Draft genome sequence of a carbapenemase-producing (NDM-1) and multidrug-resistant, hypervirulent Klebsiella pneumoniae ST11 isolate from Pakistan, with a non-hypermucoviscous phenotype associated with rmpA2 mutation

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Genome Note

Draft genome sequence of a carbapenemase-producing (NDM-1) and multidrug-resistant, hypervirulent *Klebsiella pneumoniae* ST11 isolate from Pakistan, with a non-hypermucoviscous phenotype associated with *rmpA2* mutation

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ABSTRACT

Objectives: ST11 is a high-risk sequence type associated with carbapenem-resistant *Klebsiella pneumoniae* strains. Carbapenemase-producing hypervirulent *K. pneumoniae* (hvKp) are a major concern as they harbour a diverse range of pathogenicity traits. Here we describe the characteristics of *K. pneumoniae* strain KP75w isolated from a tertiary-care hospital in Pakistan.

Methods: Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion test and broth microdilution assay. The virulence phenotype was determined by string test as well as biofilm and cell adhesion assays. Genome sequencing was performed using MiSeq and HiSeq 2500 platforms with 30 × coverage.

Results: Antimicrobial resistance profiling characterised strain KP75w as a multidrug-resistant carbapenemase-producing strain with a meropenem minimum inhibitory concentration (MIC) of 4 μg/mL, which is above the CLSI susceptible breakpoint (≤1 μg/mL). The annotated contigs indicated a genome size of 5 644 609 bp with 5679 coding regions. KP75w (ST11) was designated as a carbapenemase-producing hvKp strain on the basis of the presence of a carbapenemase gene (bla_{NDM-1}) and hypervirulence genes (*rmpA2*, *iucABCD–iutA*, *fyuA*, *irp*, *mrk*, *ybt*, *fep* and *virB2*). KP75w was found to contain a 163-kb virulence region showing 58.8% identity to the large virulence plasmid pLVPK, supporting the hypervirulence of KP75w.

Conclusion: KP75w is a novel non-hypermucoviscous carbapenemase-producing hvKp ST11 strain that appears to represent the convergence of multidrug resistance with hypervirulence traits in clinical *K. pneumoniae* strains from the Southeast Asian region.

1. Introduction

Elevated carbapenem resistance in *Klebsiella pneumoniae* is considered a major public-health challenge [1]. There have been increasing reports of class A (KPC) and class B (IMP, VIM and NDM) carbapenemases in carbapenem-resistant Enterobacteriaceae (CRE) globally and of class D (OXA-48) and NDM-1 (class B)-type carbapenemases in CRE from the Indian subcontinent [2]. Until recently, carbapenemase-producing *K. pneumoniae* strains from multilocus sequence typing (MLST) sequence type 11 (ST11) (a single-locus variant of ST258) and hypervirulent *K. pneumoniae* (hvKp) strains from ST23 constituted the two most clinically significant *K. pneumoniae* populations [3]. This study reports the draft genome sequence of a New Delhi metallo-β-lactamase 1 (NDM-1)-producing, multidrug-resistant, hypervirulent but non-hypermucoviscous (non-HMV) *K. pneumoniae* clinical isolate (KP75w) from Pakistan with a novel 163-kb hybrid virulence gene cluster.

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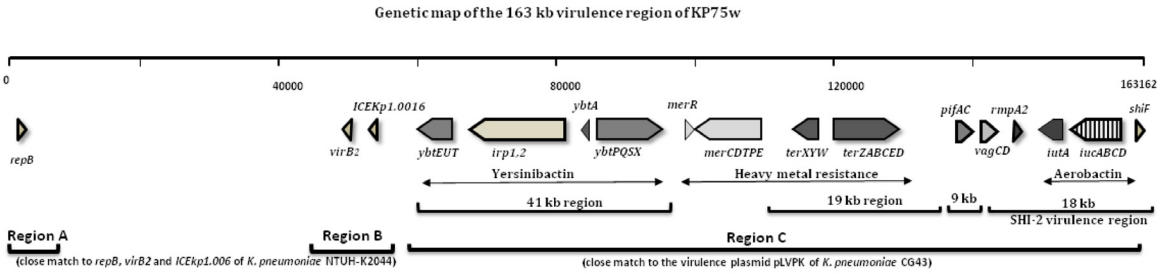

Fig. 1. Genetic map of the 163-kb virulence region of *Klebsiella pneumoniae* strain KP75w. The map shows loci identical to those of the pLVPK virulence plasmid and the hypervirulent *K. pneumoniae* strain NTUH-K2044. Regions A and B comprise *repB* (replication initiation factor in KP75w) identical to that of plasmid pK2044 and also include *virB2* and ICE*Kp1.006* determinants identical to those of NTUH-K2044. Genes of unknown function located between regions A and B are not marked. Region C is closely identical to the large virulence plasmid pLVPK of *K. pneumoniae* CG43 and includes the 18-kb SHI-2-PAI (pathogenicity island of *Shigella flexneri*) comprising *iucABCD–iutA*, *vagCD*, *shiF* and *rmpA2* (regulator of mucoid phenotype) [11] as well as other regions including the *pif*, heavy metal resistance and yersiniabactin genes.

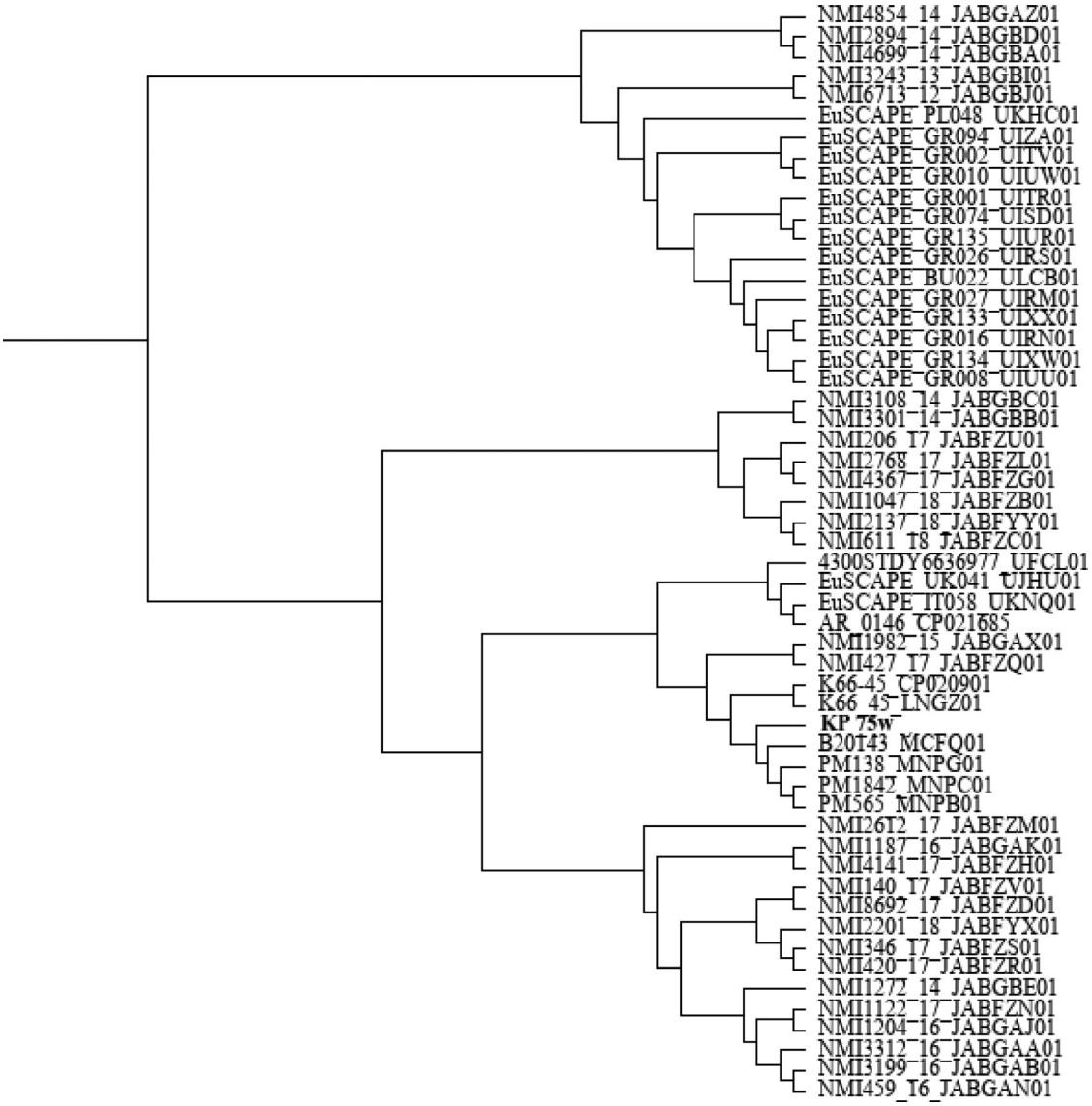

Fig. 2. Single nucleotide polymorphism (SNP)-based dendrogram generated for *Klebsiella pneumoniae* isolates closely related to strain KP75w via BacWGSTdb 2.0. Dendrogram generated by BacWGSTdb 2.0 SNP analysis showing the clonal relationship between highly similar *Klebsiella pneumoniae* isolates. Of 53 *K. pneumoniae* ST11 isolates similar to KP75w (query genome), 5 isolates (B20143, PM565, PM1842, PM138 and K66-45) were particularly closely related to KP75w. B20143 is the hypervirulent *K. pneumoniae* (hvKp) strain reported by Shankar et al. from the Indian subcontinent [12]. PM565, PM1842 and PM138 have been reported as colistin-resistant *K. pneumoniae* strains [13]. K66-45 is the multidrug-resistant bla_{NDM-1}-expressing *K. pneumoniae* isolate reported from Norway [14]. Of the 53 ST11 isolates, 30 (57%) were from Poland, 12 (23%) from Greece, 4 (8%) from India, 2 (4%) from Norway, and 1 (2%) each from Thailand, Italy and Bulgaria. The country of origin remains unspecified for two isolates (AR_0146_CP021685 and EuSCAPE_UK041_UJHU01). For the 53 isolates presented in the dendrogram, urine was found to be the most common source (24; 45%), followed by rectal swab (13; 25%), blood (4; 8%), wound secretion (4; 8%), bronchoalveolar lavage (2; 4%), pus (1; 2%) and central venous catheter (1; 2%); the source remained unspecified for 4 isolates. Of the 53 isolates, 15 were designated as clinical samples in the BacWGSTdb 2.0 record.

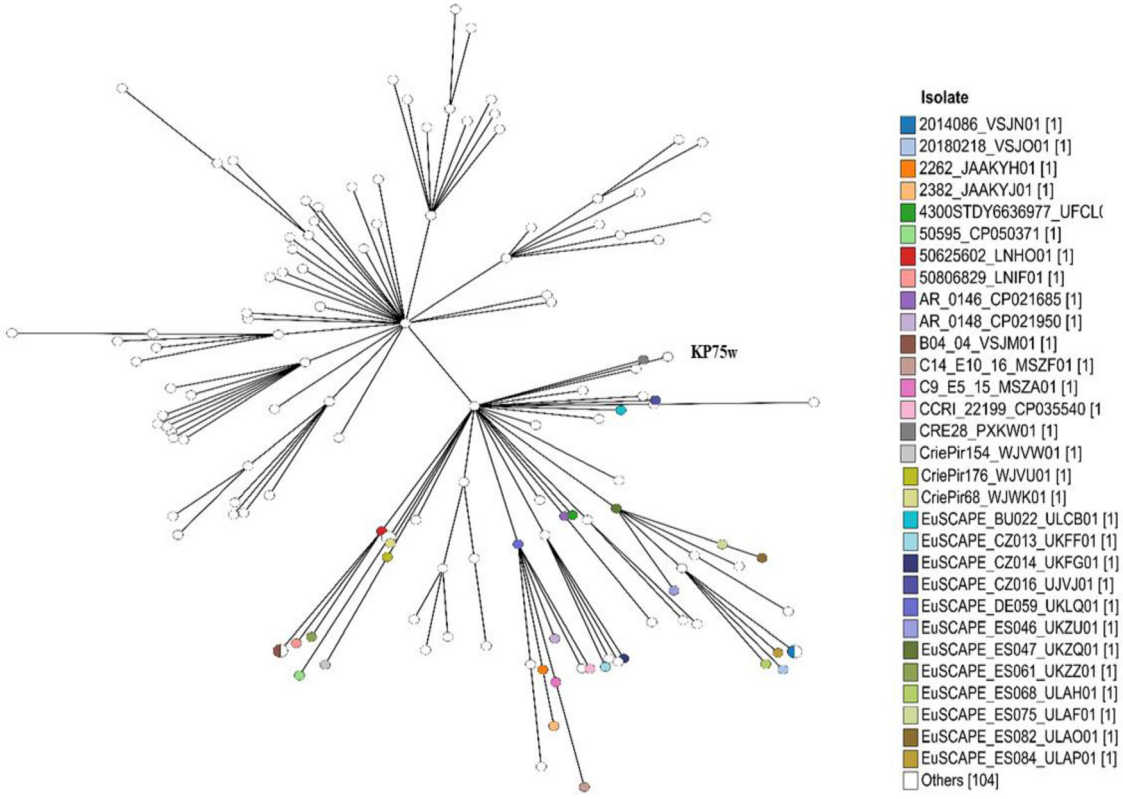

Fig. 3. Core genome multilocus sequence typing (cgMLST)-based cluster analysis generated for *Klebsiella pneumoniae* isolates closely related to strain KP75w by BacWGSTdb 2.0. cgMLST is an extension of the conventional seven-gene MLST used to the track evolutionary history of bacterial pathogens. A total of 134 *K. pneumoniae* ST11 isolates found related to KP75w are represented by nodes in the grape tree cluster. Epidemiologically, the most prevalent *K. pneumoniae* isolates originated from Poland (n = 68), followed by Spain (n = 16), Greece (n = 14), Russia (n = 5), Norway (n = 4), Czech Republic (n = 4), the UK (n = 3), Vietnam (n = 3), Slovakia (n = 2), the USA (n = 2), Tunisia (n = 2), Malaysia (n = 2) and Hungary, Thailand, Bulgaria, Canada, Italy and Germany (n = 1 each); for the remaining 3 isolates the country/state of origin has not been indicated. Urine was identified as the most common sample source of the *K. pneumoniae* isolates (n = 54), followed by rectal swab (n = 23), blood (n = 14), wound infection (n = 9), wound secretion (n = 4), puncture fluid (n = 3), bronchoalveolar lavage (n = 3) and respiratory tract (n = 3). Less prevalent sample sources included faeces/stool sample (n = 2), throat (n = 2), central venous catheter (n = 1), ureteric stent (n = 1), sputum (n = 1), anus (n = 1), pus (n = 1), vaginal swab (n = 1), abdominal fluid (n = 1) and fistula (n = 1); the sample source remained unidentified for 9 *K. pneumoniae* isolates in the BacWGSTdb 2.0 record and 42 were from clinical samples.

2. Materials and methods

Klebsiella pneumoniae strain KP75w was isolated from the urine of a 30-year-old male patient in a tertiary-care hospital in Pakistan. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The minimum inhibitory concentration (MIC) of meropenem (Sigma-Aldrich) was determined by the standard broth microdilution method [4] and was interpreted according to 2010 CLSI breakpoints (susceptible, $\leq$1 μg/mL; intermediate, 2 μg/mL; and resistant, $\geq$4 μg/mL) [5]. The string test was used to detect the hypermucoviscous phenotype ($\geq$5 mm mucoviscous string). Biofilm potential was assessed by the microtitre plate assay [6]. The adhesion potential of KP75w to HEK293^T cells at a multiplicity of infection of 10 was determined using *Escherichia coli* W3110 as a positive control [7]. Genome sequencing was performed by MicrobesNG (Birmingham, UK) on Illumina HiSeq and MiSeq sequencing platforms (30 $\times$ coverage), followed by QUAST analysis. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP), PATRIC, the Virulence Factor Database (VFDB) and the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/analyze/rgi) were used to annotate the sequences.

Whole-genome sequencing-based epidemiological comparison was performed via single nucleotide polymorphism (SNP) (sequence-based) and core genome MLST (cgMLST) (allele-based) analysis using BacWGSTdb 2.0 [8].

3. Results

Strain KP75w was found to be resistant to ampicillin, ceftriaxone, aztreonam, gentamicin, levofloxacin, imipenem and meropenem, but was susceptible to fosfomycin, polymyxin B and colistin. The MIC for meropenem (4 μg/mL) exceeded the CLSI screening breakpoint for carbapenemase production (ertapenem or meropenem MIC of 2 μg/mL) [9]. KP75w was characterised as a non-HMV isolate with strong biofilm-forming capacity and high adhesion (120 000 CFU/mL) to HEK293^T cells (compared with *E. coli* W3110 at 80 000 CFU/mL).

Next-generation sequencing analysis indicated that the genome of KP75w is 5 644 609 bp in size with a GC content of 56.97%. A total of 198 contigs were annotated, identifying 5679 protein-coding genes. MLST analysis confirmed ST11, and *wzi*-based capsule serotyping confirmed the K-type as K24. Extended-spectrum β-lactamase (ESBL) and carbapenemase genetic determinants identified in KP75w were $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-182}}$, $bla_{\text{SHV-67}}$ and $bla_{\text{TEM-1B}}$. Moreover, factors encoding resistance to aminoglycosides [*aac(6′)-Ib*-cr, *aph(3′)-Ib, aph(6′)-Id, rmtF*], fosfomycin (*fosA*) and quinolones (*oqxA, oqxB*) as well as efflux regulators (*acrA, acrB, acrR, marA, marR, soxS, soxR, envR, oqxR, rarA*) were also identified. PlasmidFinder indicated five plasmids in KP75w with replicons types IncFIB(pQil), IncFII(K), IncHI1B(pNDM-MAR), IncR and RepB. The replication initiation gene *repB* is identical to that of plasmid pK2044 from strain NTUH-K2044, an invasive, liver abscess-inducing hvKp strain [10]. KP75w was characterised

as hypervirulent based on the presence of a 163-kb virulence region showing 58.8% identity to the 219-kb virulence plasmid pLVPK of *K. pneumoniae* CG43 [11], which is associated with hypervirulence (Fig. 1) [3]. Furthermore, SNP-based analysis via BacWGSTdb 2.0 confirmed KP75w to be closely related to B20143, which is a hvKp strain reported from the Indian subcontinent [12] (Fig. 2). A broader cgMLST-based cluster analysis is shown in Fig. 3.

4. Discussion

Klebsiella pneumoniae strain KP75w displayed 99.4%, 99.8%, 99.9% and 100% identity with four regions (41, 19, 18 and 9 kb, respectively) of the pLVPK plasmid (Fig. 1). These highly identical regions included the yersiniabactin siderophore genes (*ybtPQSX*–ybtAUTE, irp1, irp2), a tellurite resistance cluster (*terZABCED–terXYW*), *vagCD, rmpA2*, the aerobactin siderophore system (*iucABCD–iutA*), *shiF* and *pif* of the pLVPK plasmid (Fig. 1) [11]. The 638-bp regulator of mucoid phenotype A gene (*rmpA2*) found in the KP75w virulence cluster shows 99.7% identity to the 639-bp *rmpA2* of pLVPK. However, two point mutations were detected in *rmpA2* of KP75w: a deletion (G at position 276) and a C→A transversion (in the frameshift region) at position 301. The deletion results in a predicted translational truncation and frameshift, similar to that reported for plasmid pVir-SCNJ1 (which is 99.7% identical to pLVPK) [15]. The deletion mutation likely explains the non-HMV phenotype, despite the presence of *rmpA2*. This view is supported by the recent identification of a similar *rmpA2* frameshift mutation in a carbapenem-resistant *K. pneumoniae* ST11 strain that also displayed a negative string test [16]. The 58.8-kb region of pLVPK harbouring *rmpA, iroBCDN* (salmochelin), *fecIRA* (ferric citrate uptake genes) and 40 other genes [3] is absent in the 163-kb KP75w virulence cluster. Interestingly, three other genetic loci within the 163-kb virulence region are identical to genes of hvKp strain NTUH-K2044 (Fig. 1): the replication initiation factor (*repB*) of plasmid pK2044 and the *virB2* (294 bp) and ICE*Kp1.0016* (582 bp) of ICE*Kp1*, a 76-kb integrative conjugative element (ICE; **KY454627.1**). The *virB2* gene encodes a putative type IV secretion system [17]. This finding indicates that the 163-kb virulence region is part of a virulence plasmid derived by recombination between pLVPK- and pK2044-like plasmids and a ICE*Kp1*-like element. Thus, KP75w is a novel non-HMV carbapenemase-producing hvKp ST11 strain [18] that appears to represent the convergence of multidrug resistance with hypervirulence traits in clinical *K. pneumoniae* strains from the Southeast Asian region.

GenBank accession no.

The whole-genome sequence of *Klebsiella pneumoniae* strain KP75w has been deposited in GenBank under accession number **JABMDC000000000**. The version described in this paper is **JABMDC010000000**.

Data availability

Any additional information required can be requested from the corresponding author as per ethical guidelines.

Declaration of Competing Interest

None declared.

Acknowledgment

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Ethical approval

This study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Bio-Ethical Committee (BEC) of Quaid-i-Azam University under protocol number #BEC-FBS-QAU2019-148. Informed consent was obtained from all subjects involved in the study.

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