Journal of Global Antimicrobial Resistance 28 (2022) 146–150

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Detection of NDM-1/5 and OXA-48 co-producing extensively drug-resistant hypervirulent *Klebsiella pneumoniae* in Northern Italy

Giovanni Lorenzin[a,1], Floriana Gona[a,1], Simone Battaglia[a], Andrea Spitaleri[a], Francesca Saluzzo[a], Alberto Trovato[a], Federico di Marco[a], Paola Cichero[b], Anna Biancardi[c], Paola Nizzero[c], Barbara Castiglione[d], Paolo Scarpellini[d], Matteo Moro[c], Daniela Maria Cirillo[a,*]

[a] *Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[b] *Laboratory of Microbiology and Virology, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[c] *Infection Control, IRCCS San Raffaele Scientific Institute, Milan, Italy*
[d] *Unit of Infectious and Tropical Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy*

ARTICLE INFO

Article history:
Received 16 August 2021
Revised 17 December 2021
Accepted 5 January 2022
Available online 10 January 2022

Editor:Prof Andrea Endimiani

Keywords:
Hypervirulent Klebsiella pneumoniae
NDM-5
NDM-1
OXA-48
Multiple plasmids
Carbapenem-resistant Klebsiella pneumoniae

ABSTRACT

Objectives: Using a hybrid long-read sequencing approach, we aimed to fully characterise four extensively-drug resistant (XDR) hypervirulent *Klebsiella pneumoniae* isolates, one of which represented the first strain isolated in Italy co-expressing NDM-1/5 and OXA-48 carbapenemases.

Methods: Whole-genome sequencing was performed using Illumina and Oxford Nanopore Technology platforms. An assembly pipeline was used to recover the structures both of the chromosome and plasmids.

Results: Multilocus sequence typing (MLST) showed that these strains belonged to high-risk sequence types (STs) not commonly circulating in Italy (ST383, ST147 and ST15). The hybrid sequencing approach allowed to characterise three multidrug resistance plasmids, which demonstrated high homology with previously sequenced plasmids, that were simultaneously detected in one ST383 strain carrying, respectively, $bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$.

Conclusion: This is the first report in Italy of new hypervirulent XDR *K. pneumoniae* clones characterised by co-production of OXA-48, NDM-1 and NDM-5. The discovery of new high-risk clones harbouring multiple mobile elements is a growing problem that poses a great challenge for public health.

1. Introduction

Extensively drug-resistant (XDR) *Klebsiella pneumoniae* is globally recognised as a major cause of difficult-to-treat healthcare-acquired infections [1,2]. Highly transmissible and conjugable plasmids play a key role in the antimicrobial resistance emergency worldwide, enabling the horizontal transfer of acquired antibiotic resistance genes [3]. Recently, routine molecular epidemiological surveillance has been focused on chromosome analysis, even though plasmids are the main means for antimicrobial resistance genes to spread [2].

In Northern Italy, local epidemiology highlights that the main mechanism of carbapenem resistance is the plasmid-borne KPC-2/3 variant, and its carriage is associated with major clones involved in outbreaks, such as sequence types ST258/512, ST307 and ST101 [4].

None the less, carbapenem resistance has been described worldwide in other sequence types such as ST11, ST15, ST147 and ST383 [5–7]. These clones have been reported as causes of hospital outbreaks [8] and the main identified mechanism of carbapenem resistance is the presence of plasmid-mediated carbapenemases, especially metallo-β-lactamase [5]. Therefore, determination of virulence and resistance factors associated with the mobilome in new emerging clones is essential for infection prevention and control and antimicrobial stewardship protocols in all hospital wards.

* Corresponding author. Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, San Gabriele Building, Via Olgettina 58, 20132 Milan, Italy. Tel.: +39 02 2643 9121; fax: +39 02 2643 5183.
E-mail address: cirillo.daniela@hsr.it (D.M. Cirillo).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.jgar.2022.01.001

Table 1
Minimum inhibitory concentrations (MICs) of the four isolates, and MIC breakpoints according to EUCAST [10].

Antibiotic	MIC breakpoint (mg/L)		MIC (mg/L)			
	S ≤	R >	KP45-19	KP48-19	KP50-19	KP52-19
Amikacin	8	8	>16	>16	>16	>16
Cefepime	1	4	>32	>32	>32	>32
Cefotaxime	1	2	>4	>4	>4	>4
Ceftazidime	1	2	>128	>128	>128	>128
Ciprofloxacin	0.25	0.5	>2	>2	>2	>2
Colistin	2	2	<0.5	<0.5	<0.5	<0.5
Gentamicin	2	2	>4	>4	>4	>4
Imipenem	2	4	8	>16	>16	>16
Meropenem	2	8	16	>64	>64	>64
Piperacillin/tazobactam	8	8	>128	>128	>128	>128
Tigecycline	NA	NA	2	0.5	1	1
Trimethoprim/sulfamethoxazole	2	4	>320	>320	>320	1
Ceftolozane/tazobactam	2	2	>256	>256	>256	>256
Ceftazidime/avibactam	8	8	>256	>256	>256	>16

EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; R, resistant; NA, not available.

After the 2019 NDM-1 epidemic in Tuscany [9], our laboratory implemented a new mobilome surveillance protocol to better understand the epidemiology related to plasmid diffusion. This programme allowed to identify and analyse four XDR *K. pneumoniae* strains with peculiar characteristics of antimicrobial resistance and virulence.

2. Methods

Four strains of non-KPC-producing XDR *K. pneumoniae* were isolated at the end of 2019 from four different patients who were admitted to our hospital due to orthopaedic disease. All patients were colonised as identified by rectal swab by the isolated strains. Two patients developed sepsis consequent to infection of an implanted orthopaedic prosthesis, and one patient suffered from catheter-related bloodstream infection. The four strains (named KP45-19, KP48-19, KP50-19 and KP52-19) were susceptible only to colistin and tigecycline by antimicrobial susceptibility testing. Minimum inhibitory concentrations (MICs) (Table 1) were determined using VITEK®2 AST-GN67 (bioMérieux SA, Marcy-l'Étoile, France) and were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [10]. The patient harbouring KP45-19 recovered from the infection after 8 weeks of treatment with tigecycline and fosfomycin; the patient infected by KP48-19 was treated for 4 days with ceftazidime/avibactam, aztreonam and tigecycline before being transferred to another hospital; the patient harbouring KP52-19 was treated with ceftazidime/avibactam, aztreonam and tigecycline for 8 weeks and asked to be discharged before complete recovery; and finally, the last patient was colonised on the rectal swab by KP50-19, but as no infection was reported no treatment was required during hospitalisation.

All four isolates tested positive by immunochromatographic test (NG-Test® CARBA 5; Hardy Diagnostics, Santa Maria, CA, USA) for the co-production of a New Delhi metallo-β-lactamase (NDM) and OXA-48-like carbapenemases and displayed a hypermucoviscous phenotype by positive string test (defined as the presence of a viscous filament >5 mm from the agar plate). Therefore, the isolated strains could be defined as hypervirulent as they presented both clinical and phenotypical (hypermucoviscous phenotype) characteristics associated with hypervirulence [11] as well as genotypic characteristics whose in-depth analysis will be presented in the results section.

The isolates underwent whole-genome sequencing using an Illumina MiniSeq platform (Illumina Inc., San Diego, CA, USA) with paired-end Nextera XT library preparation following the manufacturer's instructions. Long-read sequencing was performed using a MinION Mk1B platform (Oxford Nanopore Technologies, Oxford, UK) with a FLO-MIN106 R9.4.1 flow cell and using Rapid Barcoding Kit (SQK-RBK004) for library preparation. To obtain the best possible assembly consensus, a hybrid assembly approach was chosen. The hybrid assembly pipeline consists of generating long-read assemblies using Flye; polishing with Racon and Medaka; ten Minimap-Racon iterations for short-read polishing; and ten iterations of Bowtie2-Pilon for accurate base calling. Annotation of the plasmids was done using Prokka software tool with a tailor-made plasmid database, and the resulting output was visualised using Circos software. Basecalling was performed using Guppy software v.3.3.3 from fast5 files. Demultiplexing and trimming was performed using Porechop v.0.2.4. Fastq files are available in the NCBI database as reported in Table 2.

Table 2
Accession numbers of Illumina and Oxford Nanopore Technology (ONT) sequences.

Isolate	Accession Illumina	Accession ONT
KP45-19	SAMN23803868	SAMN23805233
KP52-19	SAMN23803869	SAMN23805235
KP48-19	SAMN23803870	SAMN23805236
KP50-19	SAMN23803871	SAMN23805234

All of the samples have been uploaded on NCBI SRA, project no. **PRJNA787449**.

3. Results

Klebsiella pneumoniae KP45-19 belonged to ST147, KL64 capsular type (*wzi* allele 64), KP48-19 belonged to ST15, KL112 capsular type (*wzi* allele 93), and the other two strains (KP50-19 and KP52-19) belonged to ST383, KL51 capsular type (*wzi* allele 298) as determined using multilocus sequence typing (MLST) and *wzi* allele databases.

We focused on plasmids carrying genes encoding carbapenemases as carbapenem resistance is the major issue related to the treatment of these pathogens. Using AMRFinderPlus, we characterised KP45-19 and KP48-19 as carrying $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$ carbapenemases genes, KP50-19 as carrying $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$ genes, and KP52-19 as carrying the three carbapenemases $bla_{\text{OXA-48}}$, $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-1}}$ gene (Fig. 1).

Molecular analysis revealed no mutations in *pmrAB, phoPQ* or *mgrB* genes that may confer a reduced/non-susceptible phenotype to polymyxins.

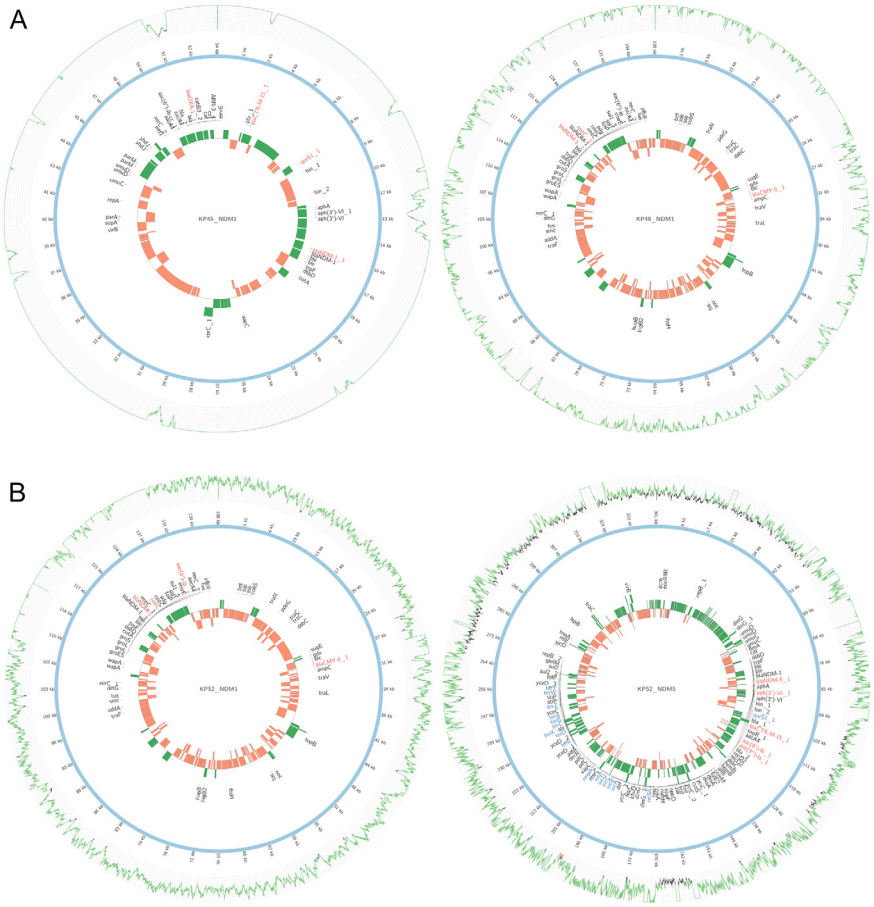

Fig. 1. (A.B) Genomic Circos plots of de novo assembled contigs for the KP45 and KP48 (A) and KP52 (B) plasmids, with annotated genes. Inward: coverage (green >30; black, >10; grey, <10; red, 0). Plasmid genomic co-ordinates (blue), annotated genes with name (red, antimicrobial resistance genes; blue, virulence genes) and annotated genes (green, forward; red, reverse).

The plasmids carrying the bla_{OXA-48} gene are very similar to previously sequenced plasmids (Table 3). These plasmids carry only bla_{OXA-48}, and no other virulence factors are present.

Plasmids carrying bla_{NDM-1} were identified in KP45-19, KP48-19 and KP52-19. KP45-19-NDM1 [IncFIB(pQil), 54 064 bp] has 100% homology to **NZ_CP021947.1** that was previously identified in a Syrian patient in a hospital in Rome [12]. KP48-19-NDM1 and KP52-19-NDM1 share the same plasmid: an IncC plasmid that shows 100% homology between each other and has >99% homology with the conjugative plasmid **CP022126.1** reported in Nevada (USA) in a patient who had a history of recent travel to India (Table 3).

The bla_{NDM-5} gene was detected only in the ST383 isolates. This gene is carried, respectively, in two large hybrid plasmids. KP50-19-NDM5 and KP52-19-NDM5, both IncFIB/IncHI1B, are two large (>350 kb) hybrid plasmids that carry many antimicrobial resistance and virulence genes (Table 3). They both display >99.9% homology with **NZ_CP034201.1** identified in a patient in Southeast England [5]. KP52-19-NDM5 lacks two transposable islands, one of ~9 kb and one of ~24 kb (Table 3).

ST147/KP45 and ST15/KP48 isolates carry more virulence genes then the ST383/KP50-52 clones. The main difference is correlated to the strain genetic background. All investigated isolates harboured *mrkABCDF, iucABCD* and *iutA* and, in addition, KP45-19 and KP48-19 carry yersiniabactin markers (*irp* and *ybt*) siderophores and *fyuA*, and only KP48-19 carries *kfuA*.

All of the strains carry the major virulence gene *rmpA*/*rmpA2* that encodes for capsule upregulation and regulates the hypermucoviscous phenotype. In KP48-19 and KP45-19, *rmpA* and *rmpA2* genes were located on a large virulence plasmid [KP45-pVir-IncFIB(Mar)/IncHIB, 340 204 bp, >99.9% homology with **NZ_CP040726.1**, 339 117 bp). In KP50-19 and KP52-19 the *rmpA* gene is located on the bla_{NDM-5}-carrying plasmid with many other virulence genes (Table 3).

Other virulence determinants, such as OmpK35 loss, OmpK36, AcrAB efflux pump, ABC iron transporter, putative allantoin permease and periplasmic iron-binding protein were identified (Table 3).

4. Discussion

Despite the presence of multiple conjugative plasmids carrying virulence and resistance genes, some mobile elements are large (>250 kb) and therefore these are supposed to be considered non-transferable [13]. This paradigm is slowly changing and there is multiple evidence of carbapenem-resistant strains that acquire transposons or plasmids of virulence once considered non-conjugative [6,14,15]. It is worrisome to see how the facilitated process of conjugation [14,16,17] can generate large plasmids in different STs and how this can lead, in an evolutionary process, to new plasmids that have had a wide diffusion internationally (from Syria [12], to England [5], to Italy) in just a few years.

These plasmids not only carry antimicrobial resistance genes but also virulence factors in strains not usually recognised as hypervirulent. Although ST15, ST147 and ST383 were considered non-hypervirulent types [11], these have been recognised as hypervirulent in the recent literature [7,16]. Failure to recognise the viru-

Table 3
Description of the genetic content of the carbapenemase-carrying plasmids for each isolate.

Isolate	ST	Plasmid	Length (bp)	AMR genes	Virulence genes	Reference sequence[a]	Differences[b]
KP45-19	ST147	KP45; IncL/M(pOXA-48)	63 675	*bla*$_{OXA-48}$	NA	**NZ_LR025091.1**, 63 589 bp	(Incomplete in N-terminus RelB antitoxin); 100% homology
		KP45; IncFIB(pQil)_NDM-1	54 064	*bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *qnrS1*	NA	**NZ_CP021947.1**, 54 064 bp	100% homology, 100% coverage
KP48-19	ST15	KP48; IncL/M(pOXA-48)	63 589	*bla*$_{OXA-48}$	NA	**NZ_LR025091.1**, 63 589 bp	100% homology, 100% coverage
		KP48; IncA/C2_NDM-1	138 371	*aac(6′)-Ib*, *bla*$_{CMY-6}$, *bla*$_{NDM-1}$, *rmtC1*	NA	**CP022126.1**, 140 133 bp	TraN, IS*6*-like transposase, other transposase ~2000 bp, it is an insertion element
KP50-19	ST383	KP50; IncL_OXA48	72 606	*bla*$_{OXA-48}$, *bla*$_{CTX-M-14}$, *aph(3′′)*, *aph(3′′)-Ib*, *aph(6)-Id*	NA	**NZ_CP034202**, 720 57 bp	Single IS*1*-like transposase insertion
		KP50; IncFIB(pNDM-Mar)/IncHI1B(pNDM-MAR)_NDM5	376 576	*aac(6′)-Ib*, *ant(3′′)-Ia*, *bla*$_{CTX-M-15}$, *bla*$_{NDM-5}$, *bla*$_{TEM-1}$, *sul1*	*iucA*, *iucB*, *iucC*, *iucD*, *iutA*, *qnrS*, *rmpA*, *rmpA2*, *terA*, *terB*, *terC*, *terD*, *terE*, *terW*, *terX*, *terY*, *terZ*	**NZ_CP034201.1**, 372 826 bp	>99.99% homology (it acquired a IS*6*–transposase–*sul1*–IS*6* island but the *sul2* island is lost; it acquired a group II intron reverse transcriptase/maturase)
KP52-19	ST383	KP52; IncL_OXA48	73 124	*bla*$_{OXA-48}$, *bla*$_{CTX-M-14}$, *aph(3′′)*, *aph(3′′)-Ib*, *aph(6)-Id*	NA	NZ_CP034202, 72 057 bp	99.98% homology (loss of IS*1*-like transposase, replication protein RepC)
		KP52; IncA/C2_NDM-1	138 371	*aac(6′)-Ib*, *bla*$_{CMY-6}$, *bla*$_{NDM-1}$, *rmtC1*	NA	**CP022126.1**, 140 133 bp	TraN, IS*6*-like transposase, other transposase ~2000 bp, it is an insertion element
		KP52; IncFIB(pNDM-Mar)/IncHI1B(pNDM-MAR)_NDM5	341 944	*aac(6′)-b*, *ant(3′′)-Ia* *aph(3′)-Ia*, *armA*, *bla*$_{CTX\_M-15}$, *bla*$_{NDM-5}$, *bla*$_{TEM-1}$	*iucA*, *iucB*, *iucC*, *iucD*, *iutA*, *qnrS*, *rmpA*, *rmpA2*, *terA*, *terB*, *terC*, *terD*, *terE*, *terW*, *terX*, *terY*, *terZ*	**NZ_CP034201.1**, 372 826 bp	>99.99% homology, missing two large transposable islands

ST, sequence type; AMR, antimicrobial resistance; NA, not applicable.
[a] The reference sequence column represents the closest match using MASH (https://ccb-microbe.cs.uni-saarland.de/plsdb/).
[b] Homology and coverage were determined using the BLAST+ algorithm (https://blast.ncbi.nlm.nih.gov/).

lence and resistance factors could lead to an underestimation of the dangerous circulation of these strains.

Regarding the identified plasmids, IncL/M OXA-48 have been recognised to be very stable, highly conserved, with a high conjugation rate and are associated with global dissemination/epidemic [13]. These plasmids have very efficient conjugation not only among high-risk *Klebsiella* clones but also between different species [13]. New Delhi metallo-β-lactamases are usually not linked to a specific plasmid as the NDM-carrying element (integron or transposon) is highly mobile and can be integrated in many plasmids of different Inc groups [18]. This translates to date to a non-highly epidemic plasmid clonality, as expected in new evolving clones. The high recombination rate of these plasmids in association with high mobility of the NDM genetic element poses a serious challenge in hospital settings, where selective pressure may lead to the spreading of an epidemic, more stable, hybrid resistance/virulence, plasmid–clone lineage.

Due to the fitness and genetic cost, usually only one mechanism of resistance to carbapenems is carried by a single clone. Despite this, it has already been described that multiple carbapenemases may emerge in *K. pneumoniae* strains [19]. Recent studies also demonstrated the co-existence of NDM and other resistance genes in different pathogens, e.g. NDM-1 and OXA-232 in *Acinetobacter* spp., NDM-7 and OXA-48 in *Escherichia coli* and *K. pneumoniae*, and NDM-1 and VIM-1 in *K. pneumoniae* [20]. It has been speculated that some strains may be more 'tolerant' to multiple plasmid carriage than others. This translates to 'middle host' emerging clones capable of causing outbreaks and transferring highly mobile elements to other, more successful, clones [18].

The co-existence of OXA-48 in association with NDM-1 and NDM-5, identified in KP52-19, on three different plasmids in a single strain has, to the best of our knowledge, not been previously described. This may be a 'missing link' to the rise and evolution of non-KPC carbapenemases in the era of the 'new' KPC selective inhibitors as first-line treatment for carbapenem-resistant *K. pneumoniae*. Short-read sequencing assembly was not resolutive in the identification of the two NDM genes, as the *bla*$_{NDM}$ island is identical in both plasmids. Erroneous assembly of short reads is still an issue for this technology [21]. Only using a hybrid approach with long-read sequencing were we able to obtain the full structure and carriage of the NDM-carrying plasmids.

The discovery of new high-risk clones harbouring multiple mobile elements is a growing problem that poses a great challenge. Surveillance of the spread of virulence/resistance plasmids in new emerging clonal types of *K. pneumoniae* is of absolute importance especially in highly epidemic regions where the main mechanism of carbapenem resistance is linked to KPC enzyme production.

Conflict of Interest

None declared.

Funding

None.

Ethical approval

Not required.

References

[1] de Man TJB, Lutgring JD, Lonsway DR, Anderson KF, Kiehlbauch JA, Chen L, et al. Genomic analysis of a pan-resistant isolate of *Klebsiella pneumoniae*, United States 2016. mBio 2018;9 e00440-18. doi:10.1128/MBIO.00440-18.
[2] 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. doi:10.1038/S41564-019-0492-8.
[3] Martin J, Phan HTT, Findlay J, Stoesser N, Pankhurst L, Navickaite I, et al. Covert dissemination of carbapenemase-producing *Klebsiella pneumoniae* (KPC) in a successfully controlled outbreak: long- and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission. J Antimicrob Chemother 2017;72:3025–34. doi:10.1093/jac/dkx264.
[4] Giani T, Antonelli A, Caltagirone M, Mauri C, Nicchi J, Arena F, et al. Evolving β-lactamase epidemiology in Enterobacteriaceae from Italian nationwide surveillance, October 2013: KPC-carbapenemase spreading among outpatients. Euro Surveill 2017;22:30583. doi:10.2807/1560-7917.ES.2017.22.31.30583.
[5] Turton J, Davies F, Turton J, Perry C, Payne Z, Pike R. Hybrid resistance and virulence plasmids in 'high-risk' clones of *Klebsiella pneumoniae*, including those carrying $bla_{\text{NDM-5}}$. Microorganisms 2019;7:326. doi:10.3390/MICROORGANISMS7090326.
[6] Starkova P, Lazareva I, Avdeeva A, Sulian O, Likholetova D, Ageevets V, et al. Emergence of hybrid resistance and virulence plasmids harboring New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* in Russia. Antibiotics (Basel) 2021;10:691. doi:10.3390/ANTIBIOTICS10060691.
[7] Xie M, Yang X, Xu Q, Ye L, Chen K, Zheng Z, et al. Clinical evolution of ST11 carbapenem resistant and hypervirulent *Klebsiella pneumoniae*. Commun Biol 2021;4:650. doi:10.1038/S42003-021-02148-4.
[8] Guo L, An J, Ma Y, Ye L, Luo Y, Tao C, et al. Nosocomial outbreak of OXA-48-producing *Klebsiella pneumoniae* in a Chinese hospital: clonal transmission of ST147 and ST383. PLoS One 2016;11:e0160754. doi:10.1371/journal.pone.0160754.
[9] European Centre for Disease Prevention and Control (ECDC) Regional outbreak of New Delhi metallo-β-lactamase producing carbapenem-resistant Enterobacteriaceae, Italy: ECDC; 2019. 2018–2019.
[10] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 11, 2021. http://www.eucast.org [accessed 4 October 2021].
[11] Turton JF, Payne Z, Coward A, Hopkins KL, Turton JA, Doumith M, et al. Virulence genes in isolates of *Klebsiella pneumoniae* from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and 'non-hypervirulent' types ST147, ST15 and ST383. J Med Microbiol 2018;67:118–28. doi:10.1099/jmm.0.000653.
[12] Angeletti S, Cella E, Lai A, Lo Presti A, Antonelli F, Conti A, et al. Whole-genome sequencing of *Klebsiella pneumoniae* MDR strain isolated in a Syrian refugee. Pathog Glob Health 2017;111:212–15. doi:10.1080/20477724.2017.1316918.
[13] Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother 2012;56:559–62. doi:10.1128/AAC.05289-11.
[14] Xu Y, Zhang J, Wang M, Liu M, Liu G, Qu H, et al. Mobilization of the nonconjugative virulence plasmid from hypervirulent *Klebsiella pneumoniae*. Genome Med 2021;13:119. doi:10.1186/S13073-021-00936-5.
[15] Yang X, Dong N, Chan EW-C, Zhang R, Chen S. Carbapenem resistance-encoding and virulence-encoding conjugative plasmids in *Klebsiella pneumoniae*. Trends Microbiol 2021;29:65–83. doi:10.1016/J.TIM.2020.04.012.
[16] Lam MMC, Wyres KL, Wick RR, Judd LM, Fostervold A, Holt KE, et al. Convergence of virulence and MDR in a single plasmid vector in MDR *Klebsiella pneumoniae* ST15. J Antimicrob Chemother 2019;74:1218–22. doi:10.1093/JAC/DKZ028.
[17] Lan P, Jiang Y, Zhou J, Yu Y. A global perspective on the convergence of hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*. J Glob Antimicrob Resist 2021;25:26–34. doi:10.1016/J.JGAR.2021.02.020.
[18] Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis 2017;17:153–63. doi:10.1016/S1473-3099(16)30257-2.
[19] Ben Nasr A, Decré D, Compain F, Genel N, Barguellil F, Arlet G. Emergence of NDM-1 in association with OXA-48 in *Klebsiella pneumoniae* from Tunisia. Antimicrob Agents Chemother 2013;57:4089–90. doi:10.1128/AAC.00536-13.
[20] Khan AU, Maryam L, Zarrilli R. Structure, genetics and worldwide spread of New Delhi metallo-β-lactamase (NDM): a threat to public health. BMC Microbiol 2017;17:101. doi:10.1186/S12866-017-1012-8.
[21] Yahav T, Privman E. A comparative analysis of methods for de novo assembly of hymenopteran genomes using either haploid or diploid samples. Sci Rep 2019;9:6480. doi:10.1038/s41598-019-42795-6.