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EPIDEMIOLOGY AND SURVEILLANCE

# Genomic Epidemiology of Carbapenem-Resistant *Klebsiella* in Qatar: Emergence and Dissemination of Hypervirulent *Klebsiella pneumoniae* Sequence Type 383 Strains

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**ABSTRACT** The emergence of carbapenem-resistant, hypervirulent *Klebsiella pneumoniae* is a new threat to health care. We studied the molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* isolates in Qatar using whole-genome sequence data. We also characterized the prevalence and genetic basis of hypervirulent phenotypes and established the virulence potential using a *Galleria mellonella* model. Of 100 *Klebsiella* isolates studied, NDM and OXA-48 were the most common carbapenemases. Core genome single-nucleotide polymorphism (SNP) analysis indicated the presence of diverse sequence types and clonal lineages; isolates belonging to *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* sequence type 196 (ST196) and ST1416 may be disseminated among several health care centers. Ten *K. pneumoniae* isolates carried *rmpA* and/or truncated *rmpA2*, and 2 isolates belonged to KL2, indicating low prevalence of classical hypervirulent isolates. Isolates carrying both carbapenem resistance and hypervirulence genes were confined mainly to ST231 and ST383 isolates. One ST383 isolate was further investigated by MinION sequencing, and the assembled genome indicated that $bla_{NDM}$ was located on an IncHI1B-type plasmid (pFQ61_ST383_NDM-5) which coharbored several virulence factors, including the regulator of the mucoid phenotype (*rmpA*), the regulator of mucoid phenotype 2 (*rmpA2*), and aerobactin (*iucABCD* and *iutA*), likely resulting from recombination events. Comparative genomics indicated that this hybrid plasmid may be present in two additional Qatari ST383 isolates. Carbapenem-resistant, hypervirulent *K. pneumoniae* ST383 isolates pose an emerging threat to global health due to their simultaneous hypervirulence and multidrug resistance.

**KEYWORDS** carbapenem resistance, genomics, molecular epidemiology, virulence, hybrid plasmid, *Klebsiella*

*Klebsiella pneumoniae* is a Gram-negative bacterial pathogen that is widely present in nature and in the human intestine. *K. pneumoniae* is well known to cause hospital-acquired infections in immunocompromised patients (1, 2), but infections caused by *K. pneumoniae* can also occur in long-term-care facilities, such as nursing homes,



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and in the community. Types of infection vary and include hospital-acquired pneumonia, lung abscesses, bloodstream infections, catheter-related infections, wound or surgical site infections, upper and lower urinary tract infections, liver abscesses, and meningitis (1). Based on the genome sequencing data, various related species and subspecies, such as *K. aerogenes*, *K. oxytoca*, *K. quasipneumoniae*, and *K. variicola*, have been recognized (3–5). In our previous study, in which we had studied 149 carbapenem-resistant *Enterobacterales* (CRE) isolates in Qatar, *K. pneumoniae* (54%) and *K. quasipneumoniae* (16%) isolates were prevalent (6).

CRE infections are a global health priority and are among the most serious antimicrobial resistance (AMR) threats (7). Carbapenem resistance in *K. pneumoniae* is primarily driven by production of carbapenemases, with extended-spectrum $\beta$-lactamases (ESBL) such as CTX-M-2 playing a supplementary role in hydrolyzing cephalosporins in combination with decreased membrane permeability in the cell wall (6, 8–11). In the aforementioned study in Qatar, genes encoding metallo-$\beta$-lactamases were detected in 45.8% of the isolates and OXA-48-like enzymes in 40.3% (6).

Hypervirulent *K. pneumoniae* (hvKp) can cause serious life-threatening infections, such as liver abscesses, and is associated with high mortality and morbidity (12). Several virulence factors contribute to the pathogenicity, including hypermucoviscosity-specific capsular antigens (i.e., K1 and K2 serotypes) and virulence loci. such as mucoid phenotype regulator, encoded by *rmpA*, and aerobactin (12, 13). Traditionally, multidrug-resistant (MDR) and hypervirulent phenotypes in *K. pneumoniae* have been associated with distinct lineages. However, MDR lineages acquiring virulence traits or hypervirulent lineages acquiring AMR genes have increasingly been reported in the last decade, especially in South and Southeast Asia (14–18), mostly through dissemination of conjugative and hybrid plasmids harboring both resistance and virulence genes. This may lead to widely disseminated community-acquired infections in healthy people that are difficult to treat.

While carbapenem resistance in *Klebsiella* is increasingly documented in the Middle East region, there is limited information on hypervirulence and how it intersects with carbapenem resistance. We therefore conducted an in-depth analysis of *Klebsiella* genomes that were sequenced in the study of CRE in Qatar during 2014 to 2017 (6), with the following three aims: (i) to describe the genetic diversity, AMR genes, and virulence determinants of *Klebsiella* isolates, (ii) to investigate the molecular epidemiology data of selected sequence types (ST) that had $\geq$4 isolates, and (iii) to characterize the genetic context and virulence potential of a carbapenem-resistant hvKp strain belonging to ST383.

## RESULTS

**Epidemiology of sequence types and AMR genes.** Whole-genome sequencing (WGS) had been carried out on 100 carbapenem-resistant *Klebsiella* isolates, which was part of a larger-scale epidemiology study that included all CRE isolates retrieved from the Hamad Medical Corporation's microbiology department (Doha, Qatar) from 1 April 2015 to 30 November 2017 (6). As previously described, the species included *K. pneumoniae* ($n$ = 80), *K. quasipneumoniae* subsp. *quasipneumoniae* ($n$ = 14), *K. quasipneumoniae* subsp. *similipneumoniae* ($n$ = 2), *K. aerogenes* ($n$ = 3), and *K. oxytoca* ($n$ = 1). Among the 40 different STs reported, 23 were represented by a single isolate; 4 isolates were not reported elsewhere and were submitted for assignment of new ST numbers, and 1 isolate (FQ156, ST25-1LV) did not meet the criteria for assignment (see Table S1 in the supplemental material). Common *K. pneumoniae* STs included ST147 ($n$ = 13), ST231 ($n$ = 7), and ST11 ($n$ = 5). ST147 and ST11 belong to widespread clonal group 147 (CG147) and CG258 (Table 1; Table S1). ST147 isolates were identified from all specimen types (blood, pus, sterile body fluid, urine, and respiratory tract), while ST231 isolates were collected from all specimen types except sterile body fluid. Out of the 14 *K. quasipneumoniae* subsp. *quasipneumoniae* isolates (14%) (6), 9 belonged to ST196 and 4 belonged to ST1416. These isolates were identified from blood, pus, and urine specimens (Table 1).

As previously reported, carbapenemase genes identified included those encoding

**TABLE 1** Comparison of key features of *Klebsiella* isolates from different specimens in Qatar

| Group or feature | No. (%) of isolates from: | | | | | |
|---|---|---|---|---|---|---|
| | Blood ($n$ = 20) | Pus ($n$ = 12) | Fluid and other sources ($n$ = 6) | Urine ($n$ = 37) | RT[a] ($n$ = 23) | Total |
| *Species* | | | | | | |
| *K. pneumoniae* | 13 (65) | 8 (66.7) | 5 (83.3) | 32 (86.4) | 22 (95.7) | 80 |
| *K. quasipneumoniae* | 6 (30) | 3 (25) | 1 (16.7) | 5 (13.5) | 1 (4.3) | 16 |
| *K. aerogenes* | | 1 (8.3) | | 2 (5.4) | | 3 |
| *K. oxytoca*[b] | 1 (5) | | | | | 1 |
| Major STs (CG) | | | | | | |
| ST147 (CG147) | 3 (15) | 1 (8.3) | 1 (16.7) | 5 (13.5) | 3 (13) | 13 |
| ST231 (CG2321) | 1 (5) | 1 (8.3) | | 1 (2.7) | 4 (17.4) | 7 |
| ST11 (CG258) | | 1 (8.3) | | 2 (5.4) | 2 (8.7) | 5 |
| ST14/15 (CG15) | | 1 (8.3) | | 3 (8.1) | | 4 |
| ST383 | | 1 (8.3) | 1 (16.7) | 2 (5.4) | | 4 |
| ST196 | 3 (15) | 2 (16.7) | | 4 (10.8) | | 9 |
| ST1416 | 2 (10) | | 1 (16.7) | | 1 (4.3) | 4 |
| AMR | | | | | | |
| NDM | 10 (50) | 5 (43) | 2 (33.3) | 22 (59.5) | 6 (26.1) | 45 |
| OXA-48 | 8 (40) | 3 (25) | 1 (16.7) | 18 (48.6) | 12 (52.2) | 42 |
| KPC | | 2 (16.7) | 1 (16.7) | | 2 (8.7) | 5 |
| CTX-M | 19 (95) | 12 (100) | 5 (83.3) | 34 (91.9) | 21 (91.3) | 91 |
| Virulence | | | | | | |
| *rmpA* | 1 (5) | 1 (8.3) | 2 (33.3) | 2 (5.4) | 2 (8.7) | 8 |
| *rmpA2* | | 1 (8.3) | 2 (33.3) | 4 (10.8) | 2 (8.7) | 9 |
| *iuc* | 2 (10) | 3 (25) | 2 (33.3) | 5 (13.5) | 4 (17.4) | 16 |
| *iro* | | 1 (8.3) | | 3 (8.1) | 1 (4.3) | 5 |
| *clb* | | 1 (8.3) | 1 (16.7) | | 1 (4.3) | 3 |
| KL2/KL20 | 1 (5) | | | 4 (10.8) | 2 (8.7) | 7 |

[a]RT, respiratory tract.
[b]As *K. michiganensis* in Kleborate.

NDM-1 ($n$ = 39), OXA-48 ($n$ = 20), OXA-232 ($n$ = 10), and OXA-181 ($n$ = 12), but KPC-2 ($n$ = 3) and KPC-3 ($n$ = 2) were rare. Seven *K. pneumoniae* isolates carried more than one carbapenemase gene, while 15 isolates did not harbor any carbapenemase gene and instead carried combinations of $bla_{\text{CTX-M}}$ genes with mutations in porin genes *ompK35* and *ompK36* (Table S1), which have previously been linked to carbapenem resistance in *Klebsiella* (19). In total, 68 out of 100 *Klebsiella* isolates had *ompK35* or *ompK36* loss/truncation/mutation, which may contribute to reduced susceptibility to carbapenems (Table S1). $bla_{\text{CTX-M}}$ was coharbored by 75 isolates (75/85 [88.2%]), including 66 isolates (77.6%) harboring $bla_{\text{CTX-M-15}}$, 7 isolates (8.2%) harboring $bla_{\text{CTX-M-14b}}$, and 2 isolates (2.3%) harboring $bla_{\text{CTX-M-27}}$. Cocarriage of $bla_{\text{NDM}}$ and/or $bla_{\text{OXA-48-type}}$ and $bla_{\text{CTX-M}}$ was reported for 6 isolates, including 3 ST383 isolates (Table S1).

We then studied the genetic relationships among species/isolates using core genome single-nucleotide polymorphism (cgSNP) analysis based on whole-genome alignment. The cgSNP alignment containing 403,061 bases indicated that 93% identity was shared by isolates among *K. pneumoniae* and *K. quasipneumoniae*, as well as *K. oxytoca* and *K. aerogenes* (Fig. 1; Table S2). cgSNP analysis illustrated that *K. pneumoniae* and *K. quasipneumoniae* isolates shared around 98.2% identity (>7,000 cgSNP differences), and they each formed monophyletic clades. There were genetic variations within isolates of *K. pneumoniae* (0 to 905 cgSNPs), *K. quasipneumoniae* subsp. *quasipneumoniae* (0 to 868 cgSNPs), *K. quasipneumoniae* subsp. *similipneumoniae* (859 cgSNPs), and *K. aerogenes* (10 to 1,142 cgSNPs) (Table S2). Genetic variations were also detected within prevalent *K. pneumoniae* isolates in terms of STs and presence or absence of certain AMR genes. For example, ST147 isolates differed by 2 to 56 cgSNPs, and out of 13 isolates, 6 had $bla_{\text{NDM}}$ while 8 had $bla_{\text{OXA-48-like}}$. Similarly, ST231 isolates differed by 2 to 25 cgSNPs, and out of 7 isolates, only 5 had $bla_{\text{OXA-48}}$. ST383 isolates differed by 3 to 12 cgSNPs, and out of 4 isolates, 3 had $bla_{\text{NDM-5}}$. In contrast, *K. quasipneumoniae* subsp.

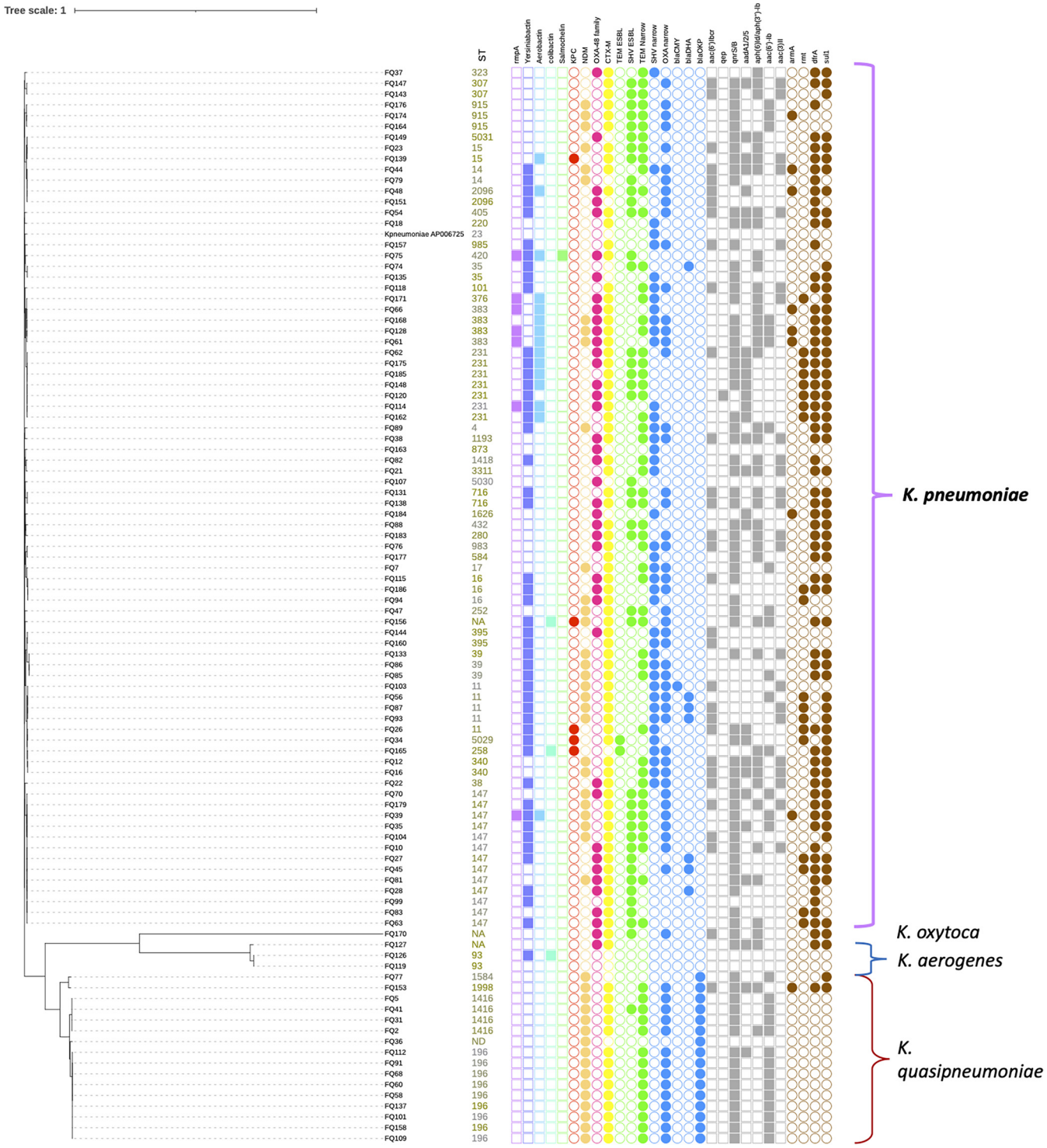


**FIG 1** Genetic relationship of *Klebsiella* isolates inferred from cgSNPs (Parsnp) overlaid with the presence/absence of AMR and virulence phenotypes.

*quasipneumoniae* ST196 was highly clonal (0 to 1 cgSNPs), and all isolates carried $bla_{\text{NDM-1}}$ (Fig. 1).

Although short-read assemblies were fragmented due to repetitive mobile genetic elements like insertion sequences (ISs), we attempted to gain an overview on the spectrum of plasmids associated with carbapenemase genes based on the replicon sequences detected from the same contigs as the carbapenemase genes. $bla_{\text{NDM-1}}$ was associated with the IncFII replicon, while $bla_{\text{OXA-48-like}}$ ($bla_{\text{OXA-181}}$ and $bla_{\text{OXA-232}}$) were commonly associated with ColKP3

**TABLE 2** Plasmid replicons linked to carbapenemase genes in 24 isolates of *K. pneumoniae* and *K. quasipneumoniae* subsp. *quasipneumoniae* (from Plasmidfinder)

| Isolate | Species | ST | Date of isolation (day/mo/yr) | Carbapenemase | Replicon |
|---|---|---|---|---|---|
| FQ103 | *K. pneumoniae* | 11 | 16/3/17 | NDM-1 | IncA/C2 |
| FQ7 | *K. pneumoniae* | 17 | 20/5/15 | NDM-1 | IncFIB (pQil) |
| FQ94 | *K. pneumoniae* | 16 | 26/2/17 | OXA-181 | ColKP3 |
| FQ115 | *K. pneumoniae* | 16 | 27/4/17 | OXA-181 | ColKP3 |
| FQ186 | *K. pneumoniae* | 16 | 24/12/17 | OXA-232 | ColKP3 |
| FQ22 | *K. pneumoniae* | 38 | 7/7/15 | OXA-232 | ColKP3 |
| FQ27 | *K. pneumoniae* | 147 | 22/7/15 | OXA-181 | ColKP3 |
| FQ28 | *K. pneumoniae* | 147 | 25/7/15 | OXA-181 | ColKP3 |
| FQ45 | *K. pneumoniae* | 147 | 11/11/15 | OXA-181 | ColKP3 |
| FQ70 | *K. pneumoniae* | 147 | 21/10/16 | OXA-181 | ColKP3 |
| FQ114 | *K. pneumoniae* | 231 | 25/4/17 | OXA-232 | ColKP3 |
| FQ120 | *K. pneumoniae* | 231 | 17/5/17 | OXA-232 | ColKP3 |
| FQ148 | *K. pneumoniae* | 231 | 26/8/17 | OXA-232 | ColKP3 |
| FQ144 | *K. pneumoniae* | 395 | 20/8/17 | OXA-232 | ColKP3 |
| FQ138 | *K. pneumoniae* | 716 | 27/7/17 | OXA-48 | ColKP3 |
| FQ151 | *K. pneumoniae* | 2096 | 5/9/17 | OXA-232 | ColKP3 |
| FQ107 | *K. pneumoniae* | 5030 | 26/3/17 | OXA-232 | ColKP3 |
| FQ149 | *K pneumoniae* | 5031 | 28/8/17 | OXA-181 | ColKP3 |
| FQ58 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 3/4/16 | NDM-1 | IncFII_1_pKP91 |
| FQ60 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 22/4/16 | NDM-1 | IncFII_1_pKP91 |
| FQ91 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 12/2/17 | NDM-1 | IncFII_1_pKP91 |
| FQ101 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 14/3/17 | NDM-1 | IncFII_1_pKP91 |
| FQ117 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 28/4/17 | NDM-1 | IncFII_1_pKP91 |
| FQ137 | *K. quasipneumoniae* subsp. *quasipneumoniae* | 196 | 26/7/17 | NDM-1 | IncFII_1_pKP91 |

plasmids (Table 2). Most contigs carrying $bla_{NDM}$ were divergent and had different mobile genetic elements. While $bla_{NDM-1}$ was linked to IncFIB and IncA/C2 in *K. pneumoniae* in one isolate each, it was found to be associated with IncFII_1_pKP91 in 7 out of 9 *K. quasipneumoniae* ST196 isolates. When we aligned the contig carrying $bla_{NDM-1}$ and $bla_{CTX-M-15}$ in ST196 to the two homologous contigs in *K. quasipneumoniae* subsp. *quasipneumoniae* ST1416 isolates, we found that the overlapping region was highly similar (>90%) and included 6 AMR genes and ISs, suggesting that mobile genetic elements may spread these AMR genes among these STs. The contig bearing $bla_{KPC-3}$ contained a commonly reported mobile genetic element, Tn*4401a*, based on annotation (Fig. S1). Overall, among all *Klebsiella* isolates, FIB was the most common F replicon, found in 89 isolates (89%), followed by FII, in 72 isolates (72%), and FIA, in 21 isolates (21%) (Table S1).

**Prevalence of virulence markers.** We used (i) the presence of *rmpA* or *rmpA2* and/or (ii) the presence of aerobactin (*iuc*) and salmochelin (*iro*) biomarkers that are associated with hypervirulence to qualify strains that may demonstrate a hypervirulent phenotype (20). According to Kleborate results, 10 isolates (12.5%) had *rmpA* and/or *rmpA2* (*rmpA2* was truncated in all isolates), and four of them belonged to ST383. The prevalences of the *iuc* or *iro* and *rmpA* combination in ST147, ST383, ST420, and ST231 were 7.7%, 75%, 100%, and 14.3%, respectively (Table 3). Sixteen (20%) *K. pneumoniae* isolates carried the aerobactin *iuc* locus, while only 2 (2.5%) isolates harbored the salmochelin *iro* locus, and they all belonged to ST420. Two (2.5%) isolates carried the colibactin *clb* locus, one of which belonged to ST258. The *ybt* locus, encoding the acquired siderophore yersiniabactin, was detected in 49 (61.3%) *K. pneumoniae* isolates, representing 20 different STs. Five different *ybt* locus types and their associated integrative conjugative elements (ICE) were identified, and the most prevalent locus was *ybt14* ($n$ = 18 [18.8%]), with ICE*Kp5* detected in 8 STs, followed by *ybt9* with ICE*Kp3* ($n$ = 12 [12.5%]) and *ybt16* with ICE*Kp12* ($n$ = 11 [11.5%]). All three *K. aerogenes* isolates carried *iro*, and one of them (FQ126) also harbored *ybt* and *clb* (Table S1). The virulence loci, such as *ybt*, *clb*, *iro*, *rmpA*, and *rmpA2*, were not detected in any of the *K. oxytoca* or *K. quasipneumoniae* isolates. The capsule biosynthesis (KL) were identified for all isolates, spanning 91 distinct KL types (Table S1). hvKp serotype KL1 was not detected in this

**TABLE 3** Notable hypervirulent isolates in this investigation (virulence loci from Kleborate)[a]

| Isolate | Specimen | Infection | ST | *rmpA* | *rmpA2* | *ybt* | *iuc* | *iro* | *clb* | K_locus | Carbapenemase(s) | ESBL | Other $\beta$-lactamases | Omp mutation(s) and variants | Clinical outcome | Travel (30 days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FQ44 | Urine | UTI | 14 | | | *ybt14*; ICE*Kp5* | | | | KL2 | NDM-1 | CTX-M-15 | | *ompK35*, 88%; *ompK36* GD | Alive | No |
| FQ139 | Pus | IAI | 15 | | | | *iuc5* | | | KL102 | KPC-2 | CTX-M-14, SHV-28.v1 | | *ompK35*, 21% | Alive | Yes |
| FQ39 | Other | SSI | 147 | *rmp1*; KpVP-1 | *rmpA2_6**, 47% | *ybt9*; ICE*Kp3* | *iuc1* | | | KL64 | NDM-1 | CTX-M-15 | SHV-11.v1 | *ompK35*, 25% | Alive | Yes |
| FQ148 | Pus | SSI | 231 | | | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | OXA-232 | | SHV-1 | *ompK35*, 30% | Alive | No |
| FQ162 | Urine | UTI | 231 | | | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | | CTX-M-15 | | *ompK35*, 30%; *ompK36* GD | Alive | Yes |
| FQ62 | Blood | BSI | 231 | | | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | OXA-48 | CTX-M-14; CTX-M-15 | SHV-1 | *ompK35*, 30%; *ompK36*, 72% | Dead | No |
| FQ114 | RT | RTI | 231 | *rmp1*; KpVP-1 | *rmpA2_3**, 47% | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | OXA-232 | CTX-M-15 | | *ompK35*, 30%; *ompK36* GD | Dead | No |
| FQ175 | RT | RTI | 231 | | | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | OXA-232 | CTX-M-15 | SHV-1 | *ompK35*, 30%; *ompK36* GD | Alive | Yes |
| FQ185 | RT | RTI | 231 | | | *ybt14*; ICE*Kp5* | *iuc* unknown | | | KL51 | | CTX-M-15; SHV-106 | | *ompK35*, 30%; *ompK36* GD | Alive | Yes |
| FQ171 | Blood | BSI | 376 | *rmp1*; KpVP-1 | | | *iuc1* | | | KL2 | OXA-48 | CTX-M-14 | | | Dead | Yes |
| FQ168 | Urine | UTI | 383 | | *rmpA2_6**, 47% | | *iuc1* | | | KL30 | NDM-5; OXA-48 | CTX-M-14; CTX-M-15 | SHV-1 | *ompK35*, 10% | Alive | No |
| FQ128 | Urine | UTI | 383 | *rmp1*; KpVP-1 | *rmpA2_6*, 60% | | *iuc1* | | | KL30 | NDM-5; OXA-48 | CTX-M-14; CTX-M-15 | SHV-1 | *ompK35*, 10% | Alive | No |
| FQ66 | Other | SSTI | 383 | *rmp1*; KpVP-1 | *rmpA2_6**, 60% | | *iuc1* | | | KL30 | OXA-48 | CTX-M-14 | SHV-1 | *ompK35*, 10% | Alive | No |
| FQ61 | Pus | SSTI | 383 | *rmp1*; KpVP-1 | *rmpA2_6*, 60% | | *iuc1* | | | KL30 | NDM-5; OXA-48 | CTX-M-14; CTX-M-15 | SHV-1 | *ompK35*, 10% | Alive | No |
| FQ75 | Urine | UTI | 420 | *rmp1*; KpVP-1 | *rmpA2_3*, 47% | *ybt9*; ICE*Kp3* | *iuc1* | *iro1* | | KL20 | OXA-48 | CTX-M-14 | SHV-75 | | Alive | No |
| FQ72 | RT | RTI | 420 | *rmp1*; KpVP-1 | *rmpA2_3*, 47% | *ybt9*; ICE*Kp3* | *iuc1* | *iro1* | | KL20 | OXA-48 | CTX-M-14 | SHV-75 | | Alive | No |
| FQ48 | Urine | UTI | 2096 | | *rmpA2_8*, 60% | *ybt14*; ICE*Kp5* | *iuc1* | | | KL64 | OXA-232 | CTX-M-15, SHV-28.v1 | | *ompK36* GD | Alive | Yes |
| FQ156 | RT | RTI | ST25-1LV | | | *ybt14*; ICE*Kp5* | | | *clb3* | KL2 | KPC-3; NDM-1 | CTX-M-15 | SHV-11.v1 | *ompK36*, 50% | Alive | No |
| FQ135 | Urine | UTI | ST35 | | | *ybt16*; ICE*Kp12* | | | | KL2 | OXA-181 | | | | Alive | No |
| FQ133 | Urine | UTI | ST39 | | | *ybt16*; ICE*Kp12* | | | | KL2 | NDM-1 | CTX-M-15 | SHV-11.v1 | *ompK35*, 40%; *ompK36*, 55% | Alive | No |

[a]UTI, urinary tract infection; IAI, intrabdominal infection; BSI, bloodstream infection; RTI, respiratory tract infection; SSTI, skin and soft tissue infection; ESBL, extended-spectrum $\beta$-lactamase. *ybt*, *iuc*, *iro*, and *clb* encode yersiniabactin, aerobactin, salmochelin, and colibactin, respectively. Percentages in the columns for *ompK* and *rmpA2* represent percent amino acid length from the start codon (truncation).

study, while KL2, usually associated with invasive liver abscess syndrome, was detected in 5 (5.8%) isolates of different STs. One (FQ44) of them belonged to ST14, and the rest belonged to ST376, ST35, ST39, and ST25-SLV (Table 3). KL20 was detected in two ST420 isolates (Table 3). The most prevalent KL type in *K. pneumoniae* was KL64 ($n$ = 17 [17%]), followed by KL51 ($n$ = 10 [10%]) and KL46 ($n$ = 9 [9%]). The most prevalent O antigen-type loci were O2 variant 1 (O2v1) ($n$ = 19 [19.8%]), O1v1 ($n$ = 18 [18.8%]), and O1v2 ($n$ = 17 [17.7%]) (Table S1).

**In-depth investigation of *K. pneumoniae* ST383.** Our collection had four ST383 isolates (FQ61, FQ66, FQ128, and FQ168) collected at different hospitals from April 2016 to October 2017; this ST is not commonly reported. Two isolates (FQ61 and FQ128) carried both *rmpA* (hypermucoidy locus *rmpADC*) and truncated *rmpA2*, as well as $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$; in contrast, FQ168 had both carbapenemase genes but did not have *rmpA*, while FQ66 had *rmpA* and *rmpA2* but did not have $bla_{\text{NDM-5}}$. To understand the genetic basis and the plasmids associated with ST383 in Qatar, we sequenced isolate FQ61 using both Illumina and MinION technologies. Hybrid assembly revealed that FQ61 harbored a chromosome and five plasmids, including pFQ61_ST383_NDM-5 (IncHI1B; ~376 kb)-, pFQ61_ST383_OXA-48 (IncL; ~72 kb)-, and Col (phAD28; 5 to 23 kb)-type plasmids. $bla_{\text{NDM-5}}$ was located on pFQ61_ST383_NDM-5 (IncHI1B type), which also carried eight other AMR genes, including $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-9}}$, $bla_{\text{TEM-1}}$, *aac(6′)-Ib*, *aph(3′)-VI*, *aph(3′)-Ia*, *dfrA5*, and *armA*. Based on a BLAST search, plasmid FQ61_ST383_NDM-5 showed high similarity (>99%) with high query coverage (>99%) with pKpvST383L (GenBank accession number CP034201.2), a hybrid virulence/resistance plasmid reported for another ST383 strain in the United Kingdom and carrying multiple ISs as well as AMR and virulence genes (21). Based on the location of AMR and virulence genes, plasmid FQ61_ST383_NDM-5 was divided into three regions: MDR region 1, MDR region 2, and virulence (Fig. 2). Pairwise comparison revealed that MDR region 1 (34,570 bp) was highly similar (99 to 100%) to the homologous region in pKpvST383L (Fig. 2), while evidence of a large-scale inversion and rearrangement event was observed in MDR region 2 adjacent to the repetitive elements (45,327 bp) in comparison to pKpvST383L. The virulence region (42,300 bp) of FQ61_ST383_NDM-5, harboring virulence genes *rmpA*, *rmpA2*, *iucABCD*, and *iutA*, also exhibited high similarity (>99% identity and 100% query coverage) to pKvpvST383L (UK, 2018) (Fig. 2), as well as pKpvST147B (UK, 2019), pKP-135LU_HIB-FIB (Italy, 2019), pSI0646A-ARMA-Vir-NDM (Italy, 2019), phvKpST395 (Russia, 2019), phvKpST874 (Russia, 2019), and phvKpST147 (Russia, 2017) in various *K. pneumoniae* isolates (Fig. 2) (22, 23). Based on contig analysis using BANDAGE and BRIG, we identified contigs in FQ128 that were highly homologous to pFQ61_ST383_NDM-5, suggesting that FQ128 may also have a plasmid highly similar to that in FQ61 and carrying both $bla_{\text{NDM-5}}$ and other virulence genes (Fig. 3). FQ168 also carried a similar plasmid that harbored $bla_{\text{NDM-5}}$ and $bla_{\text{CTX-M-15}}$ and the other virulence genes except *rmpA*; in contrast, the plasmids in FQ66 appeared to be distinct in terms of organization (Fig. 3).

$bla_{\text{OXA-48}}$ was located on pFQ61_ST383_OXA-48 (IncL-type plasmid), together with AMR genes $bla_{\text{CTX-M-14}}$ and *aph(3′)-Vib*. pFQ61_ST383_OXA-48 was highly similar (>99.95%) to pKpvST383L_2 (CP034202.1) reported from the United Kingdom (21). Contigs homologous to pFQ61_ST383_OXA-48 were also detected in FQ66, FQ128, and FQ168, indicating that these three other ST383 isolates may possess highly similar plasmids that carry $bla_{\text{OXA-48}}$ and $bla_{\text{CTX-M-14b}}$ (Fig. 3).

Genomic comparison of our ST383 isolates with global ST383 isolates (Table S3) revealed the genetic compositions as well as different resistomes and virulomes of the isolates which might be linked to mobile genetic elements. Figure 4 illustrates the phylogenetic relatedness of the local ST383 strains together with publicly available ST383 assembled genomes and raw reads ($n$ = 32). The isolates in Qatar (FQ61, FQ128, and FQ168) clustered with those collected in Lebanon, the United Kingdom, and Italy, which also carried $bla_{\text{NDM-5}}$/$bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-14}}$, as well as several major virulence genes such as *iuc*, *rmpA1*, and *rmpA2*. In contrast, FQ66 nested in a clade which contains isolates from China and Germany mostly carrying $bla_{\text{OXA-48}}$ and

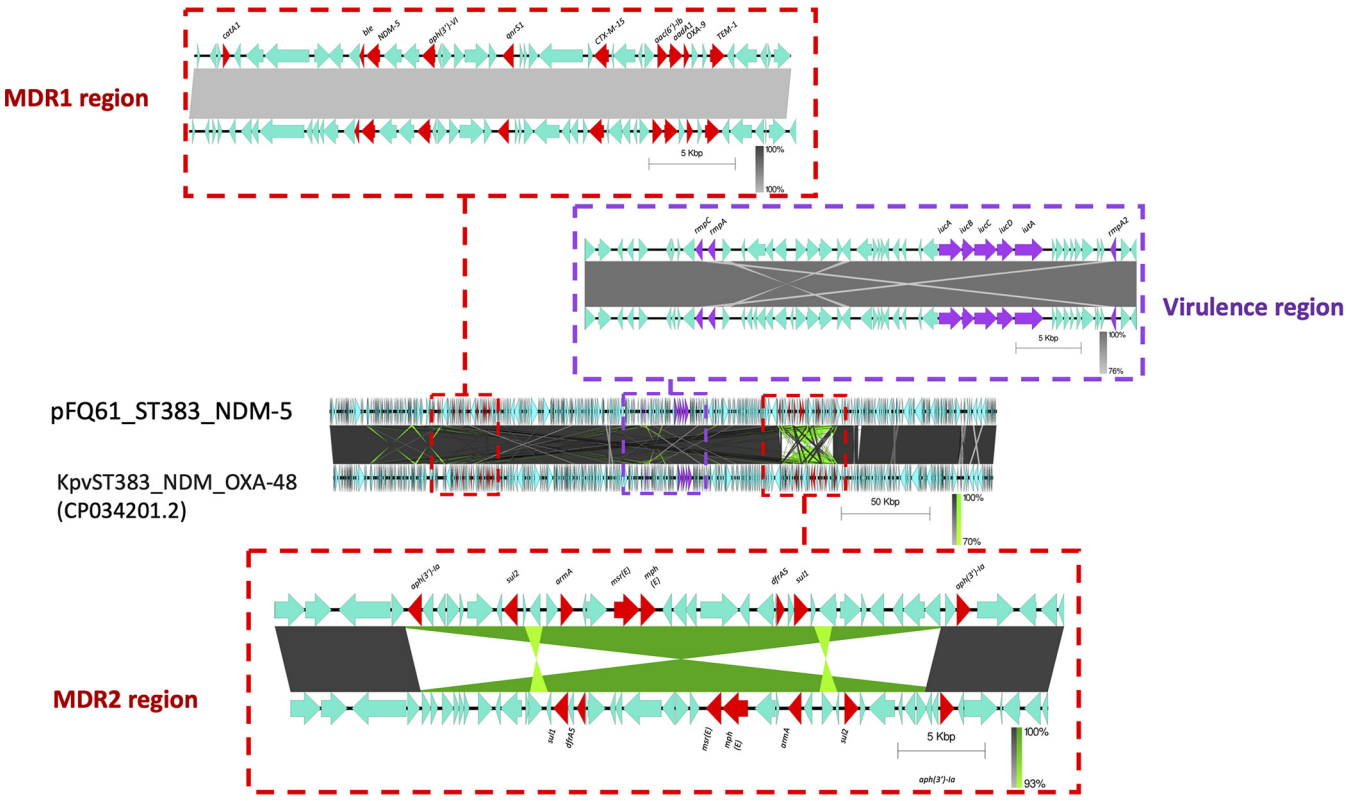


**FIG 2** Genomic comparison of hybrid plasmid harboring $bla_{NDM-5}$ and virulence phenotypes recovered from FQ61 and pKpvST383L (21).

$bla_{CTX-M-14}$. The tree suggested that there could be two independent plasmid acquisition events in the past few years: the first plasmid with $bla_{OXA-48}$ and $bla_{CTX-M-14}$, followed by a hybrid plasmid carrying $bla_{NDM-5}$ and the virulence genes (Fig. 4). However, earlier-reported ST383 isolates from Greece and France carried genes encoding carbapenemases such as KPC, OXA-48, and various VIM types (Fig. 4).

To correlate the presence of virulence genes with virulent phenotype, *Galleria mellonella* larvae were infected with selected *K. pneumoniae* isolates. In addition to the ST383 isolates, other isolates were selected based on the presence of KL2/KL20 loci such as *rmpA* and *iuc*, which are often associated with virulence. All larvae injected with 10 mM $MgSO_4$ solution only (negative control) survived. With an inoculum of $1.0 \times 10^7$ CFU, the survival rates were 0% after 72 h with a classic hypervirulent K1 isolate (BL21, control) and 10% after 96 h with two hypervirulent K2 isolates (FQ44 and FQ156) (Fig. 5). The survival rates were 10 to 30% at 96 h after infection for the ST383 isolates (FQ61, FQ128, and FQ168). Also, the survival rates were 0% after 24 h with FQ114 (ST231; $rmpA^+$ $iuc^+$) and 0% after 48 h with FQ75 (ST420; $rmpA^+$ $iuc^+$ $iro^+$ K20) and FQ179 (ST147; K64) (Fig. 5).

**Possible local outbreaks of *K. pneumoniae* and *K. quasipneumoniae*.** High-resolution SNP analysis based on read mapping and variant calling together with epidemiological investigation was performed on cases associated with *Klebsiella* isolates of prevalent STs ($n \geq 4$), including ST147, ST231, ST11, ST196, ST383, and ST1416, to identify possible outbreak and transmission events (Table S4).

The largest cluster belonged to ST147, with 13 isolates collected from May 2015 and November 2017 (Fig. 6). Over half of the isolates were from hospital-acquired infections (HAI) ($n = 7$ [54%]), while the rest was present on admission (POA) ($n = 6$ [46%]). Mean pairwise SNP difference between these 13 isolates was 87.5 SNPs (range, 0 to 139) (Table 4); however, FQ27 and FQ28 differed by 0 SNPs, and the corresponding patients were admitted to the same hospital in different units on different dates, indicating possible intrahospital transmission. Another cluster involved 7 ST231 isolates collected from July 2016 to December 2017. Although most cases were determined to be HAI ($n = 5$ [71%]) in the

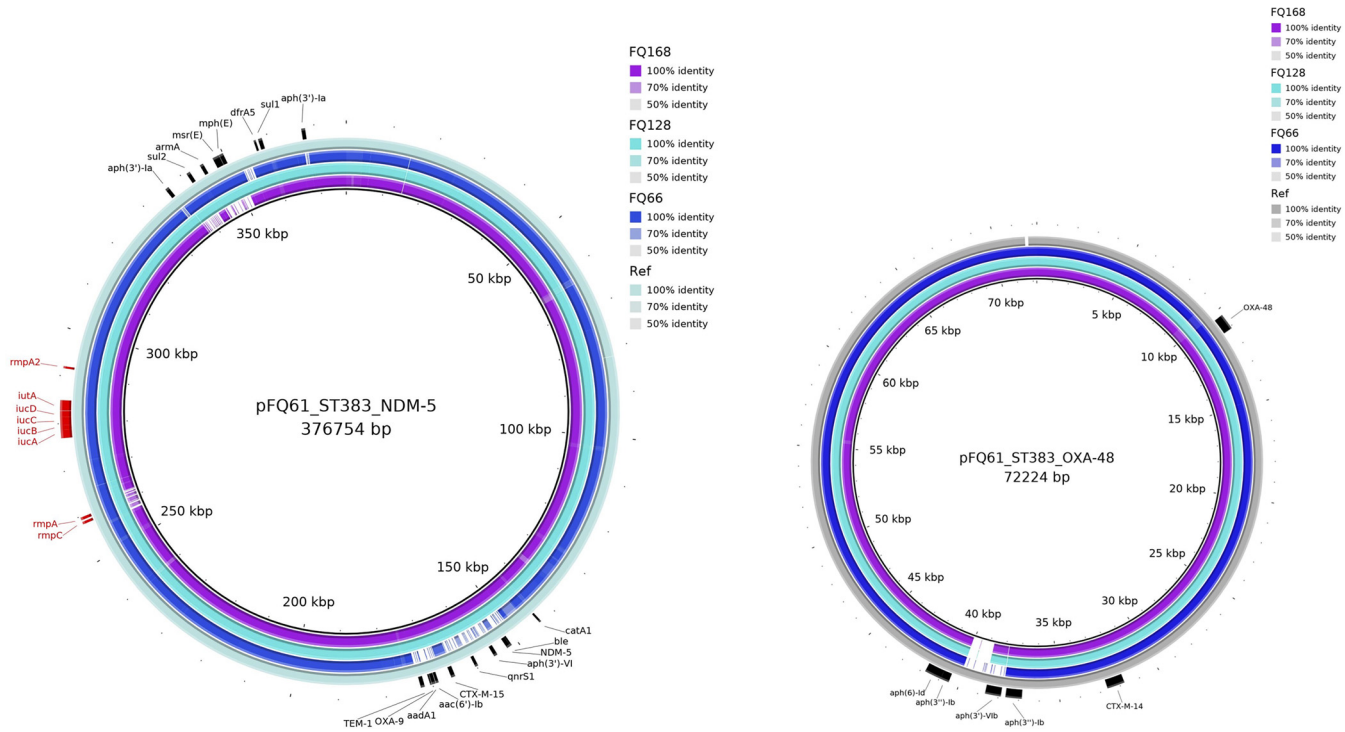


**FIG 3** Homologous contigs from ST383 isolates FQ66, FQ128, and FQ168 were compared to two major plasmids, pFQ61_ST383_NDM-5 and pFQ61_ST383_OXA-48. BRIG was used to generate a visual representation with pKpvST383_NDM_OXA48 as a reference (CP034201.2 and CP034202.1, respectively). Red and black arcs in the outer ring represent the major well-annotated AMR and virulence genes.

same hospital, the SNP differences were large (range, 68 to 105) (Table 4), which was not consistent with intrahospital transmission/dissemination. Similarly, 5 ST11 isolates were collected from July 2015 to March 2017, but the epidemiological information, such as date of admission and hospital systems, and SNP differences (range, 3 to 170) did not suggest intra- or interhospital transmission (Table 4). In contrast, 4 ST383 isolates had relatively large genetic variation (SNP range, 44 to 183), and interestingly, three of them were POA, where the patients had travel and medical histories in Egypt within 6 months of hospital admissions, suggesting that the isolates might have been acquired there (Table S4).

Isolates of *K. quasipneumoniae* subsp. *quasipneumoniae* ST196 and ST1416 were clustered together with 0 to 1 cgSNPs (among isolates within each ST) in the cgSNP tree (Fig. 1; Table S2), which prompted an investigation to identify possible disease outbreaks. All ST196 and ST1416 isolates had identical capsular and lipopolysaccharide types and similar AMR genes (Fig. 1; Table S1). The high-resolution SNP tree (from mapping and variant calling) indicated that these two subspecies may be clonal, as all ST196 isolates except FQ158 were highly similar, with 0 to 10 high-resolution SNPs (Fig. 6). Most ST196 isolates (66.7%) were associated with HAI. They were collected from various patients in 5 different hospitals in different times and units, but interhospital transmission/dissemination due to transfer may still be possible (Table S4). While four ST1416 isolates were also highly similar, with 0 to 4 high-resolution SNPs (Table 4), and were from the same hospital and were identified as HAI (Fig. 6), there were no epidemiological and clinical associations among the four patients to suspect outbreaks among the isolates, although cryptic transmission events cannot be rule out (Table S4).

The virulence-associated loci such as *iuc*, *clb*, *iro*, and *rmpA* and *rmpA2* were not detected in any of the *K. quasipneumoniae* subsp. *quasipneumoniae* isolates. We compared these *K. quasipneumoniae* subsp. *quasipneumoniae* isolates (ST1416 and ST196) to the global isolates to determine if they represented local clones. The isolates belonging to ST196 were genetically different from global ST196 isolates (Fig. S2). For instance, previously reported ST196 isolates were mostly KPC producers from Europe and the United States (Table S5), while most *K. quasipneumoniae* subsp. *quasipneumoniae* isolates in Qatar

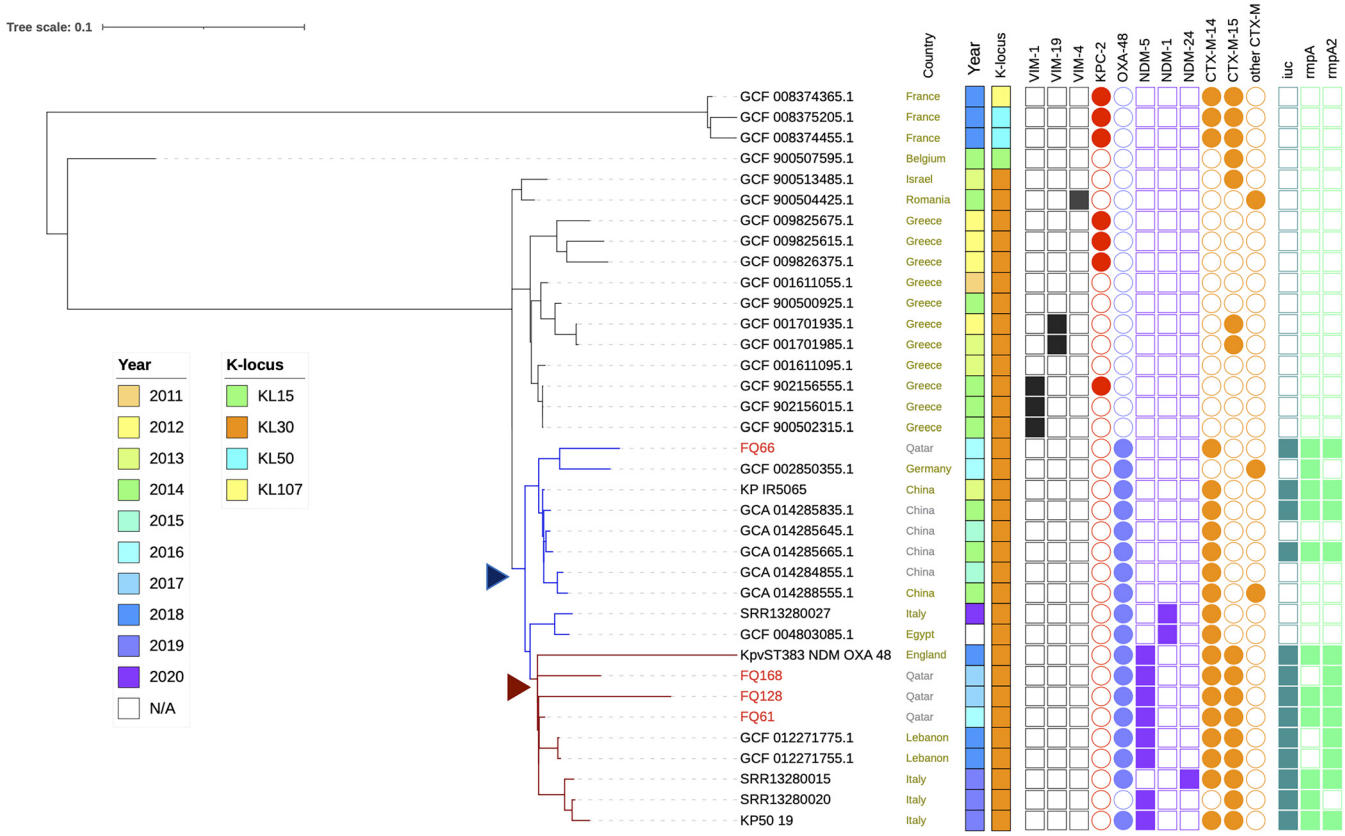


**FIG 4** Phylogenetic tree showing the relationships among *K. pneumoniae* ST383 isolates from different countries and Qatar using Parsnp (overlaid with presence/absence of key AMR genes and virulence loci). Arrowheads (blue and dark red) indicate the two possible plasmid acquisition events.

were NDM-1 producers. Similarly, *K. quasipneumoniae* subsp. *quasipneumoniae* ST1416 were not commonly reported elsewhere; the isolates in Nigeria and China were genetically divergent from the Qatari isolates (Fig. S2), despite the Chinese isolate also carrying $bla_{\text{NDM-1}}$.

## DISCUSSION

*Klebsiella* species are responsible for HAI worldwide and are also increasingly implicated in community-associated infections. The predominant species is *K. pneumoniae*, but other *Klebsiella* species, including *K. aerogenes*, *K. michiganensis*, *K. quasipneumoniae*, and *K. variicola*, also cause human infections. The key clinically relevant attributes of *K. pneumoniae* are its antimicrobial resistance and virulence, or hypervirulence. However, these aspects are less well studied in *Klebsiella* species other than *K. pneumoniae*. Recent reports suggest that these species, like *K. pneumoniae*, are also sources of antimicrobial resistance and hypervirulence (5, 24, 25). Using a data set of carbapenem-resistant *Klebsiella* clinical isolates from a hospital in Qatar, we conducted in-depth genomic analysis of carbapenem resistance and its intersection with hypervirulence.

Our data revealed the presence of diverse STs and different lineages across the *Klebsiella* species. Among *K. pneumoniae* isolates, ST231, ST147, and ST11 were the most prevalent carbapenem-resistant clones, which were different from those isolated from rectal screening swabs in local pediatric populations (9), among which ST73, ST14, and ST17 were the most common STs. ST147 (CG147) and ST11 (CG258) are international high-risk clones reported mainly from Asia and Europe and have been responsible for nosocomial transmission and various care center outbreaks (26, 27), while ST231 was considered an endemic clone associated with $bla_{\text{OXA-232}}$ in India (28). Based on previous studies, *K. pneumoniae* CG258 (ST11 and ST258) is the predominant KPC-producing clone reported globally (29); however, only six isolates were reported in our study. In Qatar, substantial proportions of the population are migrant workers from the

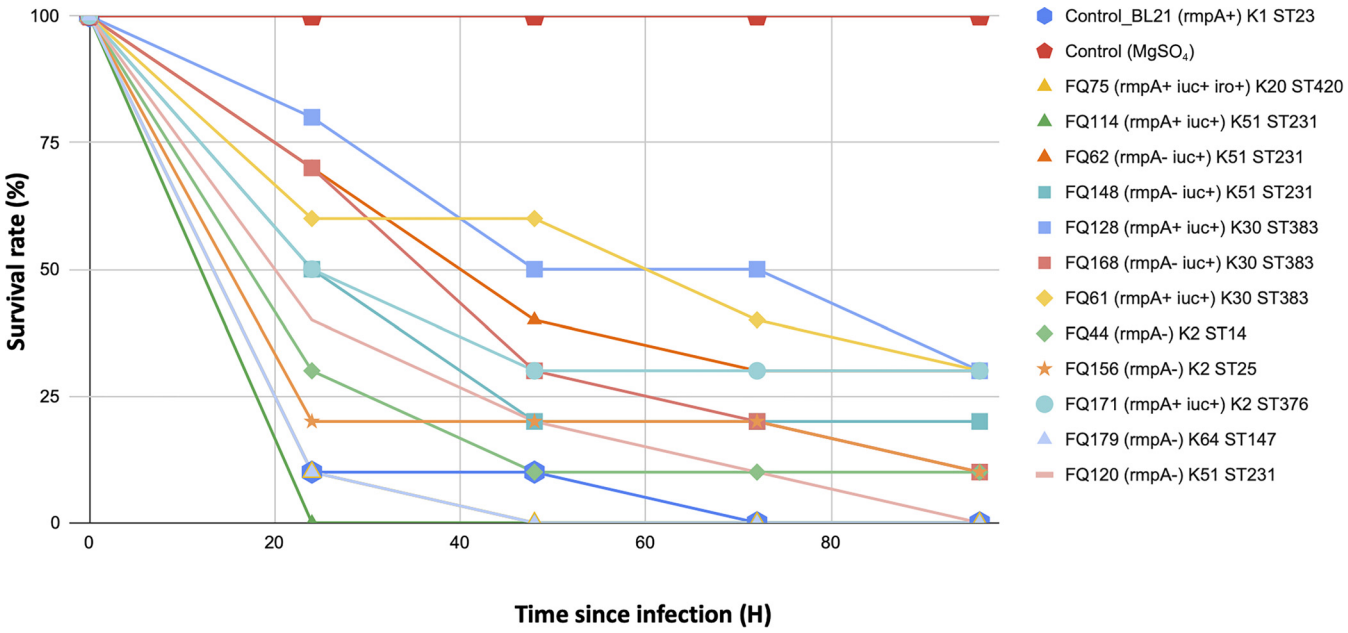


**FIG 5** Virulence potential of selected *K. pneumoniae* isolates in a *Galleria mellonella* infection model.

Indian subcontinent. Pérez-López et al. (9) suggested that CRE in pediatric populations in Qatar were mainly introduced sporadically by asymptomatic carriers who received health care in some nearby countries in which they are endemic. Moreover, consistent with other CRE studies of pediatric and adult patients, genes encoding NDM and OXA-48-type carbapenemases were widely prevalent (9, 27, 30).

Isolates of the next common species, *K. quasipneumoniae* subsp. *quasipneumoniae*, mostly belonged to ST196 or ST1416 and carried $bla_{NDM}$. Outbreaks caused by *K. quasipneumoniae* have been rare, but ST334 has been reported as a potentially

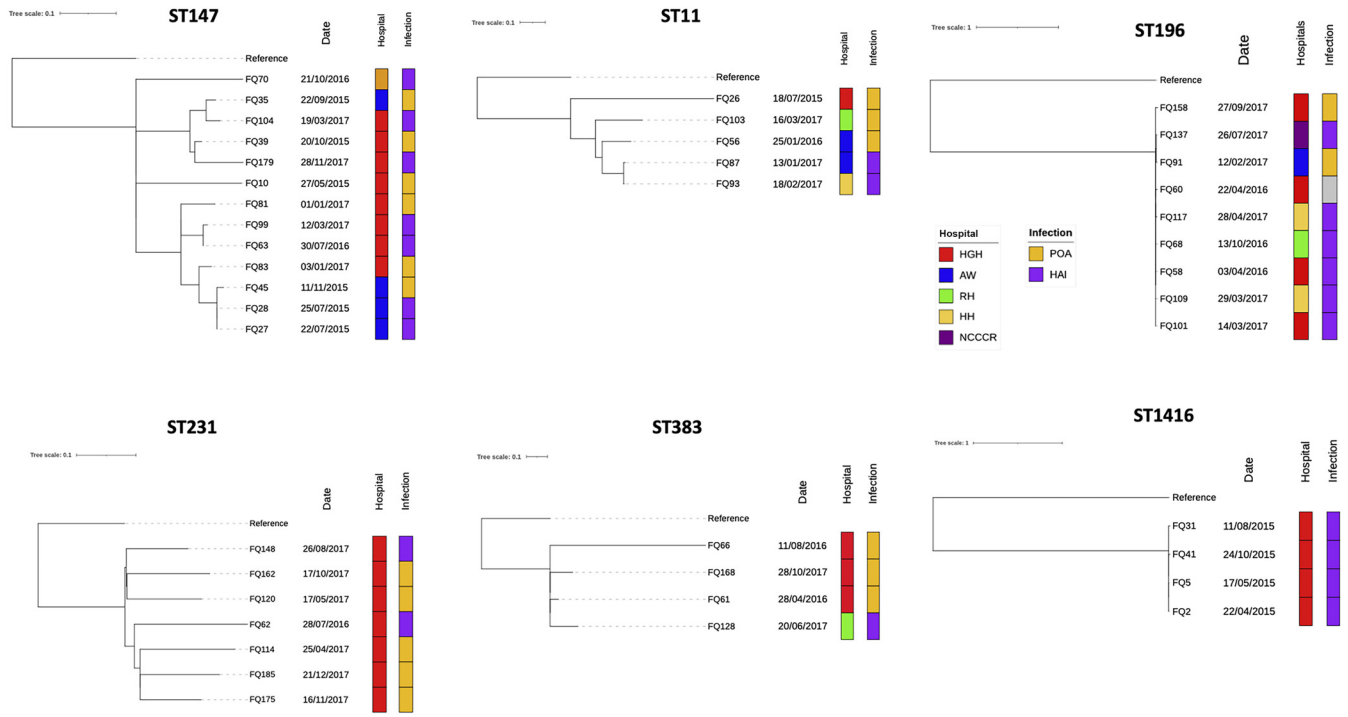


**FIG 6** Phylogenetic tree generated from high-resolution SNPs and linked epidemiological data of prevalent *K. pneumoniae* and *K. quasipneumoniae* subsp. *quasipneumoniae* isolates.

**TABLE 4** Pairwise SNPs differences among *K. pneumoniae* and *K. quasipneumoniae* subsp. *quasipneumoniae* isolates based on high-resolution SNP analysis (using Snippy workflow)

| Organism and sequence type (no. of samples) | No. of characters | Mean pairwise SNPs among samples in Qatar (range, distance to reference genome) | Reference genome used in Snippy pipeline |
|---|---|---|---|
| *K. pneumoniae* | | | |
| ST147 (13) | 485 | 87.5 (0–139) | NZ_CP012745.1.fasta |
| ST231 (7) | 361 | 86.7 (68–105) | GCF_002909775.1_ASM290977v2_genomic.fna |
| ST11 (5) | 348 | 101.6 (3–170) | GCF_003931835.1_ASM393183v1_genomic.fna |
| ST383 (4) | 331 | 112.8 (44–183) | GCF_001611055.1_ASM161105v1_genomic.fna |
| *K. quasipneumoniae* subsp. *quasipneumoniae* | | | |
| ST196 (9) | 227 | 3.9 (0–10) | GCF_003146655.1_ASM314665v1_genomic.fna |
| ST1416 (4) | 137 | 2 (0–4) | GCF_005503875.1_ASM550387v1_genomic.fna |

emerging outbreak-associated MDR clone in Pakistan and Cambodia (31, 32, 33). Our *K. quasipneumoniae* subsp. *quasipneumoniae* isolates were highly clonal within the STs across hospitals; thus, their potential for regional spread merits monitoring. Two *K. quasipneumoniae* subsp. *similipneumoniae* isolates (ST1584 and ST1998) also could raise public health concern on antimicrobial resistance, as they carried $bla_{\text{NDM-7}}$.

Out of three *K. aerogenes* isolates in Qatar, two belonged to ST93, one of the prevalent STs and the founder genotype in the world (4). The remaining isolate belonged to ST206, which was reported only from Singapore according to the pubMLST database. Though these isolates did not carry known carbapenemase genes, the chromosomal *ampC* coupled with outer membrane porin alteration may be responsible for the development of carbapenem resistance (34).

In terms of hypervirulent traits, the classical hvKp lineages, including CG23 and CG86, were not among the carbapenem-resistant *K. pneumoniae* clinical isolates in Qatar. The proportion of hvKp isolates among *K. pneumoniae* isolates was 10% (*rmpA* and/or *rmpA2*) and could be up to 20% when solely aerobactin (*iuc*)-bearing isolates are also included. The prevalence of *rmpA*- or *rmpA2*-positive isolates among *K. pneumoniae* isolates was lower than in China and Vietnam (>20%) (12) but higher than in the United States or the United Kingdom (35, 36). *rmpA*- and/or *rmpA2*-mediated overproduction of capsular polysaccharide has been shown to contribute to hypervirulence (truncation of these loci may reduce virulence) (37), while *iuc*, *iro*, *ybt*, and *ent* mediate increased siderophore production under iron-limiting conditions (18). In addition, KL1 and KL2 hypermucoviscosity-specific capsular serotypes have been associated with invasive infections and accounted for approximately 70% of hvKp global isolates; however, they were not common in this collection. Convergence of carbapenem resistance and hypervirulence was found in limited STs such as ST231, ST383, and ST410, of which ST231 and ST410 are globally emerging hypervirulent clonal groups associated with virulence plasmids (28, 38, 39).

Our study indicates the emergence and transmission of carbapenem-resistant hvKp ST383 among patients worldwide. Three patients in Qatar infected with ST383 isolates had histories of travel to Egypt, suggesting that they likely acquired the isolates there. This may be in parallel with Kpv_ST383_S1, another ST383 isolate carrying hybrid virulence/resistance plasmids from a patient in Scotland, who had medical treatment in Cairo (21). International travel may play a role in the spread/dissemination of this hybrid plasmid. The wax moth larva virulence study indicated that these ST383 isolates were virulent, even though to a slightly lower degree than the classical hypervirulent KL1 isolate. Russo et al. (40) demonstrated that not all hvKp strains shared the same pathogenic potential in murine model infections. Previously, it was common for *K. pneumoniae* ST383 isolates to be resistant to carbapenems but not hypervirulent. Due to the acquisition of a hybrid plasmid that contains a fraction of the hvKp virulence plasmid during the evolution of conventional ST383 isolates, these new circulating isolates have become both MDR and hypervirulent and should be regarded as isolates of a superbug that could pose a serious threat to public

health. Early *K. pneumoniae* ST383 isolates carrying $bla_{VIM-4}$, $bla_{KPC-2}$, and $bla_{CMY-2}$ were reported in a Greek hospital during 2009 to 2010 (41). A later study from the Czech Republic demonstrated the presence of $bla_{VIM-19}$ in isolates of suspected Greek origin (42). More recent studies indicated the presence of $bla_{OXA-48}$ in clinical isolates in Germany and China, which then went on to acquire another hybrid plasmid that carried both $bla_{NDM}$ and virulence genes (21, 43). Sabivora et al. proposed that the plasticity of the accessory genomes in ST383 isolates may benefit the acquisition of different plasmids (44). Turton et al. (35) studied 31 ST383 isolates in the United Kingdom and identified 5 isolates harboring *rmpA* or *rmpA2* and *iutA*, which is part of the aerobactin *iuc* locus. These ST383 isolates have also emerged in Italy recently (43, 45) and in the Middle East, such as in Qatar, Egypt, and Lebanon (Fig. 3). Recently, an ST383 isolate harboring $bla_{KPC}$ and genes encoding various virulence factors was reported in Saudi Arabia (46); however, assembled genome data are not available in the public domain. Tracking the evolution and distribution of ST383 is of major importance due to its ability to acquire carbapenemase genes of different types as well as genes associated with the hv phenotype, but also the ability of mobile genetic elements to spread these genes to different ST types and species. Increasing reports of the presence of hybrid, mosaic plasmid carrying both carbapenem resistance and virulence genes suggest that carbapenem-resistant hvKp isolates are no longer confined to selected clones (16, 22, 23, 47), which will make containment of such isolates challenging.

In conclusion, our study has provided insights into the dynamics and epidemiology of carbapenem-resistant *Klebsiella* species in Qatar. Analysis of WGS data demonstrated the presence of clonal lineages. Our comparative genomic data also confirmed the emergence of carbapenem-resistant hvKp ST383 in the Middle East and worldwide. Acquisition of virulence-associated loci was reported for at least 10% of the carbapenem-resistant *K. pneumoniae* isolates, and one of the mechanisms was through the transfer of a carbapenem resistance and hypervirulence hybrid plasmid possibly mediated by mobile genetic elements, as demonstrated for ST383 isolates. Further studies will be required to understand the relationship between the hypervirulent phenotypes, carriage of the hybrid MDR-virulence plasmid, and capsular types.

## MATERIALS AND METHODS

**Hospital settings, cultures, and antimicrobial susceptibility tests.** Hamad Medical Corporation (HMC) is the major provider of secondary and tertiary health care in Qatar and has 12 hospitals—9 specialist hospitals and 3 community hospitals—as well as the National Ambulance Service and home and residential care services. The isolates were collected, maintained, and identified in the Department of Microbiology in HMC as previously described (6). Also, the antimicrobial susceptibility testing for various antibiotics was performed on BD Phoenix (Becton, Dickinson and Company, USA) using the Clinical and Laboratory Standards Institute breakpoints (6). The study was approved by the institutional review board (MRC-16134/16).

**Whole-genome sequencing (WGS) and data analysis.** Briefly, genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Germany), and the DNA libraries were sequenced on the Illumina NextSeq 550 platform using 2 × 150 paired-end reads (PE) at the Microbial Genome Sequencing Center (MiGS; Pittsburgh, PA, USA) as previously described (6). In addition, long reads for isolate FQ61 were generated using an Oxford Nanopore MinION sequencer (SQK-LSK109 and flow cell R9.4.1) at MiGS. MinION reads were generated based on the Guppy software (v4.0.11) available from Oxford Nanopore Technologies (Oxford, UK). The raw reads from Illumina NextSeq were assembled *de novo* using SPAdes v3.9.0 (48) implemented in shovill (https://github.com/tseemann/shovill) (49). *De novo* Illumina-Nanopore assemblies were generated with Unicycler v0.4.7 (50).

STs, plasmid replicons, and AMR genes were predicted from the assembled contigs using multilocus sequence typing (MLST) (https://github.com/tseemann/mlst), pubMLST (51), Plasmidfinder v2.1 (52), and ResFinder v3.2 databases implemented in ABRicate v0.9 (https://github.com/tseemann/abricate), based on >70% coverage and 90% sequence identity. Kleborate v2 was used to detect the virulence genes and capsule synthesis (K) and lipopolysaccharide (O) loci, as well as AMR genes and known chromosomal mutations associated with resistance to fluoroquinolones, colistin, and carbapenems (14). Previously unreported STs were submitted to BIGSDB (https://bigsdb.pasteur.fr/klebsiella/) for ST assignment.

Genome assemblies were annotated with Prokka v1.13.3 (53), and the completed plasmid annotations were curated before deposit in GenBank. To clarify and study the plasmid variation among 4 ST383 isolates, assembled genomes of FQ66, FQ128, and FQ168 were manually explored in their assembly graphs using Bandage v0.8.1 (54) and the completed FQ61 plasmids built from Unicycler assembly as a reference. BRIG (55) was used to generate a visual representation of the contigs in FQ66, FQ128, and FQ168 aligned to the major plasmids in FQ61.

Core genome phylogenetic trees were generated using Parsnp (56) and visualized together with associated metadata using iTOL (57). cgSNPs were extracted from the Parsnp alignment to determine the pairwise difference between *Klebsiella* species and between STs in *K. pneumoniae* and *K. quasipneumoniae* (56). Easyfig (58) was used to generate the diagram to compare the plasmid against highly homologous plasmids in the NCBI database. To generate high-resolution SNPs for epidemiological investigation within prevalent STs, the quality-trimmed reads for ST147, ST231, ST11, ST196, and ST1416 were mapped against their respective reference genome and high-quality SNPs were called by using the Snippy pipeline (https://github.com/tseemann/snippy). FastTree was used for phylogenetic analysis (59).

**Outbreak analysis.** Cases associated with prevalent *Klebsiella* STs (≥4 isolates) in this study and forming clusters in the core genome phylogenetic tree were carefully inspected as potential outbreaks. Epidemiological data, antimicrobial susceptibility testing results, and clinical information such as date of patient admission were reviewed to determine whether those isolates represented hospital-acquired infection (HAI) or community-acquired infection based on the National Health Care Safety Network (NHSN) definition published in January 2021 by HMC.

An infection was defined as an HAI if the NHSN site-specific infection occurred on or after the third calendar day of admission to an inpatient location where day of admission was calendar day 1. If the infection was identified within 2 days before admission or the day of admission to an inpatient location (calendar day 1) or calendar day 2 after admission, the infection was considered present on admission (POA).

**Virulence study.** The virulence of selected *K. pneumoniae* isolates, including 3 ST383 isolates, was tested in wax moth (*Galleria mellonella*) larvae. Briefly, overnight cultures of *K. pneumoniae* strains were prepared with 10 mM $MgSO_4$ solution and further adjusted to concentrations of $1 \times 10^5$ CFU/mL, $1 \times 10^6$ CFU/mL, and $1 \times 10^7$ CFU/mL. We infected the *G. mellonella* with the bacteria as described previously (60), and the survival rate of the *G. mellonella* was recorded every 24 h for 4 days.

**Data availability.** Raw sequence reads are available on the NCBI website under BioProject accession number PRJNA656934. The genome sequence of FQ61 was submitted to GenBank under accession numbers CP091813 to CP091818.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, XLSX file, 0.1 MB.

**SUPPLEMENTAL FILE 2**, PDF file, 0.2 MB.

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