PATHOGENS AND GLOBAL HEALTH, 2017
VOL. 111, NO. 4, 166–175
https://doi.org/10.1080/20477724.2017.1314069

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Genome sequencing and comparative analysis of an NDM-1-producing *Klebsiella pneumoniae* ST15 isolated from a refugee patient

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ABSTRACT

The escalating problem of antibiotic resistance, specifically cabarpenemase and extended-spectrum β-lacatamase (ESBL) producing *K. pneumoniae* strains, is directly correlated with increased patient morbidity and mortality and prolonged hospitalization and costs. In this study, a comprehensive genomic analysis encompassing the resistomics, virulence repertoire and mobile genetic elements of an NDM-1 positive ESBL-producing *K. pneumoniae* EA-MEH ST15 isolated from a urine sample collected from a Syrian refugee was conducted. Illumina paired-end libraries were prepared and sequenced resulting in 892,300 high-quality reads. The initial assembly produced 329 contigs with a combined 5,954,825 bp and a 56.5% G+C content. Resistome analysis revealed the presence of several β-lactamases including NDM-1, SHV-28, CTX-M-15 and OXA-1 in addition to 18 other genes encoding for resistance, among which are *aph(3')-Ia, aac(6')Ib-cr, armA, strB, strA* and *aadA2* genes. Additionally, five plasmids IncFIB(Mar), IncHI1B, IncFIB(pKPHS1), IncFIB(K) and IncFII(K) and four integrated phages were detected. *In silico* MLST analysis revealed that the isolate was of sequence type ST15. To our knowledge this is the first in-depth genomic analysis of a NDM-1 positive *K. pneumoniae* ST15 in Lebanon associated with the recent population migration. The potential dissemination of such MDR strains is an important public health concern.

KEYWORDS

ESBL; MDR; *Klebsiella pneumoniae*; draft-genome sequencing; ST15; NDM-1; SHV-28; Middle East

Introduction

Klebsiella pneumoniae are non-motile, encapsulated, lactose-fermenting, Gram-negative, facultative anaerobic rods of the *Enterobacteriacae* family [1]. They have been linked to both community-acquired and nosocomial infections worldwide, especially in patients with diabetes mellitus, alcoholism [2], immunocompromised patients [3], and in intensive care units [4]. The escalating burden of antibiotic resistance and specifically the dissemination of extended-spectrum β-lactamases (ESBLs) producing *K. pneumoniae* strains are directly correlated with increased patient morbidity and mortality [5], prolonged hospitalization time and costs [6]. ESBLs confer antimicrobial resistance against a wide range of β-lactams such as penicillins, aztreonam, and second and third generation cephalosporins [7].

Carbapenemases-producing *K. pneumoniae* is a major worldwide health concern [8,9]. Two particularly alarming carbapenemases are class B New Delhi Metallo-β lactamase-1 (NDM-1) [10] and class A *K. pneumoniae* carbapenemase (KPC) [11]. NDM-1 is capable of hydrolyzing all β-lactams, including carbapenems, with the exception of monobactams [12]. It is transmitted through mobile genetic elements and has been observed in several *Enterobacteriacae* species, in at least 40 countries particularly in poor resource settings [13]. KPC-producing *K. pneumoniae* are associated with mortality rates as high as 50%, due to limited treatment choices [14].

Some *K. pneumoniae* sequence types (STs) such as ST11, 15, 101, 147, 336 and ST258 have been implicated in worldwide epidemics/endemics. Particularly, ST258 is linked to the global dissemination of *K. pneumoniae* KPCs [14,15].

With the availability of six complete *K. pneumoniae* genomes [16–20] and several ongoing sequencing projects [21], the whole-genome sequencing (WGS) of *K. pneumoniae* has given insights into its pathogenicity, resistomics and genome plasticity [16]. Yet, little evidence exists on the complex relationship between ESBL production and pathogenesis in *K. pneumoniae* [22].

In this study we report the first comprehensive comparative genome analysis of an NDM-1-producing *K. pneumoniae* ST15 isolated from a urine sample collected from a Syrian refugee in Lebanon.

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Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/20477724.2017.1314069.

Materials and methods

Ethical approval

Ethical approval was not required as the clinical isolate was collected and stored as part of routine clinical care. Clinical isolate and patient record/information were anonymized and de-identified prior to analysis.

Sample collection

The isolate was recovered from the urine sample of an 84-year old Syrian male patient suffering from pneumonia admitted to the Middle East Institute of Health (MEIH) in Lebanon in October 2015. The patient arrived in Lebanon in 2014 when he was hospitalized for having hypertension, atrial fibrillation, moderate chronic pericardial effusion, chronic obstructive pulmonary disease and morbid obesity. He has previously undergone craniotomy and was treated for inguinal hernia.

Antimicrobial testing

Antimicrobial susceptibility test by the disk diffusion method was performed to determine the resistance patterns of the isolate to 27 different antibiotics: Penicillins (Amoxicillin, ticarcillin, amoxicillin + clavulanic acid and piperacillin + tazobactam), cephalosporins (Cefalotin, cefoxitin, cefuroxime, cefixime, cefotaxime, ceftriaxone, ceftazidime and cefepime), monobactams (Aztreonam), carbapenems (Imipenem, meropenem and ertapenem), aminoglycosides (Amikacin, gentamycin and tobramycin), quinolones (Norfloxacin, ciprofloxacin, levofloxacin and ofloxacin), tetracyclines (Tetracycline and tigecycline), sulfamides (sulfamethoxazole + trimethoprim) and nitrofurans (Nitrofurantoïn) (Oxoid, England). All antimicrobial testing was performed on Mueller-Hinton agar by the flooding technique and data interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [23].

DNA isolation and sequencing

DNA extraction was performed using the Nucleospin DNA extraction Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

Genome sequencing

Genomic DNA (gDNA) was used as input for library preparation using the Illumina Nextera XT DNA library preparation kit (Illumina). 1 µg of sample DNA was used as input for library preparation. The gDNA was subjected to end-repair, A-tailing, ligation of adaptors including sample-specific barcodes as recommended in the manufacturer's protocol. The resulting library was quantified using Qubit. The library was sequenced on an Illumina MiSeq with paired-end 500 cycles protocol to read a length of 250 bp.

Genome assembly and annotation

Genome assembly was performed *de novo* using Spades Genome Assembler Version 3.6.0 [24]. Quality control checks of raw sequence data was performed using FastQC version 1.0.0 [25]. For comparison purposes, the assembled genome was annotated using two different tools: the RAST server (http://rast.nmpdr.org) [26] using the Glimmer default option for open reading frame (ORF) calling and the Prokka Genome Annotation software v1.0.0 [27]. The number of tRNAs and rRNAs were identified using ARAGORN v1.2.36 [28], and the RNAmmer Prediction Server 1.2 [29], respectively. The classification of predicted genes was performed using the Clusters of Orthologous Groups of proteins database [30]. The obtained ORFs were subjected to BLAST analysis against the Antibiotic Resistance Database [31] and the Comprehensive Antibiotic Resistance Database [32]. Resfinder was also used to identify resistant determinants, using a 98% identity threshold (ID) [33]. Virulence factors (VFs) were identified through BLAST search against VFDB [34]. The multilocus sequence type (MLST) was determined using the MLST 1.8 server [35] available on www.genomicepidemiology.org. The MLST profiles were determined based on PubMLST.org. The clonal group cgMLST was determined using the publically available cgMLST database [36]. The presence of plasmids was determined using PlasmidFinder 1.3 [37]. Plasmid contigs were identified using BLASTn against the nucleotide sequence database. PHAST was used to determine putative phage sequences in the genome [38]. The function linked to annotated genes was inferred by UniProtKB [39] and QuickGO [40]. IslandViewer 3 was used to identify putative genomic islands (GIs) using three independent methods for island prediction: IslandPick, IslandPath-DIMOB and SIGI-HMM [41]. Insertion sequences (ISs) were identified using IS-finder [42].

Comparative genome analysis

For comparative analysis, the following genomes were included: *K. pneumoniae* EA-MEH, *K. pneumoniae* Ecl8 (accession # HF536482.1), a streptomycin-resistant reference strain for targeted gene manipulations [43], LAU-KP1 (accession # AYQE00000000.1) producing OXA-1 and SHV-11 isolated previously from a patient in Lebanon [44], *K. pneumoniae* KGM-IMP216, positive for OXA-1, NDM-1, SHV-28 among other resistance genes and isolated from a urine sample collected from a patient in Lebanon (accession # LJOI01000001.1) [45], and six of the closest matches to *K. pneumoniae* EA-MEH obtained through BLASTn: *K. pneumoniae* NTUH-K2044 (accession # AP006725.1), *K. pneumoniae* strain XH209 (accession # CP009461.1), *K. pneumoniae* strain U25 (accession # CP012043.1), *K. pneumoniae* strain NUHL24835 (accession # CP014004.1), *K. pneumoniae* strain BR (CP015990.1) and *K. pneumoniae* strain KP36 (accession # CP017385.1).

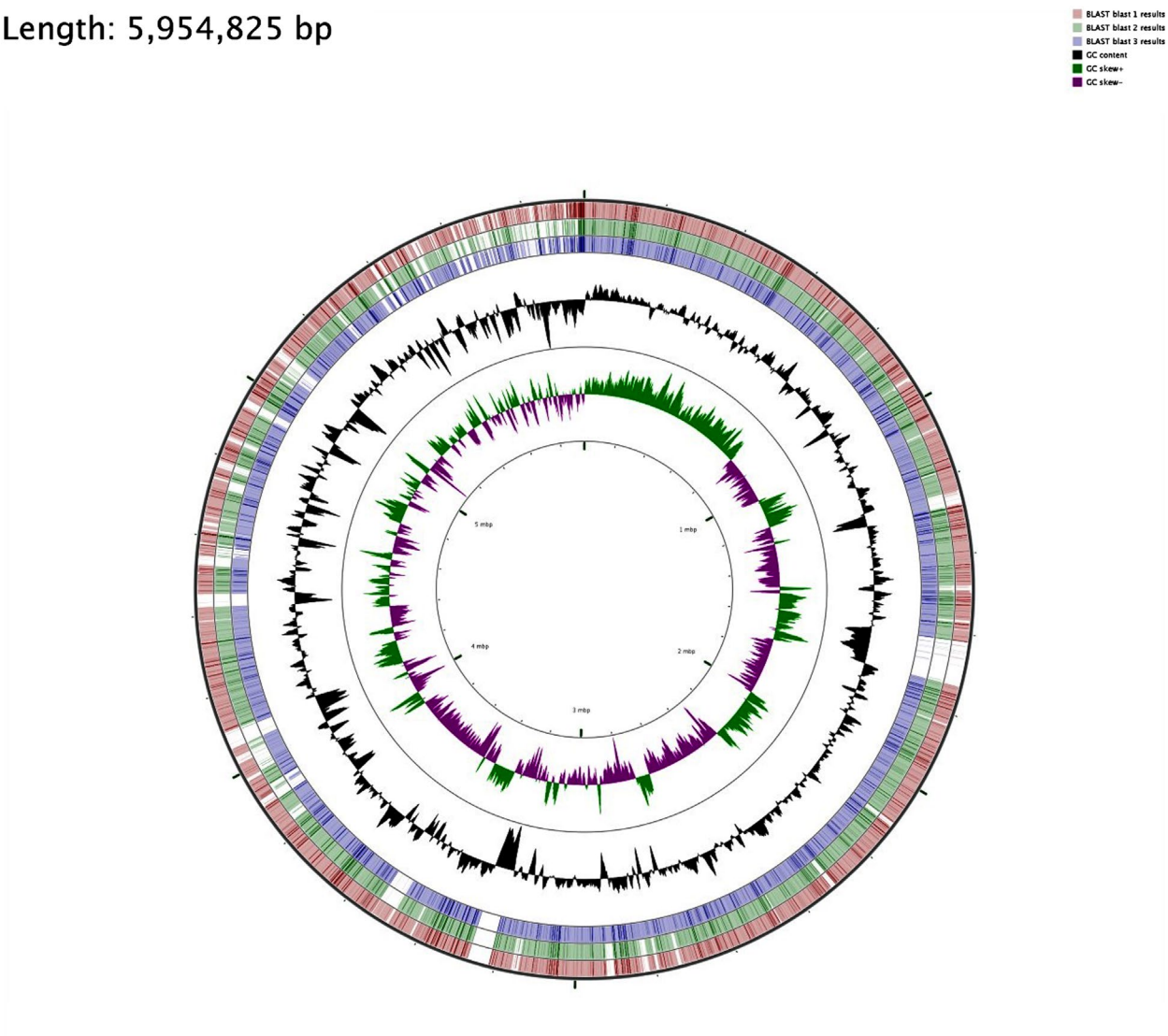

Figure 1. Circular genome representation of *K. pneumoniae* EA-MEH compared with *K. pneumoniae* Ecl8 (HF536482.1) [43], PMK-1 (CP008929), [46] and LAU-KP1 (AYQE00000000.1) (Tokajian et al., 2015). Starting from the outermost ring the feature rings depict: *K. pneumoniae* Ecl8 Blast results (red), PMK-1 Blast results (green) and LAU-KP1 Blast results (blue) representing the positions covered by the BLASTN alignment, G+C content (black), G+C positive skew (green) and G+C negative skew (purple). Image created using CGview Server V 1.0 [49].

K. pneumoniae PMK-1 (accession # CP008929), a ST15 NDM-1 producing isolate linked to a nosocomial outbreak in Nepal in 2012, was used as a reference for genome comparisons [46].

K. pneumoniae EA-MEH contigs were reordered in ProgressiveMauve [47] using reference strain *K. pneumoniae* PMK-1 (accession # CP008929) [46]. Phylogenetic analysis based on 37 marker genes was generated using Phylosift [48].

For the visualization of sequence feature information based on the sequence analysis results, circular genome representations of *K. pneumoniae* EA-MEH was obtained using CGview Server V 1.0 [49] through comparison with *K. pneumoniae* Ecl8, LAU-KP1 and PMK-1 (Figures 1 and 2).

Capsule typing

The capsular type *wzi* was determined by a PCR assay followed by sequencing using the primers and PCR conditions described in Table 1. Briefly, the obtained PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific, USA). The amplicons were sequenced using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, USA). PCR products were sequenced on the Genetic Analyzer 3500 (Life Technologies, USA) using the BigDye XTerminator purification kit (Applied Biosystems, USA). The capsular genotype was determined by uploading the *wzi* gene sequence to the Institut Pasteur *K. pneumoniae* database (http://bigsdb.web.pasteur.fr/).

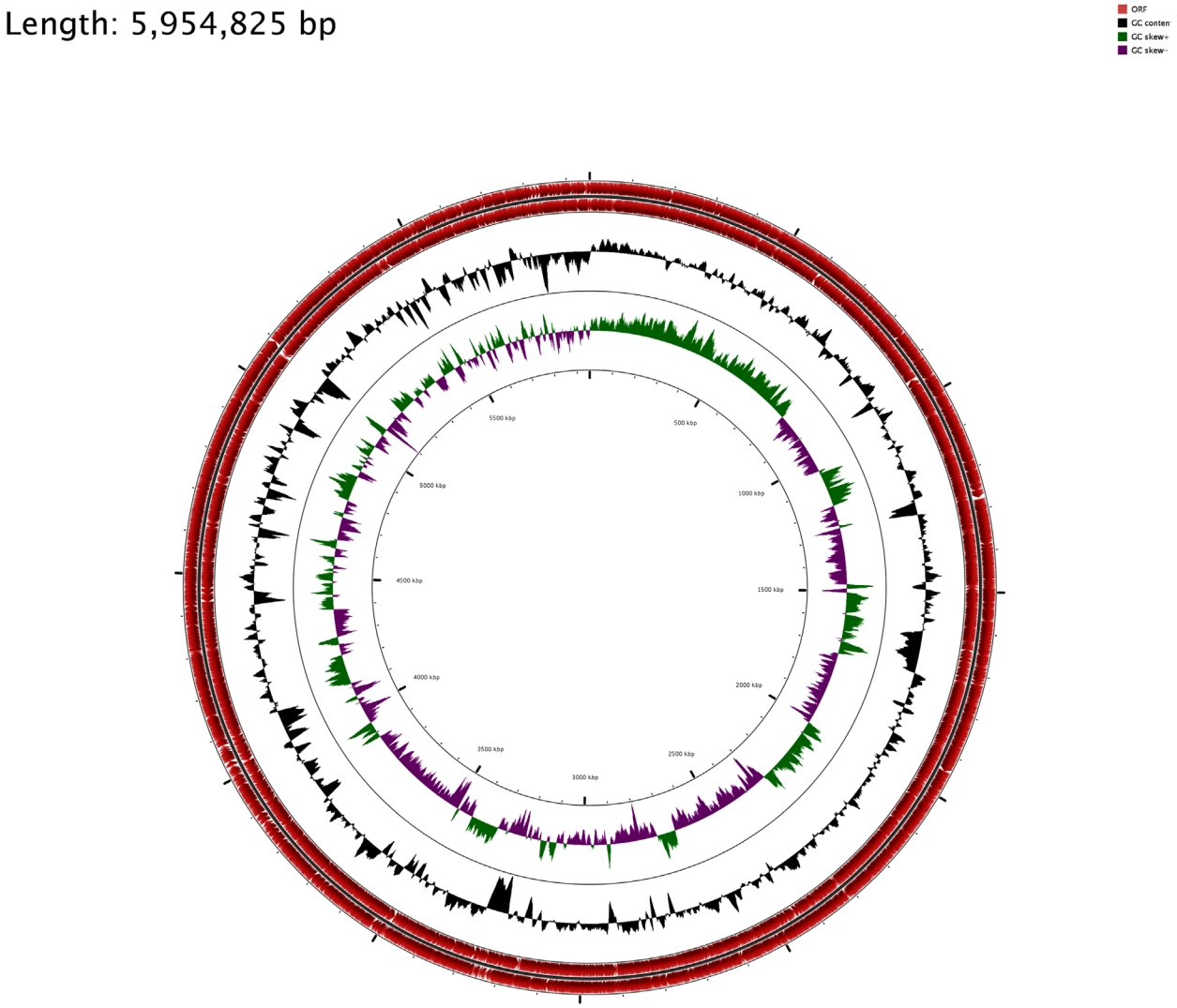

Figure 2. *K. pneumoniae* EA-MEH circular genome. Starting from the outermost rings the tracks represent: Open reading frames (ORF) (red), G+C skew (black), G+C plot (green positive skew and purple negative skew). The image was generated using CGview Server V 1.0 [49].

Table 1. Primers sequences and PCR conditions used for the amplification of bla_{SHV}, bla_{NDM} and wzi genes.

Gene	Primers	Sequence 5′-3′	Ta °C	Product size (bp)	References
wzi	*wzi*-F	GTG CCG CGA GCG CTT TCT ATC TTG GTA TTC C	55	587 bp	[50]
	wzi-R	GAG AGC CAC TGG TTC CAG AA[C or T] TT[C or G] ACC GC			
NDM	NDM-1-F	GAC CGC CCA GAT CCT CAA	55	700 bp	[51]
	NDM-1-R	CGC GAC CGG CAG GTT			
SHV	SHV-F	GCC CGG GTT ATT CTT ATT TGT CGC	62	792 bp	[52]
	SHV-R	TCT TTC CGA TGC CGC CGC CAG TCA			

Resistance genes typing

The presence of bla_{SHV} and bla_{NDM} genes was confirmed by individual PCR assays followed by sequencing, as previously described (Table 1). Sequences were identified using BLASTn.

Nucleotide sequence accession number

This draft genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number: LSBZ00000000.1.

Results

Genomic features

The draft genome sequence of *K. pneumoniae* EA-MEH was 5,954,825 bp in size with a G+C content of 56.5% in 329 contigs. Annotation of this assembly using RAST identified 5615 coding sequences (CDS), 84 tRNAs and 15 rRNAs. Prokka identified 85 tRNAs, 12 rRNAs, three CRISPRs and 5530 CDS. Genes linked to carbohydrates (873), amino acid derivatives (558), cofactors, vitamins, prosthetic groups and pigments (377) were the most

abundant among the SEED subsystem categories. Comparison of *K. pneumoniae* EA-MEH to the reference strain PMK-1 based on sequence similarity (percent identity ≥ 80) showed that 546 genes are unique for EA-MEH and that most of the functional genes including metal transport are also conserved in the reference strain.

Isolates typing

MLST

In silico MLST analysis based on seven house-keeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*) classified the *K. pneumoniae* EA-MEH as sequence type ST15 belonging to CG15. *In silico* pMLST based on FII, FIA and FIB loci typed the isolate as [FII+:A–:B–].

Capsular genotyping

The capsular genotype of the clone was determined to be *wzi*93 associated with serotype K60.

Resistance profile

Antimicrobial susceptibility testing revealed that *K. pneumoniae* EA-MEH was resistant to all tested antibiotics and had intermediate resistant for two carbapenems: imipenem and meropenem. The minimal inhibitory concentration (MIC) was also determined for the following major antimicrobial agents: 16 mg/l for amoxicillin, 16 mg/l for amoxicillin-clavulanic acid, 32 mg/l for tazobactam, 8 mg/l for imipenem, 6 mg/l for cefotaxime, 16 mg/l for amikacin, 2 mg/l for ciprofloxacin, and 0.125 mg/l for colistin (S). The ResFinder confirmed the observed resistance patterns and identified the presence of six distinct genes conferring resistance to aminoglycosides (*aph(3')-Ia, aac(6') Ib-cr, armA, strB*, strA and *aadA2*), a number of β-lactamases (bla_{SHV-28}, $bla_{CTX-M-15}$, bla_{NDM-1} and bla_{OXA-1}) and genes encoding for fluoroquinolone, macrolide, lincosamide and streptogramin (MLS), phenicol, sulphonamide and trimethoprim resistance. The isolate was susceptible to colistin, fosfomycin, fusidic acid, nitroimidazole, oxazolidinone, rifampicin and glycopeptide (Table 2). Despite the presence of the tetracycline resistance gene *tet(D),* antimicrobial susceptibility testing showed that *K. pneumoniae* EA-MEH was sensitive to tetracycline and tigecycline. The Resistance Gene Identifier based on BlastP Hist in the Comprehensive Antibiotic Resistance Database further elucidated the presence of various CTX-M types, OXA types and NDM types and the streptomycin resistance protein B encoded by *aph(6')-Id* (Table 2).

Mobile genetic elements and phage-associated regions

Five plasmids of the IncF family were identified: IncFIB(Mar), IncHI1B, IncFIB(pKPHS1), IncFIB(K) and IncFII(K) (Table 2).

PHAST identified four phage-associated regions in *K. pneumoniae* EA-MEH genome: *Vibrio* phage henriette 12B8 (NC_021073), *Sphingomonas* phage PAU (NC_019521), *Bacillus* phage G (NC_023719) and *Salisaeta* icosahedral phage 1 (NC_017983) (Table 2), with many phage-related proteins unique to EA-MEH compared to the reference; Only two phage-associated regions were identified in PMK1 (Entero P1 (NC_005856); Entero P88 (NC_026014)).

A total of 38 GIs were also detected ranging in size from 4,095 bp to 186,289 bp (Average: 19,274 ± 31,298 bp) with at least three, seven and nine islands conferring resistance to mercury, antimicrobial agents and heavy metals, respectively. Two GIs were related to defective phages, one to cell division and one to sugar transport and metabolism. GIs encoded ABC-type multidrug transport system, bleomycin resistance protein (*ble*), streptomycin resistance, aminoglycosides resistance and β-lactamases (Table S1).

A total of 105 ISs were identified in *K. pneumoniae* EA-MEH using IS-finder with 120 open-reading frames (ORFs) related to ISs. Some of the detected ISs included: IS*1* (IS*1B*, IS*1D*, IS*1G*, IS*1R*, IS*1SD*, IS*1X2*, IS*1X3*, IS*1X4*), IS*3* (IS*Ec36*, IS*Kpn8*, IS*Sen4*, IS*Yen3*), IS*Kpn21*, IS*Kpn25*, IS*6* and others. Tn*3* was detected downstream of IS*Aba125*, which in turn was downstream of bla_{NDM-1}. Another Tn*3* resided further upstream of bla_{NDM-1}. The downstream Tn*3* was not detected in PMK-1. This order was interrupted in other isolates as well (Figure 3).

Virulence factors

A BLAST search revealed 173 VFs in *K. pneumoniae* EA-MEH, classified into 18 different categories. Among the identified VFs, two were related to capsular polysaccharide (*wcbF; siaD/synD*) (Table S2).

Phylogenetic tree

Phylogenetic analysis indicated a close association between *K. pneumoniae* EA-MEH and PMK-1 (Figure 4). LAU-KP1 clustered with XH209, Ecl8 and TUH-K2044 forming a single clade. KGM-IMP216 clustered more closely to U25 and UHL24835. *K. pneumoniae* strain BR and KP36 formed two branches distinct from the other isolates.

Discussion

To the best of our knowledge, this is the first in-depth comparative genomic analysis of ST15 NDM-1 and ESBL-producing *K. pneumoniae* in Lebanon. ST15 is part of the epidemic CG15 within the MLST clonal complex 14 (CC14) [53]. ST15 has been previously implicated in numerous hospital outbreaks worldwide [13,46]. Its success is partly attributed to the accumulation of various

Table 2. Resistance genes, plasmids and phages identified in K. pneumoniae EA-MEH.

Predicted phenotype	Resistance gene	Position in contig
Aminoglycoside resistance	*aph(3')-Ia*	143..956
	aac(6')Ib-cr	1878..2477
	armA	3442..4215
	strB	3710..4546
	strA	4546..5349
	aadA2	634..1425
Beta-lactam resistance	bla_{SHV-28}	12372..13232
	$bla_{CTX-M-15}$	176..1051
	bla_{NDM-1}	4496..5308
	bla_{OXA-1}	917..1747
Quinolone resistance	*QnrB66*	13226..13870
	aac(6')Ib-cr	1878..2477
	oqxB	77466..79915
	oqxA	79939..81114
MLS - Macrolide, Lincosamide and Streptogramin B resistance	*msr(E)*	6514..7989
	mph(E)	8045..8929
Phenicol resistance	*catB3*	338..779
	catA1	750..1409
Sulphonamide resistance	*sul1*	25..861
Tetracycline resistance	*tet(D)*	1979..3163
Trimethoprim resistance	*dfrA12*	1833..2330
	dfrA14	5978..6460
Others	Elongation factor G	2687…4789
	Fluoroquinolone resistant (*gyrA)* (Ser83 → Phe & Asp87 → Ala)	46525…44111
	Fosfomycin resistance protein *(FosA)*	15585…16004
Plasmids	*IncFIB(Mar)*	1579..2017
	IncHI1B	2932..3501
	IncFIB(pKPHS1)	32186..32745
	IncFIB(K)	4153..4712
	IncFII(K)	8938..9085
Phages	*Vibrio* phage henriette 12B8 (NC_021073)	111576-120734
	Sphingomonas phage PAU (NC_019521)	57324-63593
	Bacillus phage G (NC_023719)	94476-106610
	Salisaeta icosahedral phage 1 (NC_017983)	73831-84962

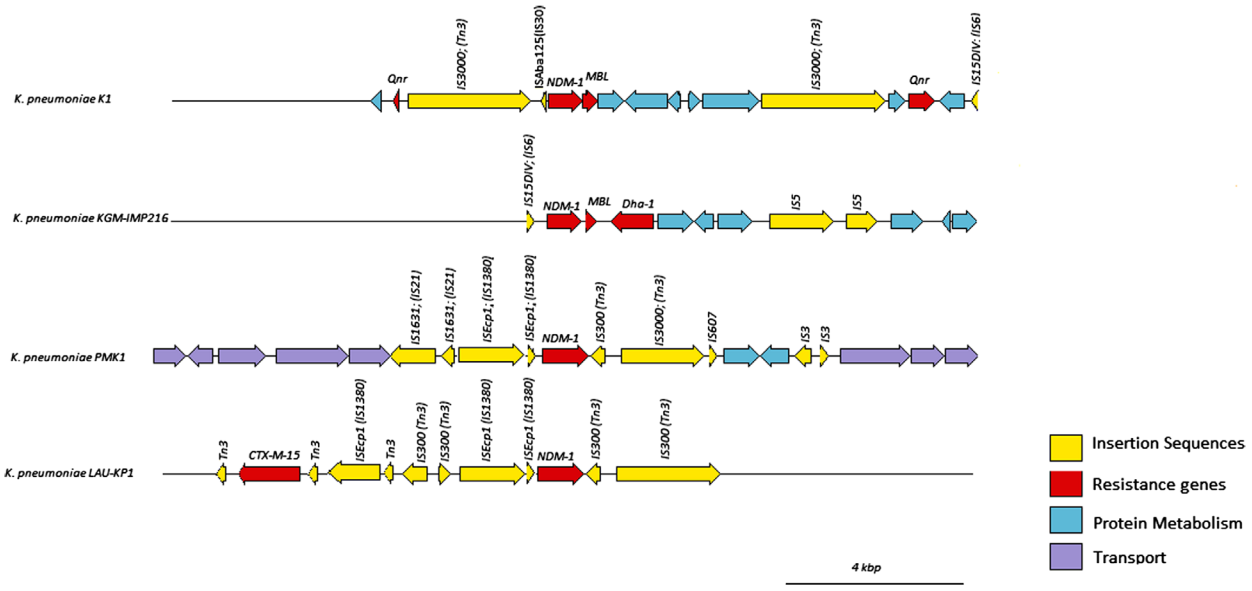

Figure 3. Structure of the bla_{NDM-1} locus in *K. pneumoniae* EA-MEH compared to PMK1, KGM-IMP216 and LAU-KP1 reference strains as inferred from RAST. Transposases were identified and labeled using the IS Finder database.

resistance genes with no fitness cost, including several β-lactamases [54]. Additionally, *K. pneumoniae* harbors all three drug resistance mechanisms found in Gram-negative bacteria [55], which are the acquisition of antibiotic catalytic genes, mutations in antibiotic targets and membrane proteins and the differential expression of efflux systems and pumps [56], and all were detected in *K. pneumoniae* EA-MEH.

K. pneumoniae EA-MEH was found to harbor several plasmid-borne resistance genes including four conferring β-lactam resistance (bla_{SHV-28}, $bla_{CTX-M-15}$, bla_{NDM-1}, bla_{OXA-1}) that were previously identified in *Enterobacteriacae* disseminating in Europe [57,58]. Additionally, 18 other enzymes conferring resistance to aminoglycosides, quinolone, MLS, phenicol, sulfonamide, tetracycline and trimethoprim were detected.

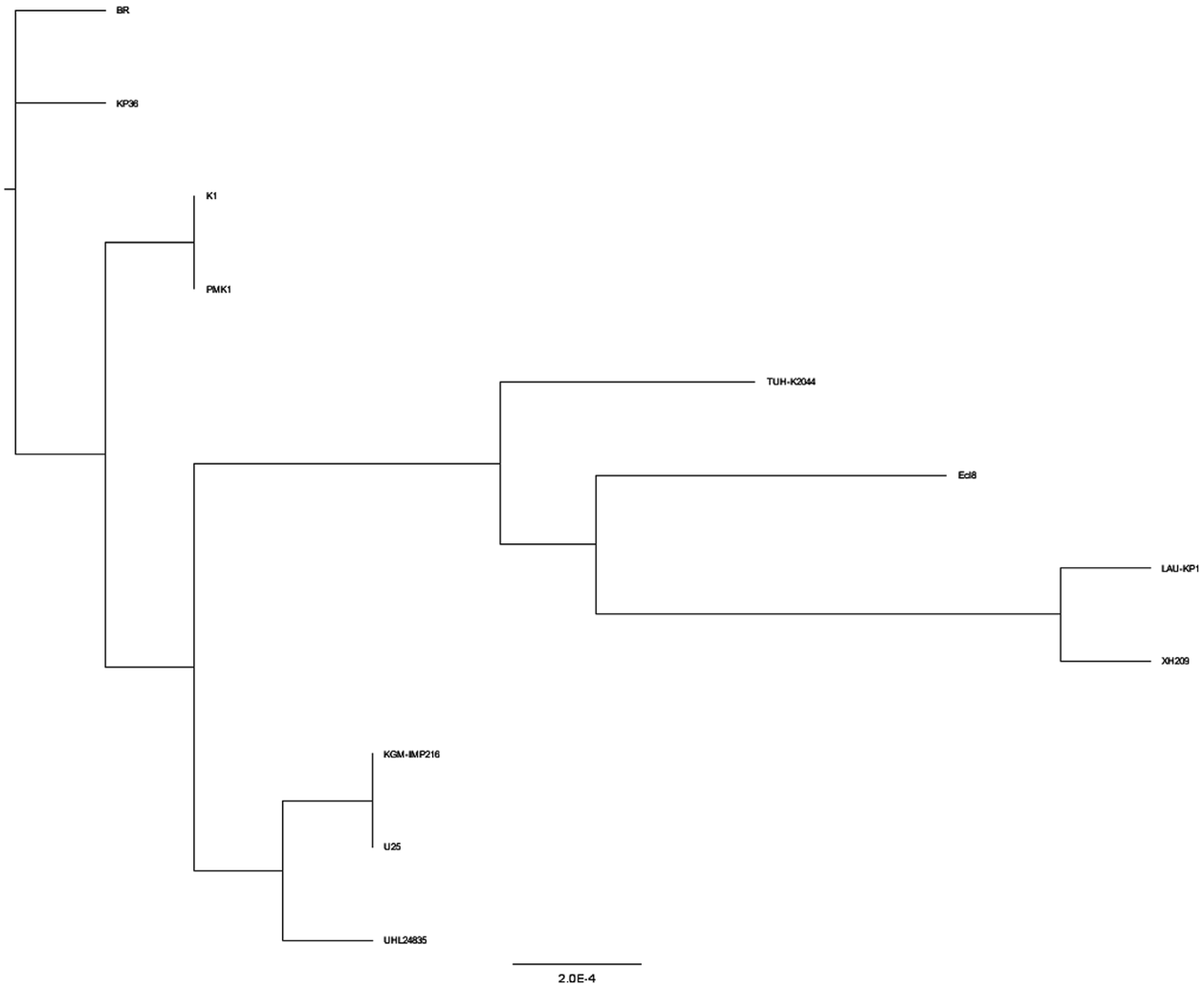

Figure 4. Phylogenetic tree of *K. pneumoniae* EA-MEH compared to the reference strains.

K. pneumoniae is intrinsically resistant to ampicillin with the bla_{SHV} being part of its core genome [59]. Despite the presence of the tetracycline resistance gene *tet(D),* antimicrobial susceptibility testing showed that the isolate was sensitive to tetracycline and tigecycline. Previous studies showed no correlation between the presence of tetracycline resistance determinants *tet*(A) or *tet*(E) and the MIC of tigecycline in *Enterobacteriaceae* [60]. The transcription repressor of the multidrug efflux pump *acrAB* operon, *acrR*, and which was found in EA-MEH, could be accounted for this genotype/phenotype discrepancy [61]. Although nitroimidazole, oxazolidinone and glycopeptides have poor activity against Gram-negative bacteria [62], these antimicrobials are not commonly used in Lebanon. Susceptibility to glycopeptides was also previously described *Escherichia coli* isolated from Lebanon [63].

bla_{NDM-1} is frequently coupled with several antibiotic resistance genes located on the same conjugative plasmids [13], narrowing and sometimes excluding all therapeutic options [14]. Concomitant detection of $bla_{CTX-M-15}$ and bla_{SHV} in MDR *K. pneumoniae* was linked to nosocomial outbreaks involving ST15, ST147 and ST336 [15]. *K. pneumoniae* EA-MEH also carried *oqxA* and *oqxB* genes as part of its core genome, encoding for quinolone/fluoroquinone resistance and *armA* encoding for aminoglycoside resistance [59]. All are among the major resistance determinants in MDR *K. pneumoniae* causing severe infections in hospitals and communities worldwide [13].

Plasmids of the IncF family are known to acquire novel virulence traits through replicon diversification and horizontal gene transfer [64], which contributes to the adaptability of *K. pneumoniae* to different health care setting [65]. IncFIB(K) plasmid was detected with high homology to plasmid pKPN3, conferring copper and silver resistance [66], additionally IncFIB(K) and IncFII(K) were previously linked with the global dissemination of CTX-M-15 [64].

The numerous Tn*3* ISs detected upstream and/or downstream of bla_{NDM-1} in *K. pneumoniae* EA-MEH, LAU-KP1 and PMK1 mediate genomic plasticity and the acquisition of resistance genes through homologous recombination [67]. bla_{NDM-1} detected in this study has a chimeric structure forming one bla_{NDM}-ble_{MBL} operon with the bleomycin resistance gene ble_{MBL} [68]. The bla_{NDM}-ble_{MBL} operon along with few neighboring genes,

were preassembled in *Acinetobacter spp*. before transmission to *Enterobacteriaceae*. The bla_{NDM}-ble_{MBL} operon was previously detected on transposon Tn*125* having two ISs (IS*Aba125*) [65]. The absence of the upstream IS*Aba125* in *K. pneumoniae* EA-MEH could suggest deletion linked to Tn*3* insertion, which is common in *Enterobacteriaceae* and is in contrast to *A. baumannii* [69].

Prophages contribute to genome plasticity and favor the transfer of antimicrobial resistance genes and VFs [70]. They were additionally used as potential therapeutic agents and as markers for diagnosis and epidemiological typing in *K. pneumoniae* [71]. Although *K. pneumoniae* PMK1 was the closest to EA-MEH on BLASTn showing the highest sequence similarity (Figures 1 and 4), their prophage DNA profile did not match.

Among the 173 VFs identified in *K. pneumoniae* EA-MEH, 40 genes were related to metal uptake. Siderophores and iron-metabolism are critical for virulence in *K. pneumoniae* [72], playing an important role in modulating the immune response and protecting against reactive oxygen species [73]. Moreover, the capsule is an essential virulence determinant in *K. pneumoniae*. The polysaccharide capsule helps the pathogen to evade phagocytosis [74] and complement-mediated killing [75], and to suppress β-defensin expression [76]. Different capsular serotypes were linked to biofilm formation in carbapenem-resistant *K. pneumoniae* [72]. The capsular genotype of *K. pneumoniae* EA-MEH was *wzi*93 related to serotype K60, and was recently connected to the transmission of a CTX-M-15-producing ST15*K. pneumoniae* between patients treated in a single center in the Netherlands followed by inter-institutional spread [58]. The role of the capsular serotypes in the pathogenesis of *K. pneumoniae* requires further attention and could be considered as a novel therapy target [58].

Phylogenetic analysis revealed the clustering of related STs into three major clades. *K. pneumoniae* EA-MEH ST15 formed a separate clade with PMK1 ST15. *K. pneumoniae* LAU-KP1 ST336 clustered with XH209 ST17, an MDR strain isolated from the blood of a patient in China [57]. The latter two STs are closely associated, since ST336 is a single-locus variant (SLV) of ST17 with both belonging to the same clonal group (CG) 17 [77]. Similarly, *K. pneumoniae* KGM-IMP216 ST14 clustered with U25 ST14, an MDR strain isolated from a tertiary care hospital in India [77], and with NUHL24835, NDM-5-producing *K. pneumoniae* ST14 strain from China [78]. The observed phylogenetic heterogeneity among the three isolates recovered from Lebanon (*K. pneumoniae* EA-MEH ST15, LAU-KP1 ST336 and KGM-IMP216 ST14) underlined the diversity in their molecular features.

The identification of NDM-1 producing ST15 *K. pneumoniae* in a hospital setting in Lebanon is alarming and poses a public health concern. It is compulsory to expand our current understanding of the genome plasticity in *K. pneumoniae* spp. through in-depth functional genomic analysis. This requires the effective cooperation between clinicians and public health practitioners in the region for adequate monitoring coupled with the implementation of effective control measure. Understanding the dynamics of transmission and shifting disease control away from policies focused on geopolitical borders would help in better understanding and controlling communicable diseases especially the ones associated with population displacement.

Acknowledgements

We thank the staff of the Middle East Institute of Health (MIEH) for their cooperation in sample collection.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Fang CT, Lai SY, Yi WC, et al. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis. 2007;45:284–293.
[2] Tsay RW, Siu LK, Fung CP, et al. Characteristics of bacteremia between community-acquired and nosocomial *Klebsiella pneumoniae* infection: risk factor for mortality and the impact of capsular serotypes as a herald for community-acquired infection. Arch Intern Med. 2002;162:1021–1027.
[3] Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci Trans Med. 2012;4:148ra116.
[4] Lin MY, Lyles-Banks RD, Lolans K, et al. The importance of long-term acute care hospitals in the regional epidemiology of *Klebsiella pneumoniae* carbapenemase–producing *Enterobacteriaceae*. Clin Infect Dis. 2013;14:cit500.
[5] Gupta N, Limbago BM, Patel JB, et al. Carbapenem-resistant enterobacteriaceae: epidemiology and prevention. Clin Infect Dis. 2011;53:60–67.
[6] Giske CG, Cornaglia G. Supranational surveillance of antimicrobial resistance: the legacy of the last decade and proposals for the future. Drug Resis Update. 2010;13:93–98.
[7] Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933–951.
[8] Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9:228–236.
[9] Richter SN, Frasson I, Franchin E, et al. KPC-mediated resistance in *Klebsiella pneumoniae* in two hospitals in Padua, Italy, June 2009-December 2011: massive spreading of a KPC-3-encoding plasmid and involvement of non-intensive care units. Gut Pathog. 2012;4:1. DOI:10.1186/1757-4749-4-7.
[10] Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–5054.

[11] Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2009;45:1151–1161.
[12] Li H, Zhang J, Liu Y, et al. Molecular characteristics of carbapenemase-producing *Enterobacteriaceae* in China from 2008 to 2011: predominance of KPC-2 enzyme. Diagn Microbiol Infect Dis. 2014;78:63–65.
[13] Chung PY. The emerging problems of *Klebsiella pneumoniae* infections: carbapenem resistance and biofilm formation. FEMS Microbiol Lett. 2016;363:fnw219. DOI:10.1093/femsle/fnw219
[14] Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis. 2013;13:785–796.
[15] Rodrigues C, Machado E, Ramos H, et al. Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFII K). Int J Med Microbiol. 2014;304:1100–1108.
[16] Wu KM, Li LH, Yan JJ, et al. Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. J Bacteriol. 2009;191:4492–4501.
[17] Ogawa W, Li DW, Yu P, et al. Multidrug resistance in *Klebsiella pneumoniae* MGH78578 and cloning of genes responsible for the resistance. Biol Pharm Bull. 2005;28:1505–1508.
[18] Liu P, Li P, Jiang X, et al. Complete genome sequence of *Klebsiella pneumoniae* subsp. pneumoniae HS11286, a multidrug-resistant strain isolated from human sputum. J Bacteriol. 2012;194:1841–1842.
[19] Shin SH, Kim S, Kim JY, et al. Complete genome sequence of the 2, 3-butanediol-producing *Klebsiella pneumoniae* strain KCTC 2242. J Bacteriol. 2012;194:2736–2737.
[20] Lin AC, Liao TL, Lin YC, et al. Complete genome sequence of *Klebsiella pneumoniae* 1084, a hypermucoviscosity-negative K1 clinical strain. J Bacteriol. 2012;194:6316.
[21] Ramos PI, Picão RC, de Almeida LG, et al. Comparative analysis of the complete genome of KPC-2-producing *Klebsiella pneumoniae* Kp13 reveals remarkable genome plasticity and a wide repertoire of virulence and resistance mechanisms. BMC Genomics. 2014;15:1. DOI:10.1186/1471-2164-15-54
[22] Carasso E, Salmon-Divon M, Carmeli Y, et al. Draft Genome sequences of two multidrug-resistant extended-Spectrum-β-Lactamase-producing Klebsiella pneumoniae strains causing bloodstream infections. Genome A. 2016;4:e01533–15.
[23] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 24th informational supplement. CLSI document M100-S24. Wayne (PA): CLSI; 2014.
[24] Bankevich A, Nurk S, Antipov D, et al. Spades: a new genome assembly algorithm and its applications to single-cell sequencing. J Comp Biol. 2012;19:455–477.
[25] Andrews S. FastQC: a quality control tool for high throughput sequence data. 2010. http://www.bioinformatics.babraham.ac.uk
[26] Aziz RK, Devoid S, Disz T, et al. SEED servers: high-performance access to the SEED genomes, annotations, and metabolic models. PLOS One. 2012;7:e48053.
[27] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068–2069.
[28] Laslett D, Canback B. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 2004;32:11–16.
[29] Lagesen K, Hallin P, Rødland EA, et al. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007;35:3100–3108.
[30] Tatusov RL, Fedorova ND, Jackson JD, et al. The COG database: an updated version includes eukaryotes. BMC Bioinformatics. 2003;4:1. DOI:10.1186/1471-2105-4-41
[31] Liu B, Pop M. ARDB – antibiotic resistance genes database. Nucleic Acids Res. 2009;37:D443–D447.
[32] Jia B, Raphenya AR, Alcock B, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 2017;45:D566–D573.
[33] Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640–2644.
[34] Chen L, Zheng D, Liu B, et al. VFDB 2016: hierarchical and refined dataset for big data analysis – 10 years on. Nucleic Acids Res. 2016;44:D694–7.
[35] Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total genome sequenced bacteria. J Clin Microbiol. 2012;50:1355–1361.
[36] Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics. 2010;11:1.
[37] Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–3903.
[38] Zhou Y, Liang Y, Lynch KH, et al. PHAST: a fast phage search tool. Nucleic Acids Res. 2011;39:W347–W352.
[39] UniProt Consortium. Activities at the universal protein resource (UniProt). Nucleic Acids Res. 2013;42:D191–D198.
[40] Binns D, Dimmer E, Huntley R, et al. QuickGO: a web-based tool for gene ontology searching. Bioinformatics. 2009;25:3045–3046.
[41] Dhillon BK, Laird MR, Shay JA, et al. IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis. Nucleic Acids Res. 2015;43:W104–W108.
[42] Siguier P, Pérochon J, Lestrade L, et al. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 2006;34:D32–D36.
[43] Fookes M, Yu J, De Majumdar S, et al. Genome sequence of *Klebsiella pneumoniae* Ecl8, a reference strain for targeted genetic manipulation. Genome A. 2013;1:e00027–12.
[44] Tokajian S, Eisen JA, Jospin G, et al. Whole genome sequencing of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* isolated from a patient in Lebanon. Front Cell Infect Microbiol. 2015;5:32. DOI:10.3389/fcimb.2015.00032.
[45] Tokajian S, Eisen JA, Jospin G, et al. Draft Genome sequence of *Klebsiella pneumoniae* KGM-IMP216 Harboring $bla_{CTX-M-15}$, bla_{DHA-1}, bla_{TEM-1B}, bla_{NDM-1}, bla_{SHV-28}, and bla_{OXA-1} isolated from a patient in Lebanon. Genome A. 2016;4:e01632–15.
[46] Stoesser N, Giess A, Batty EM, et al. Genome sequencing of an extended series of NDM-*Klebsiella pneumoniae* neonatal infections in a Nepali hospital characterizes the extent of community versus hospital-associated transmission in an endemic setting. Antimicrob Agents Chemother. 2014;58:7347–7357.
[47] Darling AE, Mau B, Perna NT. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLOS One. 2010;5:e11147.
[48] Darling AE, Jospin G, Lowe E, et al. PhyloSift: phylogenetic analysis of genomes and metagenomes. PeerJ. 2014;2:e243.

[49] Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 2008;36:W181–W184.
[50] Brisse S, Passet V, Haugaard A, et al. wzi gene sequencing, a rapid method for determination of capsular type for Klebsiella strains. J Clin Microbiol. 2013;4073–4078.
[51] Centre for Disease Control and Prevention. Multiplex Real-Time PCR Detection of Klebsiella pneumoniae Carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1). https://www.cdc.gov/hai/settings/lab/kpc-ndm1-lab-protocol.html
[52] Kaur M, Aggarwal A. Occurrence of the CTX-M, SHV and TEM genes among the extended spectrum β-lactamase producing isolates of Enterobacteriaceae in a tertiary care hospital of north India. J Clin Diag Res. 2013;4:642–645.
[53] Bialek-Davenet S, Criscuolo A, Ailloud F, et al. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Diseases. 2014;20:1812–1820.
[54] Toth A, Kocsis B, Damjanova I, et al. Fitness cost associated with resistance to fluoroquinolones is diverse across clones of *Klebsiella pneumoniae* and may select for CTX-M-15 type extended-spectrum β-lactamase. Eur J Clin Microbiol Infect Dis. 2014;33:837–843.
[55] Kumar V, Sun P, Vamathevan J, et al. Comparative genomics of *Klebsiella pneumoniae* strains with different antibiotic resistance profiles. Antimicrob Agents Chemother. 2011;55:4267–4276.
[56] Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010;362:1804–1813.
[57] Hua X, Chen Q, Li X, et al. Complete genome sequence of *Klebsiella pneumoniae* sequence type 17, a multidrug-resistant strain isolated during tigecycline treatment. Genome A. 2014;2:e01337–14.
[58] Zhou K, Lokate M, Deurenberg RH, et al. Use of whole-genome sequencing to trace, control and characterize the regional expansion of extended-spectrum β-lactamase producing ST15 *Klebsiella pneumoniae*. Sci Rep. 2016;6:20840. DOI:10.1038/srep20840
[59] Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. Trends Microbiol. 2016;24:944–956.
[60] Fluit AC, Florijn A, Verhoef J, et al. Presence of tetracycline resistance determinants and susceptibility to tigecycline and minocycline. Antimicrob Agents Chemother. 2005;49:1636–1638.
[61] Li R, Han Y, Zhou Y, et al. Tigecycline susceptibility and molecular resistance mechanisms among clinical *Klebsiella pneumoniae* strains isolated during Non-Tigecycline treatment. Microb Drug Res. 2017;23:139–146. DOI:10.1089/mdr.2015.0258.
[62] Zhanel GG, Schroeder C, Vercaigne L, et al. A critical review of oxazolidinones: an alternative or replacement for glycopeptides and streptogramins? Can J Infect Dis Med Microbio. 2001;12:379–390.
[63] Tokajian S, Salloum T, Eisen JA, et al. Genomic attributes of extended-spectrum β-lactamase-producing Escherichia coli isolated from patients in Lebanon. Future Microbiol. 2017;3:213–226.
[64] Coelho A, González-López JJ, Miró E, et al. Characterisation of the CTX-M-15-encoding gene in Klebsiella pneumoniae strains from the Barcelona metropolitan area: plasmid diversity and chromosomal integration. Int J Antimicrob Agents. 2010;36:73–78.
[65] Poirel L, Labarca J, Bello H, et al. Emergence of the 16S rRNA methylase RmtG in an extended-spectrum-β-lactamase-producing and colistin-resistant *Klebsiella pneumoniae* isolate in Chile. Antimicrob Agents Chemother. 2014;58:618–619.
[66] Villa L, García-Fernández A, Fortini D, et al. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. Antimicrob Agents Chemother. 2010;65:2518–2529.
[67] Poirel L, Bonnin RA, Boulanger A, et al. Tn125-related acquisition of blaNDM-like genes in *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2012;56:1087–1089.
[68] Fiett J, Baraniak A, Izdebski R, et al. The first NDM metallo-β-lactamase-producing *Enterobacteriaceae* isolate in Poland: evolution of IncFII-type plasmids carrying the blaNDM-1 gene. Antimicrob Agents Chemother. 2014;58:1203–1207.
[69] Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! Trends Mol Med. 2012;18:263–272.
[70] Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol. 2010;28:591–595.
[71] Hsu CR, Lin TL, Pan YJ, et al. Isolation of a bacteriophage specific for a new capsular type of *Klebsiella pneumoniae* and characterization of its polysaccharide depolymerase. PLOS ONE. 2013;8:e70092.
[72] Holt KE, Wertheim H, Zadoks RN, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Nat Acad Sci. 2015;112:E3574–E3581.
[73] Holden VI, Bachman MA. Diverging roles of bacterial siderophores during infection. Metallomics. 2015;7:986–995.
[74] Lee CH, Chang CC, Liu JW, et al. Sialic acid involved in hypermucoviscosity phenotype of *Klebsiella pneumoniae* and associated with resistance to neutrophil phagocytosis. Virulence. 2014;5:673–679.
[75] Clements A, Gaboriaud F, Duval JF, et al. The major surface-associated saccharides of *Klebsiella pneumoniae* contribute to host cell association. PLoS One. 2008;3:e3817.
[76] Moranta D, Regueiro V, March C, et al. *Klebsiella pneumoniae* capsule polysaccharide impedes the expression of β-defensins by airway epithelial cells. Infect Immun. 2010;78:1135–1146.
[77] Rafiq Z, Sam N, Vaidyanathan R. Whole genome sequence of *Klebsiella pneumoniae* U25, a hypermucoviscous, multidrug resistant, biofilm producing isolate from India. Mem Inst Oswaldo Cruz. 2016;111:144–146.
[78] Liu PP, Liu Y, Wang LH, et al. Draft genome sequence of an NDM-5-producing *Klebsiella pneumoniae* sequence type 14 strain of serotype K2. Genome A. 2016;4:e01610–15.