020 were submitted to European Nucleotide Archive (ENA) under the study PRJEB41773 and received the Accession numbers ERS5489252 and ERS5489253, respectively.

FUNDING INFORMATION

The current work was supported by the European Union's Horizon 2020 grant VEO (grant number 874735).

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Patel G, Bonomo RA. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol 2013; 4: 48. https://doi.org/10.3389/fmicb.2013.00048.
2. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis 2016; 3: 15–21. https://doi.org/10.1177/2049936115621709.
3. Bush K, Bradford PA. Epidemiology of *β*-lactamase-producing pathogens. Clin Microbiol Rev 2020; 33: e00047–19. https://doi.org/10.1128/CMR.00047-19.
4. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol 2011; 19: 588–95. https://doi.org/10.1016/j.tim.2011.09.005.
5. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int 2014; 2014: 249856. https://doi.org/10.1155/2014/249856.
6. Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-*β*-lactamase (NDM)-mediated

carbapenem resistance. J Med Microbiol 2013; 62: 499–513. https://doi.org/10.1099/jmm.0.052555-0.
7. Rubin JE, Peirano G, Peer AK, Govind CN, Pitout JD. NDM-1-producing Enterobacteriaceae from South Africa: moving towards endemicity? Diagn Microbiol Infect Dis 2014; 79: 378–80. https://doi.org/10.1016/j.diagmicrobio.2014.04.003.
8. Livermore DM, Walsh TR, Toleman M, Woodford N. Balkan NDM-1: escape or transplant? Lancet Infect Dis 2011; 11: 164. https://doi.org/10.1016/S1473-3099(11)70048-2.
9. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) – annual epidemiological report 2019. Stockholm: ECDC; 2020.
10. Bassetti M, Giacobbe DR, Giamarellou H, Viscoli C, Daikos GL, Dimopoulos G, et al. Management of KPC-producing *Klebsiella pneumoniae* infections. Clin Microbiol Infect 2018; 24: 133–44. https://doi.org/10.1016/j.cmi.2017.08.030.
11. Girmenia C, Serrao A, Canichella M. Epidemiology of carbapenem resistant *Klebsiella pneumoniae* infections in Mediterranean countries. Mediterr J Hematol Infect Dis 2016; 8: e2016032. https://doi.org/10.4084/MJHID.2016.032.
12. Voulgari E, Gartzonika C, Vrioni G, Politi L, Priavali E, Levidiotou-Stefanou S, et al. The Balkan region: NDM-1-producing *Klebsiella pneumoniae* ST11 clonal strain causing outbreaks in Greece. J Antimicrob Chemother 2014; 69: 2091–7. https://doi.org/10.1093/jac/dku105.
13. Giakkoupi P, Tryfinopoulou K, Kontopidou F, Tsonou P, Golegou T, Souki H, et al. Emergence of NDM-producing *Klebsiella pneumoniae* in Greece. Diagn Microbiol Infect Dis 2013; 77: 382–4. https://doi.org/10.1016/j.diagmicrobio.2013.09.001.
14. Papagiannitsis CC, Malli E, Florou Z, Sarrou S, Hrabak J, Mantzarlis K, et al. Emergence of sequence type 11 *Klebsiella pneumoniae* coproducing NDM-1 and VIM-1 metallo-β-lactamases in a Greek hospital. Diagn Microbiol Infect Dis 2017; 87: 295–7. https://doi.org/10.1016/j.diagmicrobio.2016.12.008.
15. Protonotariou E, Meletis G, Chatzopoulou F, Malousi A, Chatzidimitriou D, Skoura L. Emergence of *Klebsiella pneumoniae* ST11 co-producing NDM-1 and OXA-48 carbapenemases in Greece. J Glob Antimicrob Resist 2019; 19: 81–2. https://doi.org/10.1016/j.jgar.2019.08.020.
16. Meletis G, Chatzopoulou F, Chatzidimitriou D, Tsingerlioti F, Botziori C, Tzimagiorgis G, et al. Whole genome sequencing of NDM-1-producing ST11 *Klebsiella pneumoniae* isolated in a private laboratory in Greece. Microb Drug Resist 2019; 25: 80–6. https://doi.org/10.1089/mdr.2017.0411.
17. Spyropoulou A, Bartzavali C, Vamvakopoulou S, Marangos M, Anastassiou ED, Spiliopoulou I, et al. The first NDM metallo-β-lactamase producing *Klebsiella pneumoniae* isolate in a University Hospital of Southwestern Greece. J Chemother 2016; 28: 350–1. https://doi.org/10.1179/1973947815Y.0000000003.
18. Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, et al. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. J Antimicrob Chemother 2010; 65: 1664–71. https://doi.org/10.1093/jac/dkq210.
19. Hatrongjit R, Kerdsin A, Akeda Y, Hamada S. Detection of plasmid-mediated colistin-resistant and carbapenem-resistant genes by multiplex PCR. MethodsX 2018; 5: 532–6. https://doi.org/10.1016/j.mex.2018.05.016.
20. Tsakris A, Pournaras S, Woodford N, Palepou MF, Babini GS, Douboyas J, et al. Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-1 carbapenemase in Greece. J Clin Microbiol 2000; 38: 1290–2. https://doi.org/10.1128/JCM.38.3.1290-1292.2000.
21. Saadatian Farivar A, Nowroozi J, Eslami G, Sabokbar A. RAPD PCR profile, antibiotic resistance, prevalence of armA gene, and detection of KPC enzyme in Klebsiella pneumoniae isolates. Can J Infect Dis Med Microbiol 2018; 2018: 6183162. https://doi.org/10.1155/2018/6183162.
22. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics 2009; 10: 421. https://doi.org/10.1186/1471-2105-10-421.
23. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012; 50: 1355–61. https://doi.org/10.1128/JCM.06094-11.
24. Clausen P, Aarestrup FM, Lund O. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 2018; 19: 307. https://doi.org/10.1186/s12859-018-2336-6.
25. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 2020; 48(D1): D517–25. https://doi.org/10.1093/nar/gkz935.
26. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. Antimicrob Agents Chemother 2014; 58: 3895–903. https://doi.org/10.1128/AAC.02412-14.
27. Karampatakis T, Antachopoulos C, Iosifidis E, Tsakris A, Roilides E. Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in Greece. Future Microbiol 2016; 11: 809–23. https://doi.org/10.2217/fmb-2016-0042.
28. Meletis G, Chatzidimitriou D, Malisiovas N. Double- and multi-carbapenemase-producers: the excessively armored bacilli of the current decade. Eur J Clin Microbiol Infect Dis 2015; 34: 1487–93. https://doi.org/10.1007/s10096-015-2379-9.
29. Pitout JDD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob Agents Chemother 2015; 59: 5873–84. https://doi.org/10.1128/AAC.01019-15.
30. Politi L, Gartzonika K, Spanakis N, Zarkotou O, Poulou A, Skoura L, et al. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Greece: evidence of a widespread clonal outbreak. J Antimicrob Chemother 2019; 74: 2197–202. https://doi.org/10.1093/jac/dkz176.
31. Spyropoulou A, Papadimitriou-Olivgeris M, Bartzavali C, Vamvakopoulou S, Marangos M, Spiliopoulou I, et al. A ten-year surveillance study of carbapenemase-producing *Klebsiella pneumoniae* in a tertiary care Greek university hospital: predominance of KPC- over VIM- or NDM-producing isolates. J Med Microbiol 2016; 65: 240–6. https://doi.org/10.1099/jmm.0.000217.
32. Galani I, Karaiskos I, Karantani I, Papoutsaki V, Maraki S, Papaioannou V, et al. Epidemiology and resistance phenotypes of carbapenemase-producing *Klebsiella pneumoniae* in Greece, 2014 to 2016. Euro Surveill 2014; 23. https://doi.org/10.2807/1560-7917.ES.2018.23.30.1700775.

33. Papadimitriou-Olivgeris M, Bartzavali C, Lambropoulou A, Solomou A, Tsiata E, Anastassiou ED, et al. Reversal of carbapenemase-producing *Klebsiella pneumoniae* epidemiology from bla_{KPC}- to bla_{VIM}-harbouring isolates in a Greek ICU after introduction of ceftazidime/avibactam. J Antimicrob Chemother 2019; 74: 2051–4. https://doi.org/10.1093/jac/dkz125.
34. Protonotariou E, Meletis G, Kachrimanidou M, Papadopoulou D, Stamou A, Arhonti M, et al. In vitro activity of ceftazidime/avibactam against KPC-producing *Klebsiella pneumoniae* in Greece: a single-centre study. J Glob Antimicrob Resist 2020; 20: 82–3. https://doi.org/10.1016/j.jgar.2019.11.021.
35. Kontopoulou K, Iosifidis E, Antoniadou E, Tasioudis P, Petinaki E, Malli E, et al. The clinical significance of carbapenem-resistant *Klebsiella pneumoniae* rectal colonization in critically ill patients: from colonization to bloodstream infection. J Med Microbiol 2019; 68: 326–35. https://doi.org/10.1099/jmm.0.000921.
36. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 2018; 73: 1121–37. https://doi.org/10.1093/jac/dkx488.
37. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 2006; 12: 83–8. https://doi.org/10.1038/nm1347.
38. Almaghrabi R, Clancy CJ, Doi Y, Hao B, Chen L, Shields RK, et al. Carbapenem-resistant *Klebsiella pneumoniae* strains exhibit diversity in aminoglycoside-modifying enzymes, which exert differing effects on plazomicin and other agents. Antimicrob Agents Chemother 2014; 58: 4443–51. https://doi.org/10.1128/AAC.00099-14.