Comparative genomic analysis of *Klebsiella pneumoniae* subsp. *pneumoniae* KP617 and PittNDM01, NUHL24835, and ATCC BAA-2146 reveals unique evolutionary history of this strain

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**Abstract**

**Background:** *Klebsiella pneumoniae* subsp. *pneumoniae* KP617 is a pathogenic strain that coproduces OXA-232 and NDM-1 carbapenemases. We sequenced the genome of KP617, which was isolated from the wound of a Korean burn patient, and performed a comparative genomic analysis with three additional strains: PittNDM01, NUHL24835 and ATCC BAA-2146.

**Results:** The complete genome of KP617 was obtained via multi-platform whole-genome sequencing. Phylogenetic analysis along with whole genome and multi-locus sequence typing of genes of the *Klebsiella pneumoniae* species showed that KP617 belongs to the WGLW2 group, which includes PittNDM01 and NUHL24835. Comparison of annotated genes showed that KP617 shares 98.3 % of its genes with PittNDM01. Nineteen antibiotic resistance genes were identified in the KP617 genome: *bla*<sub>OXA-1</sub> and *bla*<sub>SHV-28</sub> in the chromosome, *bla*<sub>NDM-1</sub> in plasmid 1, and *bla*<sub>OXA-232</sub> in plasmid 2 conferred resistance to beta-lactams; however, colistin- and tetracycline-resistance genes were not found. We identified 117 virulence factors in the KP617 genome, and discovered that the genes encoding these factors were also harbored by the reference strains; eight genes were lipopolysaccharide-related and four were capsular polysaccharide-related. A comparative analysis of phage-associated regions indicated that two phage regions are specific to the KP617 genome and that prophages did not act as a vehicle for transfer of antimicrobial resistance genes in this strain.

**Conclusions:** Whole-genome sequencing and bioinformatics analysis revealed similarity in the genome sequences and content, and differences in phage-related genes, plasmids and antimicrobial resistance genes between KP617 and the references. In order to elucidate the precise role of these factors in the pathogenicity of KP617, further studies are required.

**Keywords:** *Klebsiella pneumoniae*, OXA-232, NDM-1, Carbapenemases

**Background**

*Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, facultative anaerobic bacterium, which belongs to the family Enterobacteriaceae. *K. pneumoniae* is found in the normal flora of the mouth, skin, and intestines; however, this bacterium may act as an opportunistic pathogen, causing severe nosocomial infections such as septicemia, pneumonia, and urinary tract infections in hospitalized and immune-compromised patients with chronic ailments [1, 2].

Beta-lactam antibiotics, used as therapeutic agents against a broad range of bacteria, bind to the
penicillin-binding protein and inhibit biosynthesis of the bacterial cell membrane. However, the extended spectrum β-lactamases (ESBLs) and carbapenemases confer resistance to penicillin, cephalosporins, or carbapenem [3, 4]. The β-lactamases are divided into four classes on the basis of the Ambler scheme: class A (Klebsiella pneumoniae carbapenemase, KPC; imipenem-hydrolyzing β-lactamase, IMI; Serratia marcescens enzyme, SME; Serratia fonticola carbapenemase, SFC), class B (Verona integron-encoded metallo-β-lactamase, VIM; imipenem-resistant Pseudomonas, IMP; New Delhi metallo-β-lactamase, NDM), class C (AmpC-type β-lactamase, ACT; cephemycin-hydrolyzing β-lactamase, CMY), and class D (oxacillinase, OXA) [5] are composed of transposon, cassettes, and integrons and transferred within and between species by HGT (horizontal gene transfer). Numerous carbapenemase-producing bacteria similarly harbor resistance genes that are transferred to other strains by horizontal gene transfer [6, 7]; infections caused by such multi-drug-resistant bacteria are difficult to treat [8]. The emergence of the novel carbapenemase NDM-1 (the New Delhi metallo-β-lactamase) is of great concern, as no therapeutic agents are available to treat infections caused by NDM-1-producing bacterial strains [9]. NDM-1-producing K. pneumoniae strains were first isolated from a Swedish patient who had travelled to India in 2009 [10]. Since then, NDM-1 has been reported to be produced by various species of Enterobacteriaceae, such as K. pneumoniae, Escherichia coli, Enterobacter spp. and Acinetobacter spp., in numerous countries [11].

The carbapenem-hydrolyzing β-lactamase OXA-232, which was first reported in E. coli and two K. pneumoniae strains [12], belongs to the OXA-48-like family. Carbapenemase-producing Gram-negative bacteria are often multi-drug resistant [13]. K. pneumoniae isolates that coproduce OXA-48-like β-lactamase and NDM-1 have been isolated in numerous countries [14-16]. Recently, K. pneumoniae isolates coproducing two carbapenemases, blaNDM-1 and blaOXA-232, have been identified in several countries; of these, two isolates originating in India were recovered in the USA and Korea, in January 2013, and sequenced [16, 17] but not studied yet the characteristics in the context of genomic contents by comparing these isolates. In the present study, we performed a comparative analysis of the genomes of these isolates.

**Methods**

**Isolation and serotyping of strains**

In January 2013, a 32-year-old man was hospitalized in the Intensive Care Unit of a general hospital in Seoul, Korea, two days after suffering burns during a visit to India. K. pneumoniae was isolated from his wound and another patient in the same room became infected with the same strain [18]. The K. pneumoniae isolate was identified as the KP617 strain belonging to the sequence type (ST)14, and found to coproduce NDM-1 and OXA-232, which conferred resistance to ertapenem, doripenem, imipenem, and meropenem (MICs: >32 mg/L). The K. pneumoniae strains PittNDM01 [17], NUHL24835 [19], and ATCC BAA-2146 [20] were used as reference strains for comparative genomic analysis.

**Library preparation and whole-genome sequencing**

Whole-genome sequencing of KP617 was performed using three platforms: Illumina-HiSeq 2500, PacBio RS II, and Sanger sequencing (GnC Bio: Daejeon, Republic of Korea) [16]. Sanger sequencing was used for the construction of a physical map of the genome.

**Genome assembly and annotation**

A hybrid assembly was performed using the Celera Assembler (version 8.2) [21] and a fosmid paired-end sequencing map was used to confirm the assembly. The final assembly was revised using proovread (version 2.12) [22]. An initial annotation of the KP617 genome was generated using the RAST (Rapid Annotation using Subsystem Technology, version 4.0) server pipeline [23]. The genomes of three K. pneumoniae strains, PittNDM01, NUHL24835, and ATCC BAA-2146, were annotated using the RAST server pipeline. In order to compare the total coding sequences (CDSs) of KP617 with those of the three K. pneumoniae strains, the sequence-based comparison functionality of the RAST server was utilized.

**Phylogenetic analysis**

Concatenated whole genomes of 44 K. pneumoniae strains, including KP617, and multi-locus sequence typing (MLST) of seven genes [24, 25] were used for the calculation of evolutionary distances. The seven genes used for MLST were as follows: gapA, infB, mdh, pgi, phoE, rpoB and tonB. Multiple sequence alignments were performed using Mugsy (version 1.2.3) [26]. The generalized time-reversible model [27] + CAT model [28] (FastTree Version 2.1.7) [29] was used to construct approximate maximum-likelihood phylogenetic trees. The resulting trees were visualized using FigTree (version 1.3.1) (http://tree.bio.ed.ac.uk/software/figtree/).

**Comparison of genomic structure**

The chromosome and plasmids of KP617 and the reference strains were compared using Easyfig (version 2.2.2) [30]. Whole-genome nucleotide alignments were generated using BLASTN to identify syntenic genes. The syntenic genes and genomic structures were visualized using Easyfig. A stand-alone BLAST algorithm was used to analyze the structure of the genes of interest, i.e. the OXA-232- and NDM-1 carbapenemase-encoding genes.
Identification of the antimicrobial resistance genes

We identified the antibiotic resistance genes using complete sequences of chromosomes and plasmids of four *K. pneumoniae* isolates: KP617, PittNDM01, NUHL24835 and ATCC BAA-2146 using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/) [31].

Analysis of virulence factors and phage-associated regions

The virulence factor-encoding genes were searched against the virulence factor database (VFDB) [32] using BLAST with an e-value threshold of 1e-5. Homologous virulence factor genes with a BLAST Score Ratio (BSR) of ≥0.4 were selected. The BSR score was calculated using our in-house scripts. Phage-associated regions in the genome sequences of the four *K. pneumoniae* strains were predicted using the PHAST server [33]. Three scenarios for the completeness of the predicted phage-associated regions were defined according to how many genes/proteins of a known phage the region contained: intact (≥90 %), questionable (90–60 %), and incomplete (≤60 %).

Quality assurance

Genomic DNA was purified from a pure culture of a single bacterial isolate of KP617. Potential contamination of the genomic library by other microorganisms was assessed using a BLAST search against the non-redundant database.

Results and discussion

General features

A total of 316,881,346 (32,005,015,946 bp) paired-end reads were generated using Illumina-HiSeq 2500. Using the PacBio RS II platform, 46,134 (421,257,386 bp) raw reads were produced. The complete genome of KP617 consists of a 5,416,282-bp circular chromosome and two plasmids of 273,628 bp and 6141 bp in size. The genomic features of KP617 and the reference strains are summarized in Table 1. Based on a RAST analysis, 5024 putative open reading frames (ORFs) and 110 RNA genes on the circular chromosome (Figs. 1, 2; Additional file 1: Table S1), 342 putative ORFs on plasmid 1, and 9 putative ORFs on plasmid 2 were identified.

Comparison of KP617 and the reference strains based on sequence similarity (percent identity ≤80) showed that 32 genes are unique for KP617, and that most of the functional genes of this strain are also conserved in the reference strains. The genes unique to the KP617 strain, such as the SOS-response repressor and protease LexA (EC 3.4.21.88), integrase, and phage-related protein were identified as belonging to the genome of the prophage Salmonella phage SEN4 (GenBank accession: NC_029015). When the KP617 genome was compared with that of the PittNDM01 strain, which represents the closest neighbor of the former strain on the phylogenetic tree (Figs. 3a, b), 94 genes showed a percent similarity of below 80; most of these were phage protein-encoding genes. These results indicate that the presence of prophage DNA is an important feature of the KP617 genome.

Phylogenetic analysis

The whole-genome phylogenetic analysis indicated that KP617 is evolutionarily close to PittNDM01 and NUHL24835, and that the strains belong to the WGLW2 group. However, KP617 was found to be evolutionarily distant from ATCC BAA-2146 (Fig. 3). Concordantly, MLST-based phylogenetic analysis revealed that while KP617, PittNDM01, and NUHL24835 belong to the same group [sequence type (ST)14], ATCC BAA-2146 belongs to the HS11286 group, ST 11 [20]. The only difference between the whole-genome phylogenetic tree and the MLST-based phylogenetic tree was the divergence time within the same group; MLST-based phylogeny did not reveal the minor details of genomic evolution such as the divergence between KP617, PittNDM01 and NUHL24835 in the whole-genome phylogeny. The difference was attributed to horizontal gene transfer in regions not covered by the MLST genes.

Comparison of genome structures

The comparison of genomic structures of the chromosome indicated the presence of highly conserved structures in the KP617, NUHL24835, and PittNDM01 strains (Fig. 4a). Interestingly, a 1-Mb region (233,805–1,517,597) of the KP617 chromosome was inverted relative to its arrangement in the chromosome of PittNDM01 (1,500,972–225,619). Despite this inversion, KP617 and PittNDM01 exhibited a lower substitution rate (score 20) than NUHL24835 (score 30) (Fig. 3). However, the chromosomal structure of the ATCC BAA-2146 strain, which consisted of two large inverted regions, was significantly different from that of the other strains. In addition, a 71 Kb inversion was found in the sequence of plasmid 1 of KP617 (18,633–90,686) relative to plasmid 1 of

| Strain          | KP617 | PittNDM01 | NUHL24835 | ATCC BAA-2146 |
|-----------------|-------|-----------|-----------|---------------|
| Genome (Mb)     | 5.69  | 5.81      | 5.53      | 5.78          |
| % GC (chromo-)  | 57.4  | 57.5      | 57.4      | 57.3          |
| Total open reading frames | 5375  | 4940      | 5191      | 5883          |
| Plasmids        | 2     | 4         | 2         | 4             |

Table 1 Genomic features of *Klebsiella pneumoniae* KP617 and other strains
PittNDM01 (91,507–19,453); however, the two plasmids were highly homologous to each other (Fig. 4b).

**Antimicrobial resistance genes**

Nineteen antibiotic resistance genes were identified in the genome of KP617, 39 in the genome of PittNDM01, 29 in that of ATCC BAA-2146, and nine in that of the NUHL24385 strain (Table 2). The β-lactam resistance genes in the KP617 genome were \( \text{bla}_{\text{OXA-1}} \) and \( \text{bla}_{\text{SHV-28}} \) in the chromosome, \( \text{bla}_{\text{NDM-1}} \) in plasmid 1, and \( \text{bla}_{\text{OXA-232}} \) in plasmid 2; however, genes conferring resistance to colistin and tetracycline were not found (Table 2). Plasmid 2 of KP617, which includes the OXA-232-encoding gene, consists of a 6141-bp sequence; the sequence of this plasmid was identical to that of plasmid 4 of PittNDM01 (100 % coverage and similarity) and the plasmid of \( \text{E. coli} \) (coverage: 100 %, similarity: 99.9 %). Plasmid 2 of KP617, plasmid 4 of PittNDM01 and \( \text{E. coli} \) Mob gene cluster (GenBank accession: JX423831) [12] carried the OXA-232-encoding gene, and pKF-3 of \( \text{K. pneumoniae} \) carried the OXA-181-encoding gene. However, pKF-3 was identical to plasmid 2 of KP617, except that the insertion sequence \( \text{ISEcp1} \) was inserted upstream of OXA-181 and included in the transposon \( \text{Tn2013} \) [12, 34].

The structure of plasmid 1 (273,628 bp in size) of the KP617 strain was similar to that of plasmid 1 (283,371 bp in size) of PittNDM01. A region of about 40 kb in size within plasmid 1 of the KP617 strain, which included the NDM-1-encoding gene, was composed of various resistance genes such as \( \text{aadA2, armA, aac(3')-VI, dfrA12, msrE, mphE, sul1 and qnrB1} \), and identical (coverage: 100 %, homology: 100 %) to a 40-kb sequence of plasmid 1 of PittNDM01 (Fig. 4b). Adjacent to the NDM-1-encoding gene, a region of about 70 kb in size was inverted in plasmid 1 of KP617 relative to plasmid 1 of PittNDM01. In addition, the OXA-1-encoding gene was identified in PittNDM01 but not in KP617. Transposases were found in a part of the NDM-1-encoding gene cluster (about 10 kb) in plasmid 1 of KP617. Gram-negative bacteria are known to possess a diverse range of transposases; moreover, the sequence of the NDM-1-encoding gene cluster includes a transposon [35, 36]. The partial, or complete, transfer of NDM-1-harboring plasmids between \( \text{K. pneumoniae} \) and \( \text{E. coli} \), via conjugation, has been shown to result in the emergence of strains resistant to several antimicrobial agents [11, 32, 36, 37].

Following the initial identification of NDM-1 in a \( \text{K. pneumoniae} \) isolate from a patient who had travelled to India in 2008, most NDM-1-producing \( \text{K. pneumoniae} \) isolates have been recovered from patients associated with India; however, in some cases, these strains have been isolated from patients with no history of travelling abroad, or any association with India [38]. These observations suggest that the transfer of the NDM-1- and OXA-232-harboring plasmids between Gram-negative bacteria has resulted in the spread of carbapenem resistance and emergence of strong carbapenem-resistant strains outside the Indian subcontinent.

**Virulence factors**

\( \text{Klebsiella pneumoniae} \), a significant pathogen of human hosts, causes urinary tract infections, pneumonia,
septicemia, and soft tissue infections [1]. The clinical features of *K. pneumoniae* infections depend on the virulence factors expressed by the infecting strain [39]. Therefore, we investigated the virulence factors of the present strain and compared these with those of KP617 and the reference strains. A BLAST search was performed against VFDB to identify 117 virulence factors harbored by the KP617 strain (Table 3). All 117 virulence genes of KP617 were also harbored by the reference strains; KP617 did not possess any unique virulence factors. The PittNDM01 strain was also found to possess no unique virulence factors; however, NUHL24835 and

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**Fig. 2** Circular map of the KP617 chromosome. Circular map of the KP617 genome, generated using cgview (version 2.2.2); from outside to inside, the tracks display the following information: CDSs of KP617 on the + strand (1); CDSs of KP617 on the − strand (2); tblastx result against PittNDM01 (3), tblastx result against NUHL24835 (4), tblastx result against ATCC BAA-2146 (5), GC content (6), GC skew with + value (green) and − value (purple) (7)
ATCC BAA-2146 possessed 3 and 7 unique virulence factors, respectively. The 117 virulence genes of KP617 were classified into 31 the following categories: Iron uptake (30 genes), Immune evasion (12 genes), Endotoxin (11 genes), Adherence (10 genes), Fimbrial adherence determinants (8 genes), Toxin (7 genes), Antiphagocytosis (6 genes), Regulation (5 genes), Acid resistance (3 genes), Anaerobic respiration (2 genes), Cell surface components (2 genes) and Secretion system (2 genes). Among the 117 virulence genes identified, 8 genes were lipopolysaccharide [40]-related genes and 4 genes were capsular polysaccharide [41]-related.

KP617 and PittNDM01 were found to possess two virulence factors that were not present in the other two strains: invasion (encoded by \( \textit{ail} \), attachment invasion locus protein) [42] and Iron uptake (encoded by \( \textit{fyuA} \), Yersiniabactin siderophore) [43].

**Phage-associated regions**

Prophages contribute to the genetic and phenotypic plasticity of their bacterial hosts [44] and act as vehicles for the transfer of antimicrobial resistance genes [45] or virulence factors [46]. Six phage-associated regions (KC1–KC5) of the KP617 chromosome and one phage-associated region (KP1) in plasmid 1 of the KP617 strain were identified using the PHAST algorithm (Table 4). With regard to the reference strains, six phage-associated regions were identified in the PittNDM01 strain, six in NUHL24835, and 12 in ATCC BAA-2146.

Three of the six phages, KC1, KC2 and KC3, in the KP617 strain were intact, whereas the remaining prophages were incomplete (KC5 and KP1) or questionable (KC4) and had a low PHAST score of below 90. Based on the sequence similarity of their genomes,
KP617 and PittNDM01 were found to have high similarity to each other (Figs. 2, 3a, b). Concordantly, the profile of prophage DNA in their genomes, as determined via a BLAST search, was similar, and the two strains shared four of the six prophages, whereas two phage regions, KC2 (Entero_HK140) and KC3 (Salmon_SEN4), were specific to the KP617 genome. Furthermore, it was found that one phage-associated region of KP617, namely KC2 (Entero_HK140), exhibited a high similarity to the phage-associated region of the NUHL24835 strain, NC1, with 60% query coverage and 99% identity. It should be noted that the strains compared in the present study, i.e. KP617 and the reference strain, ATCC BAA-2146, had no prophages in common.

Investigation of the antimicrobial resistance genes harbored by the strains, which was performed using ResFinder, and comparison with the prophage-associated region, as predicted using PHAST, did not reveal the presence of a prophage-delivered beta-lactamase-encoding gene in the KP617 genome, indicating that prophages did not act as a vehicle for the transfer of antimicrobial resistance genes in this strain. This finding is consistent with previous observations that beta-lactamase-encoding genes are borne by transposons [35,
Table 2: Antimicrobial resistance genes of KP617 and the reference strains

| Antibiotics | Resistance gene | % identity | Query/HSP length | Predicted phenotype | Accession number | Position* |
|-------------|-----------------|------------|------------------|---------------------|------------------|-----------|
|             |                 |            |                  |                     | KP617 PttNDM01 BAA-2146 NHUL24385 |          |
| Aminoglycosides | aacA4          | 100        | 555/555          | Aminoglycoside      | KM278199         | P3_115183.115737 |
|              | aac(3)-Ila     | 99.77      | 861/861          |                     | XS1534           | P2_41114.41974  |
|              | aac(3)-IId     | 99.88      | 861/861          |                     | EU022314         | P3_64003.64863  |
|              | aac(6)’Ib      | 100        | 606/606          |                     | M21682           | P3_2456.3061    |
|              | aadA1          | 100        | 789/789          |                     | JQ480156         | P3_3131.3919    |
|              | aadA2          | 99.75      | 792/792          |                     | JQ414041         | P3_44412.45203  |
|              | qph(3)’-Vla    | 98.46      | 780/780          |                     | X68227           | P3_2297697.2298476 |
|              | armA           | 100        | 774/774          |                     | AY220558         | P3_2297134.277907 |
|              | rmC            | 100        | 804/804          |                     | AF321551         | P3_22970.30010  |
|              | strA           | 99.88      | 837/837          |                     | M96392           | P3_22970.30846  |
|              | strB           | 100        | 837/837          |                     | M96392           | P3_22970.30846  |
|              | aac(6)’Ib-cr   | 100        | 600/600          | Fluoroquinolone and | DQ303918         | C_1122863.1123462 |
|              | β-lactams      |            |                  | aminoglycoside      |                  | P1_136136.136762 |
|              | blaOXA-1       | 100        | 831/831          | Beta-lactam         | J02967           | P1_136136.136762 |
|              | blaOXA-9       | 100        | 840/840          | resistance         | JF703130         | P3_3964.4803    |
|              | blaOXA-232     | 100        | 798/798          |                     | JX423831         | P3_3878.4675    |
|              | blaNDM-1       | 100        | 813/813          |                     | FN396876         | P4_3878.4675    |
|              | blaNDM-5       | 100        | 813/813          |                     | JN104597         | P1_7770.8582    |
|              | blaCTX-M-15    | 100        | 876/876          |                     | DQ302097         | P3_122191.123003 |
|              | blaTEM-1A      | 100        | 861/861          |                     | HM749666         | P3_68389.69264  |
|              | blaTEM-1B      | 100        | 861/861          |                     | JF910132         | P3_5503.6363    |
|              | blaSHV-11      | 100        | 861/861          |                     | GQ407109         | P3_57446.58306  |
|              | blaSHV-2B      | 100        | 861/861          |                     | HM751101         | C_1078475.1079335 |
|              | blaCMY-6       | 100        | 1146/1146        |                     | AJ011293         | P3_72203.73348  |
Table 2 continued

| Antibiotics          | Resistance gene | % identity | Query/HSP length | Predicted phenotype                                                              | Accession number | Positiona |
|----------------------|-----------------|------------|------------------|----------------------------------------------------------------------------------|------------------|-----------|
|                      |                 |            |                  |                                                                                 |                  | KP617     | PittNDM01 | BAA-2146 | NHUL24385 |
| Fluoroquinolones     | aac(6)’/Ib-cr   | 100        | 600/600          | Fluoroquinolone and aminoglycoside resistance                                  | DQ303918         | C_612688  | 612327   | C_1122863 | 1123462   |
|                      |                 | 99.42      | 519/519          |                                                                                  | EF636461         | P1_136163 | 136762   | P3_2543   | 3061      |
|                      |                 | 99.61      |                  |                                                                                  |                  | P2_82742  | 83260    | P3_115219 | 115737    |
| QnrB1                |                 | 99.85      | 682/681          | Quinolone resistance                                                             | EF682133         | P1_130519 | 130120   | P1_130247 | 130928    |
| QnrB58               |                 | 98.68      | 681/681          |                                                                                  | JX529319         | P2_26062  | 26742    | C_4169699 | 4170874   |
| oqxA                 |                 | 100        | 1176/1176        |                                                                                  | EU730913         | C_4847144 | 4848319  | C_4793024 | 4794199   |
|                      |                 | 99.23      |                  |                                                                                  |                  | P2_26062  | 26742    | C_4843968 | 4847120   |
|                      |                 | 98.79      | 3153/3153        |                                                                                  | EU730913         | C_4843968 | 4847120   | C_4789848 | 4793000   |
|                      |                 | 98.79      |                  |                                                                                  |                  | P2_26062  | 26742    | C_4170898 | 4174050   |
|                      |                 | 98.79      |                  |                                                                                  |                  | C_4849531 | 4850706  |
| Fosfomycin           | fpaA            | 97.38      | 420/420          | Fosfomycin resistance                                                            | NZ_AFB001000747  | C_2957629 | 2958048  | C_2903507 | 2903926   |
|                      |                 | 97.14      |                  |                                                                                  |                  | C_667959  | 668378   |
| MLS—macrolide,      | etr(A)          | 95.11      | 1227/1227        | Macrolide resistance                                                             | AF099140         | P3_45289  | 46515    | P1_16503  | 17408     |
| lincosamide and     | mph(A)          | 100        | 906/906          |                                                                                  | D16251           | P3_45289  | 46515    | P1_16503  | 17408     |
| streptogramin B     | mph(E)          | 99.89      | 885/885          |                                                                                  | EU294228         | P1_271994 | 272878   | P1_281737 | 282621    |
|                      | mrr(E)          | 100        | 1476/1476        | Macrolide, Lincosamide and Streptogramin B resistance                           | EU294228         | P1_270463 | 271938   | P1_280206 | 281681    |
|                      |                 |            |                  |                                                                                  |                  | C_4846355 | 4849507  |
| Phenicol             | catB3           | 100        | 442/633          | Phenicol resistance                                                             | AJ009818         | C_614386  | 614827   | C_1121323 | 1121764   |
|                      |                 | 99.13      |                  |                                                                                  |                  | P1_137861 | 138302   | P2_39809  | 40250     |
| RIFAMPICIN           | cmrA1           | 100        | 453/453          | Rifampicin resistance                                                            | AB212941         | P3_45289  | 46515    | P1_16503  | 17408     |
| TETRACYCLINES        | tet(A)          | 100        | 1200/1200        | Tetracycline resistance                                                           | AJ517790         | P1_137861 | 138302   | P2_39809  | 40250     |
| Sulphonamides        | sul1            | 100        | 927/927          | Sulphonamide resistance                                                           | CP002151         | P1_263120 | 264046   | P3_116160 | 117086    |
|                      | sul2            | 100        | 837/837          |                                                                                  | CP002151         | P1_263120 | 264046   | P3_116160 | 117086    |
|                      |                 | 99.59      | 816/816          |                                                                                  | GO421466         | P1_263120 | 264046   | P3_116160 | 117086    |
| Trimmethoprim        | dfrA1           | 100        | 474/474          | Trimethoprim resistance                                                           | X00926           | C_3627607 | 3628080  | C_3573485 | 3573958   |
|                      | dfrA12          | 100        | 498/498          |                                                                                  | AB571791         | P1_261006 | 261503   | P1_270749 | 271246    | P1_52145  | 532450  |
|                      | dfrA14          | 99.59      | 483/483          |                                                                                  | DQ388123         | P1_144525 | 145007   | P2_82728  | 78574     |

KP617: C, CP012753.1; P1, CP012754.1, P2, CP012755.1
PittNDM01: C, CP006798.1; P1, CP006799.1, P2, CP006800.1; P3, CP006801.1, P4, CP006802.1
ATCC BAA-2146: C, CP006659.2; P1 (PCuAs), CP006663.1, P2 (PHg), CP006662.2, P3, CP006660.1, P4, CP006661.1
NUHL24385: C, CP014004.1; P1, CP014005.1, P2, CP014006.1

a C chromosome, P plasmid
| Strains                                      | Category                  | Subcategory                              | Name                        |
|---------------------------------------------|---------------------------|------------------------------------------|-----------------------------|
| KP617, PittNDM01, NUHL24385 and ATCC BAA-2146 | Acid resistance           | Urease                                   | ureA, ureB, ureF, ureG, ureH |
| Adherence                                   | Cell wall associated fibronectin binding protein |                                         |                             |
| Adherence                                   | CFAI fimbriae             |                                         |                             |
| Adherence                                   | Flagella                  | fliN, fliR, fliS                         |                             |
| Adherence                                   | Hsp60                     |                                         |                             |
| Adherence                                   | Intercellular adhesin     | icaA, icaR                               |                             |
| Adherence                                   | Listeria adhesion protein | lap                                      |                             |
| Adherence                                   | OapA                      | oapA                                     |                             |
| Adherence                                   | Omp89                     | omp89                                    |                             |
| Adherence                                   | P fimbriae                | papX                                     |                             |
| Adherence                                   | PEB1/CBF1                 | pebA                                     |                             |
| Adherence                                   | Phosphoethanolamine modification |                             |                             |
| Adherence                                   | Type I fimbriae           | fimB, fimE, fimG                         |                             |
| Adherence                                   | Type IV pili              | comE/pilQ                                |                             |
| Adherence                                   | Type IV pili biosynthesis | pilM, pilW                               |                             |
| Adherence                                   | Type IV pili twitching motility related proteins | chpD, chpE                            |                             |
| Adhesin                                     | Laminin-binding protein   | lmb                                      |                             |
| Adhesin                                     | Streptococcal lipoprotein rotamase A | slrA                                     |                             |
| Adhesin                                     | Streptococcal plasmin receptor/GAPDH |                             |                             |
| Adhesin                                     | Type IV pili              | pilD, pilN, pilR, pilS, pilT             |                             |
| Amino acid and purine metabolism            | Glutamine synthesis       | glnA1                                    |                             |
| Amino acid and purine metabolism            | Leucine synthesis         | leuD                                     |                             |
| Amino acid and purine metabolism            | Lysine synthesis          | lysA                                     |                             |
| Amino acid and purine metabolism            | Proline synthesis         | proC                                     |                             |
| Amino acid and purine metabolism            | Purine synthesis          | purC                                     |                             |
| Amino acid and purine metabolism            | Tryptophan synthesis      | trpD                                     |                             |
| Anaerobic respiration                       | Nitrate reductase         | narG, narH, narI                         |                             |
| Anaerobic respiration                       | Nitrate/nitrite transporter | narK2                                   |                             |
| Anti-apoptosis factor                       | NuoG                      | nuoG                                     |                             |
| Antimicrobial activity                      | Phenazines biosynthesis   | phzE1, phzF1, phzG1, phzS               |                             |
| Antiphagocytosis                            | Alginate regulation       | algQ, algR, algU, algW, algZ, mucB, mucC, mucD, mucP |                             |
| Antiphagocytosis                            | Capsular polysaccharide   | cpsB, wblT, wblW, wcB, wodB, wcD, wcE, wza, wzc |                             |
| Antiphagocytosis                            | Capsule                   | cpsF                                     |                             |
| Antiphagocytosis                            | Capsule I                 | gmrA, wcBN, wcBF, wcBR, wcBT, wzt2       |                             |
| Cell surface components                     | GPL locus                 | fadES, fmt, rmlB                         |                             |
| Cell surface components                     | MymA operon               | adhD, fadD13, sadH, tgs4                |                             |
| Cell surface components                     | PDIM (phthiocerol dimycocerosate) and PGL (phenolic glycolipid) biosynthesis and transport | ddrA, mas, ppsC, ppsE |                             |
| Cell surface components                     | Potassium/proton antipporter | kelB                                    |                             |
| Cell surface components                     | Proximal cyclopropane synthase of alpha mycolates | pcaA                                    |                             |
| Cell surface components                     | Trehalose-recycling ABC transporter | lpaY, sugA, sugB, sugC |                             |
| Chemotaxis and motility                     | Flagella                  | flaA, flaB                               |                             |
| Efflux pump                                 | FarAB                     | farA, farB                               |                             |
Table 3 continued

| Strains            | Category                        | Subcategory                              | Name                                                                 |
|--------------------|---------------------------------|------------------------------------------|----------------------------------------------------------------------|
| Efflux pump        | MtrCDDE                         | mtrC, mtrD                               |                                                                      |
| Endotoxin          | LOS                             | gmrA/lpcA, kdaA, kpsF, lgtF, licA, lpxH, |                                                                      |
| Endotoxin          | LPS                             | msbA, opsX/rfaC, orfM, rfaD, rfaE, rfaF, |                                                                      |
|                    | LPS-modifying enzyme            | pagP                                     |                                                                      |
| Exoenzyme          | Cysteine protease               | spbP                                     |                                                                      |
| Exoenzyme          | Streptococcal enolase           | eno                                      |                                                                      |
| Fimbrial adherence determinants | Agf/Csg                      | csgD                                     |                                                                      |
| Fimbrial adherence determinants | Fim                           | fimA, fimC, fimD, fimF, fimH, fimI       |                                                                      |
| Fimbrial adherence determinants | Lpf                           | lpfB, lpfC                              |                                                                      |
| Fimbrial adherence determinants | Stg                           | stgA                                     |                                                                      |
| Fimbrial adherence determinants | Sth                           | sthA, sthB, sthC, sthD, sthE            |                                                                      |
| Fimbrial adherence determinants | Sti                           | stiB                                     |                                                                      |
| Glycosylation system | N-linked protein glycosylation | pgI                                      |                                                                      |
| Host immune evasion | Exopolysaccharide              | galE, galU, manA, mrsA/glmM, pgi         |                                                                      |
| Host immune evasion | LPS glucosylation              | gntB                                     |                                                                      |
| Host immune evasion | Polyglutamic acid capsule       | capD                                     |                                                                      |
| Immune evasion     | LPS                             | acpX, hirB, lpxA, lpxB, lpxC, lpxD, lpxK, |                                                                      |
| Intra cellular survival | LigA                          | ligA                                     |                                                                      |
| Intra cellular survival | Lipoate protein ligase A1     | lplA1                                    |                                                                      |
| Intra cellular survival | Mip                           | mip                                      |                                                                      |
| Intra cellular survival | Oligopeptide-binding protein   | oppA                                     |                                                                      |
| Intra cellular survival | Post-translocation chaperone   | prsA2                                    |                                                                      |
| Intra cellular survival | Sugar-uptake system            | hpt                                      |                                                                      |
| Invasion           | Ail                             | ail                                      |                                                                      |
| Invasion           | Cell wall hydrolase             | iap/cwhA                                 |                                                                      |
| Iron acquisition   | Cytochrome c muturation (ccm) locus | ccmA, ccmB, ccmC, ccmE, ccmF                |                                                                      |
| Iron acquisition   | Ferrous iron transport         | feoA, feoB                              |                                                                      |
| Iron acquisition   | Iron acquisition/assimilation locus | iarB                                    |                                                                      |
| Iron and heme acquisition | Haemophilus iron transport locus | hitA, hitB, hitC                          |                                                                      |
| Iron and heme acquisition | Heme biosynthesis              | hemA, hemB, hemC, hemD, hemE, hemG, hemH, hemI, hemM, hemN, hemX, hemY |                                                                      |
| Iron uptake        | ABC transporter                 | fagD                                     |                                                                      |
| Iron uptake        | ABC-type heme transporter       | hmuZ, hmuU, hmuV                          |                                                                      |
| Iron uptake        | Achromobactin biosynthesis and transport | acsB, cbrB, cbrD                |                                                                      |
| Iron uptake        | Aerobactin transport           | iutA                                     |                                                                      |
| Iron uptake        | ciu iron uptake and siderophore biosynthesis system | ciuD                                         |                                                                      |
| Iron uptake        | Enterobactin receptors         | igrA                                     |                                                                      |
| Iron uptake        | Enterobactin synthesis         | entE, entF                               |                                                                      |
| Iron uptake        | Enterobactin transport         | fepA, fepB, fepC, fepD, fepG             |                                                                      |
| Iron uptake        | Heme transport                 | shU                                      |                                                                      |
| Iron uptake        | Hemin uptake                  | chuS, chuT, chuY                         |                                                                      |
| Iron uptake        | Iron-regulated element         | ireA                                     |                                                                      |
| Iron uptake        | Iron/managanease transport     | sitA, sitB, sitC, sitD                   |                                                                      |
| Iron uptake        | Periplasmic binding protein-dependent ABC transport systems | viuC                                         |                                                                      |
| Strains                        | Category                        | Subcategory                          | Name                               |
|-------------------------------|---------------------------------|--------------------------------------|------------------------------------|
| Iron uptake                   | Pyochelin                       | pchA, pchB, pchR                     |
| Iron uptake                   | Pyoverdine                      | pvdE, pvdH, pvdJ, pvdM, pvdN, pvdO    |
| Iron uptake                   | Salmochelin synthesis and transport | iroE, iroN                           |
| Iron uptake                   | Vibriobactin biosynthesis       | vibB                                 |
| Iron uptake                   | Vibriobactin utilization        | viuB                                 |
| Iron uptake                   | Yersiniabactin siderophore      | ybtA, ybtP                           |
| Iron uptake systems           | Ton system                      | exbB, exbD                           |
| Lipid and fatty acid metabolism | FAS-II                          | kasB                                 |
| Lipid and fatty acid metabolism | Isocitrate lyase                | icl                                  |
| Lipid and fatty acid metabolism | Pantothenate synthesis          | panC, panD                           |
| Lipid and fatty acid metabolism | Phospholipases C                | plcD                                 |
| Macrophage inducible genes    | Mig-5                           | mig-5                                |
| Magnesium uptake              | Mg2+ transport                  | mgtB                                 |
| Mammalian cell entry (mce)    | Operons                         | mce3                                 |
| Metal exporters               | Copper exporter                 | ctpV                                 |
| Metal uptake                  | ABC transporter                 | irtB                                 |
| Metal uptake                  | Exochelin (smegmatis)           | fxbA                                 |
| Metal uptake                  | Herme uptake                    | mmp2, 11                             |
| Metal uptake                  | Magnesium transport             | mgtC                                 |
| Metal uptake                  | Mycobactin                      | fadE14, mbtH, mbtI                    |
| Motility and export apparatus | Flagella                        | flhH, flhG, flhT                      |
| Nonfimbrial adherence determi- | SinH                            | sinH                                 |
| nants                        | Other adhesion-related proteins | EF-Tu                                | tuf                                 |
| Other adhesion-related proteins | PDH-B                           | pdhB                                 |
| Others                        | MspB2                           | mspB2                                |
| Others                        | Nuclease                        | nuc                                  |
| Others                        | VirK                            | virK                                 |
| Phagosome arresting           | Nucleoside diphosphate kinase   | ndk                                  |
| Protease                      | Trigger factor                  | tig/ropA                             |
| Proteases                     | Proteasome-associated proteins  | mpa                                  |
| Quorum sensing                | Autoinducer-2                  | luxS                                 |
| Quorum sensing systems        | Acylhomoserine lactone synthase | hdtS                                 |
| Quorum sensing systems        | N-(butanoyl)-L-homoserine lactone QS system | rhlR |
| Regulation                    | Alternative sigma factor RpoS   | rpoS                                 |
| Regulation                    | AtxA                            | atxA                                 |
| Regulation                    | BvrRS                           | bvrR                                 |
| Regulation                    | Carbon storage regulator A      | csrA                                 |
| Regulation                    | DevR/S                          | devR/dosR                            |
| Regulation                    | GacS/GacA two-component system  | gacA, gacS                           |
| Regulation                    | LetA/LetS two component         | letA                                 |
| Regulation                    | LisR/LisK                       | lisK                                 |
| Regulation                    | MprA/B                          | mprA, mprB                           |
| Regulation                    | PhoP/R                          | phoR                                 |
| Regulation                    | RegX3                           | regX3                                |
| Regulation                    | RelA                            | relA                                 |
| Regulation                    | SenX3                           | senX3                                |
| Regulation                    | Sigma A                         | sigA/rpoV                            |
Table 3 continued

| Strains                          | Category                      | Subcategory                                      | Name                        |
|---------------------------------|-------------------------------|--------------------------------------------------|-----------------------------|
| Regulation                      | Two-component system          | bvgA, bvgS                                       |                             |
| Secreted proteins               | Antigen 85 complex            | fbpB, fbpC                                       |                             |
| Secretion system                | Accessory secretion factor    | secA2                                            |                             |
| Secretion system                | Bsa T3SS                      | bprC                                             |                             |
| Secretion system                | Flagella (cluster I)          | fliZ                                             |                             |
| Secretion system                | Mxi-Spa TTSS effectors controlled by MxiE | ipaH, ipaH2.5                                   |                             |
| Secretion system                | P. aeruginosa TTSS            | exsA                                             |                             |
| Secretion system                | P. syringae TTSS effectors    | hopA, hopAN1, hopI                               |                             |
| Secretion system                | TTSS secreted proteins        | bopD                                             |                             |
| Secretion system                | Type III secretion system     | bscS                                             |                             |
| Secretion system                | Type VII secretion system     | essC                                             |                             |
| Secretion system                | VirB/VirD4 type IV secretion system & translocated effector Beps | bepA |                             |
| Serum resistance                | BrkAB system                  | brkAB                                            |                             |
| Stress adaptation               | AhpC                          | ahpC                                             |                             |
| Stress adaptation               | Catalase-peroxidase           | katG                                             |                             |
| Stress adaptation               | Pore-forming protein          | ompA                                             |                             |
| Stress protein                  | Catalase                      | katA                                             |                             |
| Stress protein                  | Manganese transport system    | mntA, mntB, mntC                                |                             |
| Stress protein                  | Recombinational repair protein | recN                                            |                             |
| Stress protein                  | SodCl                         | sodCl                                            |                             |
| Surface protein anchoring       | Lipoprotein diacylglyceryl trans-ferase | lgt         |                             |
| Surface protein anchoring       | Lipoprotein-specific signal peptidase II | ispA     |                             |
| Toxin                           | Beta-hemolysin/cytolysin      | cylG                                             |                             |
| Toxin                           | Enterotoxin                   | entA, entB, entC, entD                           |                             |
| Toxin                           | Hydrogen cyanide production   | hcnC                                             |                             |
| Toxin                           | Phytotoxin phaseolysin        | argD, argK, csqC1                               |                             |
| Toxin                           | Streptolysin S                | sagA                                             |                             |
| Toxins                          | Alpha-hemolysin               | hlyA                                             |                             |
| Toxins                          | Enterotoxin SenB/TieB         | senB                                             |                             |
| Two-component system            | PhoPQ                         | phoP, phoQ                                       |                             |
| Type I secretion system         | ABC transporter for dispersin | aatC                                             |                             |
| Antiphagocytosis                | Capsular polysaccharide       | cpsA                                             |                             |
| Cell surface components         | GPL locus                      | pks                                              |                             |
| Cell surface components         | Mycolic acid trans-cyclopropane synthetase | cmaA2 |                             |
| Endotoxin                       | LOS                           | lgtA                                             |                             |
| Iron uptake                     | Pyoverdine receptors          | fpvA                                             |                             |
| Iron uptake                     | Vibriobactin biosynthesis     | vibA                                             |                             |
| Iron uptake                     | Yersiniabactin siderophage    | ipr1, ipr2, ybrE, ybrQ, ybrS, ybrT, ybrU, ybrX  |                             |
| Secretion system                | EPS type II secretion system  | epsG                                             |                             |
| Secretion system                | Trw type IV secretion system  | trwE                                             |                             |
| Secretion system                | VirB/VirD4 type IV secretion system & translocated effector Beps | virB11, virB4, virB9 |                             |
| Toxin                           | RTX toxin                     | rtxA, rtxB                                      |                             |
Bacteriophages are applicable to phage therapy. In particular, bacteriophages have been used as a potential therapeutic agent to treat patients infected with multidrug resistant bacteria [47] and have been used for serological typing for diagnostic and epidemiological typing in *K. pneumoniae* [48]. However, because we did not

### Table 3 continued

| Strains          | Category                        | Subcategory                        | Name       |
|------------------|---------------------------------|------------------------------------|------------|
| KP617 and PittNDM01 | Adhesin                         | Streptococcal collagen-like proteins | sCIB       |
|                  | Chemotaxis and motility         | Flagella                           | fHc        |
|                  | Iron uptake                      | Yersiniabactin siderophore         | fYuA       |
not characterize the phages in KP617, we are not sure whether or not they are active.

**Future directions**

*Klebsiella pneumoniae* subsp. *pneumoniae* KP617, which is strongly pathogenic, is known to cause severe nosocomial infections. This strain, as well as the PittNDM01 and NUH124835 strains in the WGLW2 group, belongs to the sequence type ST14. In this study, we investigated specific antimicrobial resistance genes, virulence factors, and prophages related to pathogenicity and drug resistance in *K. pneumoniae* subsp. *pneumoniae* KP617 via a comparative analysis of the genome of this strain and those of PittNDM01, NUH124835, and ATCC BAA-2146. Significant homology was observed in terms of the genomic structure, gene content, antimicrobial resistance genes and virulence factors between KP617 and the reference strains; phylogenetic analysis indicated that KP617 is next to PittNDM01, despite the presence of large inversions. Moreover, KP617 shares 98.3% of its genes with PittNDM01. Despite the similarity in genome sequences and content, there were differences in phage-related genes, plasmids, and plasmid-harborized antimicrobial resistance genes. PittNDM01 harbors two more plasmids and 21 more antimicrobial resistance genes than KP617. In order to elucidate the precise role of these factors in the pathogenicity of KP617, further studies are required.

**Availability of supporting data**

**Nucleotide sequence accession numbers** The complete genome sequence of *K. pneumoniae* KP617 has been deposited in DDBJ/EMBL/GenBank under the accession numbers CP012753, CP012754, and CP012755 [49].

**Additional file**

**Additional file 1.** Annotated genes of KP617 and comparison of their sequences with those of the reference strains by using the RAST server.

**Abbreviations**

BSR: BLAST score ratio; CDS: coding DNA sequences; HGT: horizontal gene transfer; MLST: multi-locus sequence typing; NDM-1: New Delhi metallo-beta-lactamase 1; RAST: Rapid Annotation using Subsystem Technology; ST: sequence type; str: strain; substr: substring.

**Authors’ contributions**

DWK and WK designed and led the project and contributed to the interpretation of the results. DWK drafted the manuscript. YHJ and TK interpreted the results. YHJ, SHL, MRY, and TK performed the gene annotation and bioinformatics analysis. TK and YHJ wrote the manuscript. All authors read and approved the final manuscript before submission.

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**Competing interests**

The authors declare that they have no competing interests.

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