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**Original Article**

# Extensively Drug-Resistant *Klebsiella pneumoniae* Associated with Complicated Urinary Tract Infection in Northern India

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**ABSTRACT:** *Klebsiella pneumoniae* (Kp), which is associated with hospital-acquired infections, is extensively drug-resistant (XDR), making treatment difficult. Understanding the genetic epidemiology of XDR-Kp can help determine its potential to be hypervirulent (hv) through the presence of siderophores. We characterized the genomes of 18 colistin-resistant XDR-Kp isolated from 14 patients with complicated tract infection at an Indian healthcare facility. The 18 organisms comprised the following sequence types (STs): ST14 ($n$ = 9), ST147 ($n$ = 5), ST231 ($n$ = 2), ST2096 ($n$ = 1), and ST25 ($n$ = 1). Many patients in each ward were infected with the same ST, suggesting a common source of infection. Some patients had recurrent infections with multiple STs circulating in the ward, providing evidence of hospital transmission. β-lactamase genes ($bla_{\text{CTX-M-1}}$, $bla_{\text{SHV}}$, and $bla_{\text{ampH}}$) were present in all isolates. $bla_{\text{NDM-1}}$ was present in 15 isolates, $bla_{\text{OXA-1}}$ in 16 isolates, $bla_{\text{TEM-1D}}$ in 13 isolates, and $bla_{\text{OXA-48}}$ in 3 isolates. Disruption of *mgrB* by various insertion sequences was responsible for colistin resistance in 6 isolates. The most common K-type among isolates was K2 ($n$ = 10). One XDR convergent hvKp ST2096 mutation (*iuc*+*ybt*+$bla_{\text{OXA-1}}$+$bla_{\text{OXA-48}}$) was associated with prolonged hospitalization. Convergent XDR-hvKp has outbreak potential, warranting effective antimicrobial stewardship and infection control.

## INTRODUCTION

There is growing concern regarding the disease burden associated with the ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). These bacteria are frequently multidrug resistant (MDR) and a leading cause of hospital-acquired infections worldwide (1). *Klebsiella pneumoniae* is an emerging pathogen implicated in a wide variety of infections including meningitis, pneumonia, sepsis, intra-abdominal infections, skin and soft tissue infections, and urinary tract infections (UTIs) (2). *Klebsiella pneumoniae* acquired in hospitals in South Asia are frequently extensively drug-resistant (XDR) or pan-drug resistant (PDR), making them difficult to treat (3). The increase in XDR and PDR *K. pneumoniae* has become a global concern, and the World Health Organization has placed carbapenem- and third-generation cephalosporin-resistant *K. pneumoniae* on the priority list of pathogens requiring new antimicrobials (4).

The most dangerous form of *K. pneumoniae* is associated with invasive disease and was first isolated from hypervirulent *K. pneumoniae* (hvKp) liver abscesses (5). hvKp variants are more commonly community acquired; however, their capacity to infect healthy individuals may also facilitate their transmission in healthcare facilities. Consequently, hvKp infections are becoming more prevalent and associated with increasing mortality (6). The molecular basis of hvKp is unclear, but isolates that carry the salmochelin, enterobactin, and aerobactin siderophores appear to be better adapted for systemic spread in humans. Notably, the *iuc* locus (*iucABCD*), which encodes aerobactin, is located on a plasmid, and can theoretically be transferred horizontally from one organism to another (6). hvKp isolates are generally susceptible to the most

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clinically relevant antimicrobials; however, hvKp isolates with MDR phenotypes have been reported (7). The combination of extreme AMR phenotypes (MDR, XDR, and carbapenem resistance) and hypervirulence in *K. pneumoniae* poses a major threat to public health.

*Klebsiella pneumoniae* is the second most common cause of UTI in our tertiary care center in northern India, accounting for 25% of all UTIs, 13.9% of which are XDR (8). The antimicrobial susceptibility of these organisms necessitates that colistin is currently the last resort for treating XDR *K. pneumoniae* infections (9). However, the reported prevalence of colistin nonsusceptibility in MDR *K. pneumoniae* of 18.5% in our center is also high (8). The plasmid-mediated colistin resistance gene *mcr* and mutations in the two-component systems PmrA/B and PhoP/Q, and MgrB, a negative regulator of PhoP/Q, lead to colistin resistance and compromise the potential effectiveness of this vital drug (10–12).

Given the potential health implications of complicated UTIs caused by MDR and XDR organisms, it is essential to better understand their epidemiology and genetic composition. Whole-genome sequencing (WGS) can help identify both known and novel mechanisms of antimicrobial resistance, determine STs, and aid epidemiological investigations through phylogenetics (12). In this study, we characterized the genomes of 18 colistin-resistant XDR *K. pneumoniae* isolates from 14 patients with complicated UTIs over a 6-month period in a tertiary hospital in northern India.

## MATERIALS AND METHODS

**Study design:** We conducted a retrospective study to characterize the genome sequences of 18 colistin-non-susceptible uropathogenic XDR *K. pneumoniae* isolates from urine samples of 14 inpatients admitted to the Postgraduate Institute for Medical Education and Research (PGIMER) in Chandigarh. The clinical details of the patients were recorded from the case files after obtaining informed consent from the adult patients and the parent(s)/legal guardians of all patients aged under 18 years.

**Ethical approval:** The study was conducted at PGIMER, Chandigarh after the approval of the Institute Ethics Committee of PGIMER (reference no NK/3761/MD/386; vide letter no INT/IEC/2017/1009, dated October 12, 2017). The study was also approved by the Institute Collaborative Research Committee of PGIMER (vide letter no. 79/227-Edu-18/4997, dated December 12, 2018). All methods were performed in accordance with the relevant guidelines and regulations of the Institute Ethics Committee. Samples were included in the study after obtaining informed consent from patients, and patients and parent(s)/legal guardians of all patients aged under 18 years. Consent for publication was obtained from the patients at the time of sample collection.

**Microbiological methods:** Urine samples from patients suffering from UTI (fever >100°F [>37.8°C], burning micturition, and increased frequency of urination) admitted to PGIMER between July 2017 and January 2018 were processed according to the standard operating procedures of the Enteric Laboratory (8). Samples were inoculated on cysteine lactose electrolyte-deficient media. Yellow colonies (lactose fermenting) indicative of *K. pneumoniae* were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Those confirmed as *K. pneumoniae* were subjected to antimicrobial susceptibility testing (AST), and the patient's clinical details were retrieved from their medical records (8).

AST was performed using the Kirby-Bauer disk diffusion method for aminoglycosides (amikacin [30 µg], gentamicin [10 µg]), beta-lactams (piperacillin-tazobactam [100/10 µg]), carbapenems (ertapenem [10 µg], meropenem [10 µg], imipenem [10 µg]), cephalosporins (cefotaxime [30 µg], cefepime [30 µg], cefoperazone-sulbactam [75/30 µg]), trimethoprim/sulfamethoxazole (1.25/23.75 µg), quinolones (ciprofloxacin [5 µg], norfloxacin [10 µg], nalidixic acid [30 µg]), tetracyclines (tetracycline [30 µg], minocycline [30 µg]), nitrofurantoin (300 µg), tigecycline (15 µg) and fosfomycin (200 µg) (Becton, Dickinson and Company, Sparks, MD, USA) following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (13). MDR was defined as nonsusceptibility to at least one agent in 3 or more antimicrobial categories, and XDR was defined as nonsusceptibility to at least one agent in all but 2 or fewer antimicrobial categories (i.e., bacterial isolates remaining susceptible to only one or 2 categories). XDR isolates were further tested using the CLSI broth microdilution method to determine phenotypic resistance to colistin (Sigma Aldrich, St. Louis, MO, USA) (14). Isolates with minimum inhibitory concentration (MIC) ≥2 µg/mL against colistin were deemed as colistin non-susceptible. Colistin-susceptible *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *mcr1*-positive *E. coli* NCTC 13846 (CCUG 70662, DSM 105182), with a colistin MIC of 4 mg/L, were used as quality controls.

**Whole-genome sequencing:** Genomic DNA was extracted from all isolates using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Briefly, genomic DNA was purified from each isolate and quantified using the Qubit dsDNA High-Sensitivity Assay (Thermo Fisher Scientific, Waltham, MA, USA). Libraries were prepared using a Nextera XT DNA Library Preparation Kit and sequenced on a MiSeq platform (2 × 250 bp, V2 500 cycles) (Illumina Inc., San Diego, CA, USA). The raw sequences were subjected to quality check and analysis using an in-house pipeline, BacPipe (15). Briefly, reads were quality assessed, trimmed, and de novo assembled (SPAdes version 3.10.0) (16). The assembled sequences were subjected to MLST typing, annotation (Prokka version 1.12) (17), and antimicrobial resistance gene prediction using Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) version 3 (18). To determine the incompatibility type of the plasmids present in the sequences, the data were processed using ISfinder (19) and insertion element families were detected (20). Virulence genes were identified using VFDB (21) and capsular typing was performed using Kleborate version 0·3·0 (http://github.com/katholt/Kleborate).

Table 1. Patient clinical details

| Patient code | Age (yr) | Sex | Ward | Diagnosis | Abnormality/ surgery of urinary tract | Days of hospital stay | No. of episodes of UTI | ST | Isolate no. |
|---|---|---|---|---|---|---|---|---|---|
| P1 | 46 | F | Urology ward | Invasive devices | Yes | 22 | 2 | ST14 | 1 |
| P2 | 40 | M | Urology ward | Metabolic disorders | Yes | 15 | 1 | ST231 | 30 |
| P3 | 29 | M | Urology ward | Invasive devices | Yes | 16 | 1 | ST14, ST147 | 2, 7 |
| P4 | 6 | M | Urology ward | Metabolic disorders | Yes | 23 | 1 | ST2096 | 14 |
| P5 | 4 | M | Paediatric surgery | DJ stent | Yes | 16 | 0 | ST14 | 13 |
| P6 | 48 | F | Urology ward | Invasive devices | Yes | 22 | 1 | ST147 | 16 |
| P7 | 33 | M | Urology ward | Metabolic disorders | Yes | 25 | 3 | ST14 | 20 |
| P8 | 61 | F | Urology ward | Metabolic disorders | Yes | 15 | 0 | ST147 | 17 |
| P9 | 11 | M | Paediatric surgery | DJ stent | Yes | 90 | 0 | ST14 | 21 |
| P10 | 5 | M | Paediatric surgery | DJ stent | Yes | 8 | 5 | ST14 | 24 |
| P11 | 7 | M | Paediatric surgery | DJ stent | Yes | 20 | 5 | ST14 | 25 |
| P12 | 64 | M | Urology ward | Renal parenchymal disease | Yes | 20 | 4 | ST25, ST14, ST147, ST231 | 15, 26, 9, 19 |
| P13 | 30 | M | Urology ward | Metabolic disorders | Yes | 58 | 2 | ST14 | 28 |
| P14 | 34 | M | Urology ward | Metabolic disorders | Yes | 25 | 8 | ST147 | 27 |

ST, sequence type.

**Phylogenetic analysis:** For phylogenetic comparison, all 18 sequenced isolates, along with a reference sequence (KPN528 with accession number CP020853.1), were passed through the RedDog pipeline V1beta.10.3 (https://github.com/katholt/RedDog). Phylogenetic relationships were inferred using the maximum likelihood method, based on a general time-reversible model. A phylogenetic tree was constructed and scaled, with branch lengths measured as the number of substitutions per site. SNP-sites version 2.5.1 (22) and snp-dists version 0.7.0 (23) were used to determine the single nucleotide polymorphism (SNP) distance between paired sequenced isolates and the reference genome (GenBank assembly accession no. GCA_002156785.1) from the core gene alignment. The average nucleotide identity (ANI) in relation to the reference genome was assessed using FastANI version 1.3 (24).

## RESULTS

**Baseline characteristics:** Over the 6-month sampling period, 775 (3.1%) *K. pneumoniae* isolates were obtained from 25,046 urine samples collected from patients with clinically suspected UTIs. Following AST, 108 (13.9%) MDR *K. pneumoniae* isolates were identified. Eighteen *K. pneumoniae* isolates from 14 patients were nonsusceptible to colistin and were XDR. The majority of these *K. pneumoniae* strains were isolated from patients admitted to the urology wards ($n$ = 10), all of whom had structural abnormalities of the urinary tract or had recently undergone urinary tract surgery (Table 1).

**Antimicrobial resistance gene content and phylogenetic analysis:** The 18 colistin-resistant *K. pneumoniae* isolates were subjected to WGS. A wide variety of AMR genes (41 individual genes) were detected in these isolates. ST14 KPN528 was selected as the reference strain because ST14 is the most common local ST. The β-lactamase genes $bla_{\text{CTX-M-1}}$, $bla_{\text{SHV}}$, and $bla_{\text{AmpH}}$ were present in all isolates (Fig. 1). Because $bla_{\text{OKP-LEN}}$ is common in *Klebsiella variicola* and *Klebsiella quasipneumoniae*, and $bla_{\text{SHV}}$ is common in *K. pneumoniae*, we calculated the genome ANI to *K. pneumoniae* reference genome (GenBank assembly accession no. GCA_002156785.1) to accurately identify the species. The ANI was more than 98% in all isolates (range: 98.72% to 99.97%), confirming that the identification as *K. pneumoniae* was accurate (24).

A pairwise comparison of the sequences is shown in Fig. 2. The sequences were compared using the SNP distance matrix from the core gene SNP alignment file. The core gene alignment file was obtained from the annotated assemblies, in addition to the reference genome (GenBank assembly accession no. GCA_002156785.1) using Roaryv.3.13.0. Some of the strains had fewer SNP differences (7ST147 and 9ST147, one SNP; 2ST14 and 26ST14, 2 SNPs; 20ST14 and 24ST14, 3 SNPs; and 30ST231 and 19ST231, 3 SNPS).

Furthermore, 15 of the 18 sequences contained $bla_{\text{NDM-1}}$. Of the 15 sequences containing $bla_{\text{NDM-1}}$, 13 also contained $bla_{\text{OXA-1}}$, and 2 contained $bla_{\text{OXA-48}}$. None of the isolates was a *K. pneumoniae* carbapenemase producer (Fig. 1). With respect to colistin resistance, the WGS data showed the absence of *mcr1* to *mcr10* genes and mutations in the PhoPQ and PmrAB two-component systems. However, 7 of the 18 isolates contained deletions/mutations in the *mgrB* genes, which included a disruption of IS110/ISKpn26 (most common) and Cys28Gly (Table 2). This mutation has a deleterious effect and contributes to colistin resistance (25).

We identified 5 different STs, the most common being ST14 ($n$ = 9), followed by ST147 ($n$ = 5), ST231 ($n$ = 2), ST2096 ($n$ = 1), and ST25 ($n$ = 1) (Fig. 1). We observed 2 phylogenetically distinct variants of ST147. Three

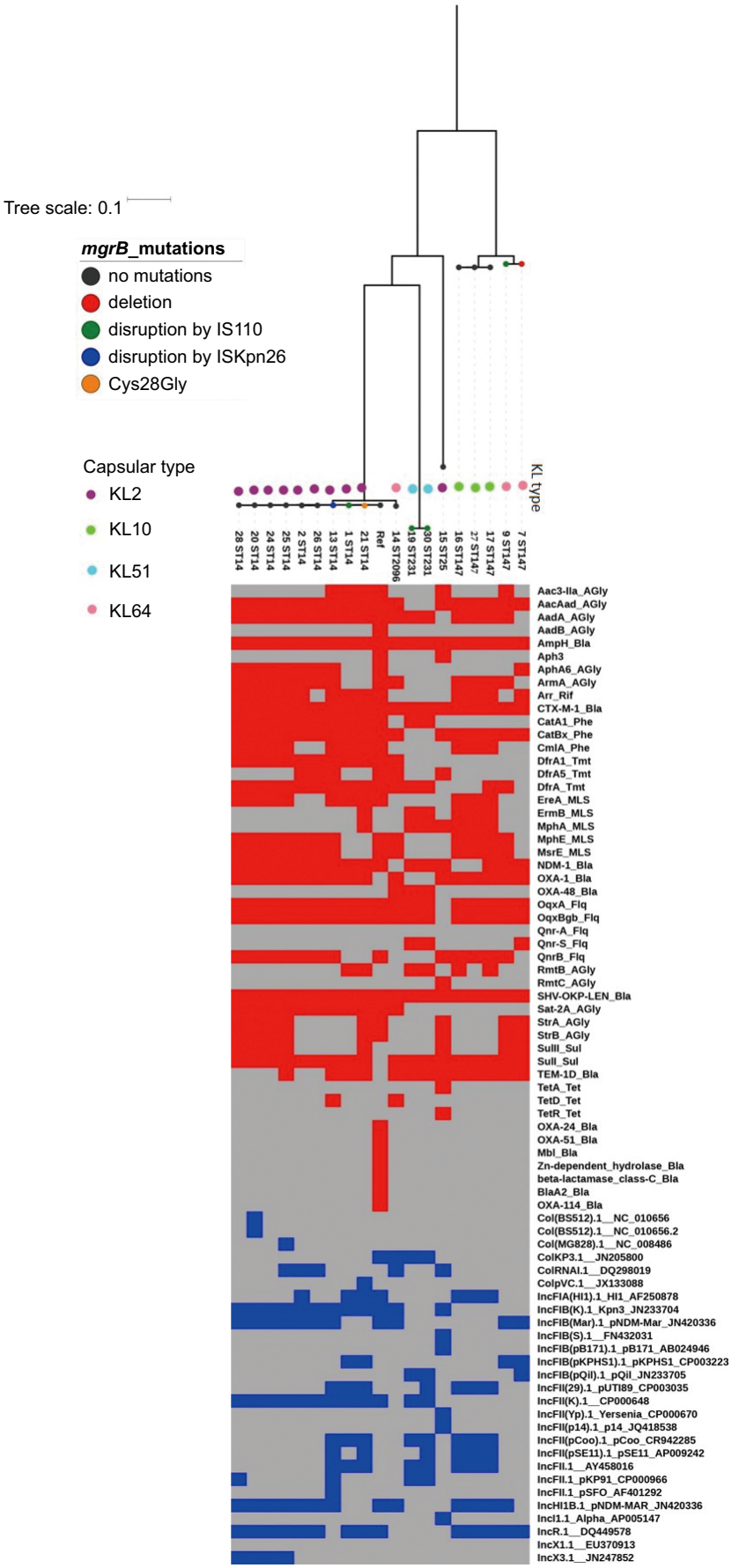


Fig. 1. (Color online) Phylogenetic structure of colistin resistant *K. pneumoniae* isolates from this study. A phylogenetic tree of *K. pneumoniae* isolates from this study was constructed based on core SNP alignment using RedDog pipeline and viewed on iTOL with a heat-map showing AMR genes (red) and plasmids replicons (blue).

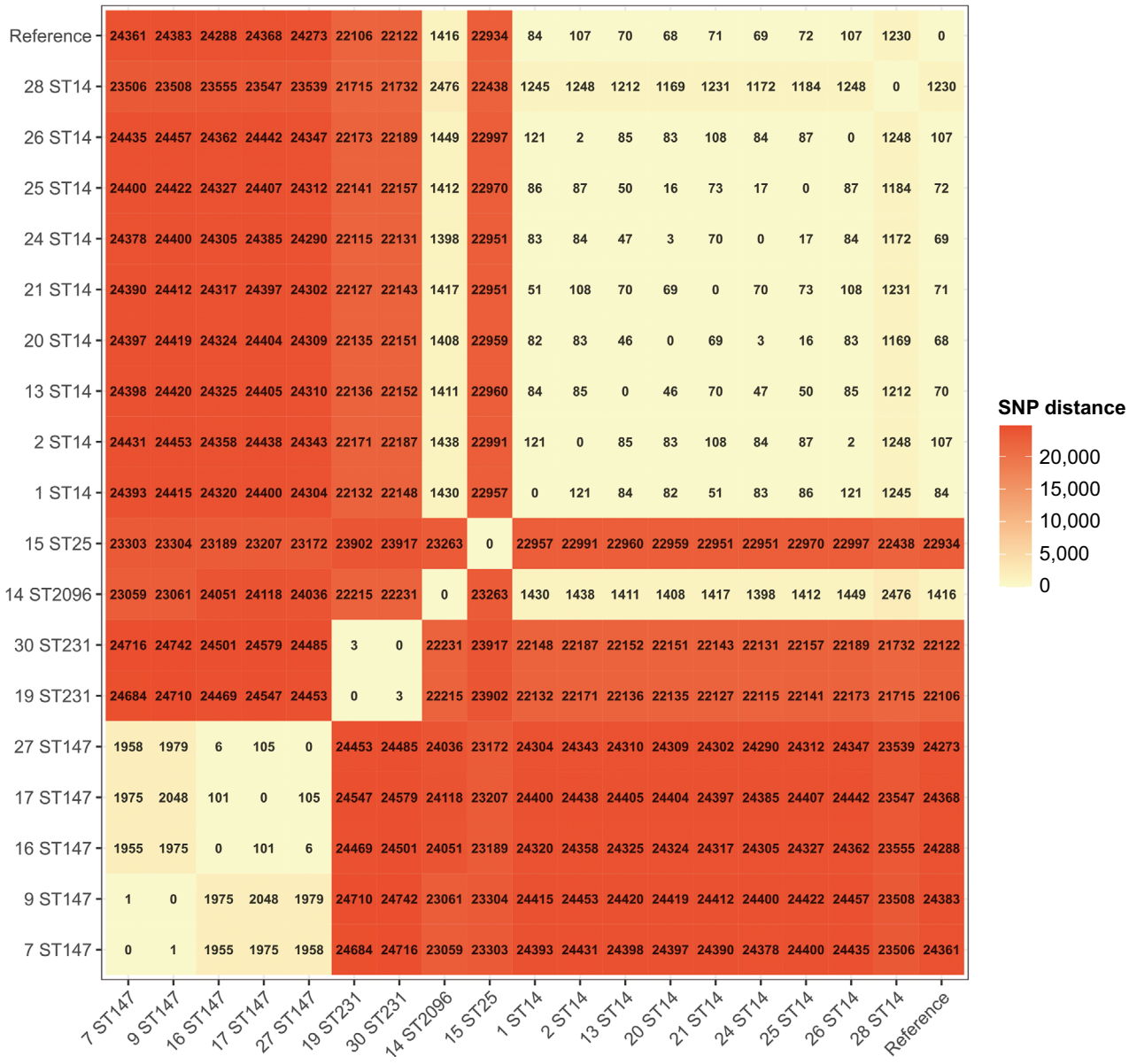


Fig. 2. (Color online) Pairwise comparison between sequences of *K. pneumoniae.*

ST147 isolates exhibited an identical compendium of AMR genes and indistinguishable plasmid profiles (Fig. 1). Furthermore, 2 ST231 isolates shared an identical plasmid containing the same AMR genes (Table 2). Multiple STs were isolated from 2 patients (P3 and P12). Patient P3, with a bulbar urethral stricture, had recurrent UTIs with ST14 and ST147; and patient P12, with autosomal dominant polycystic kidney disease and a right hydro-ureter, had multiple UTI episodes caused by ST14, ST147, ST231, and ST25 (Fig. 3, Table 1). We surmised that ST14 and ST147 probably circulated concurrently in the urology ward.

Because ST14 was the most common genotype, we analyzed the ST14 sequences in depth. We detected a wide array ($n$ = 35) of AMR genes in these ST14 isolates that were associated with resistance to several antimicrobial classes, including aminoglycosides, trimethoprim, fluoroquinolones, phenicols, sulfonamides, streptomycin, tetracycline, fosfomycin, rifampin, 16S *rRNA* methylases, and beta-lactams. Genes encoding resistance to aminoglycosides and fosfomycin (*fosA*) were present in all the isolates (Fig. 4). Additionally, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV}}$ and $bla_{\text{CTX-M-1}}$ were detected in all the isolates. We also detected $bla_{\text{TEM-1D}}$ in 4 isolates (Fig. 1).

**Virulence gene analysis and capsular typing:** We assessed the composition of the virulence-associated genes. The *ybt* (yersiniabactin) locus was the most common, present in 10 of the 18 isolates (56%), and found in multiple STs (ST231 [$n$ = 2], ST2096 [$n$ = 1], ST14 [$n$ = 4], ST147 [$n$ = 2], and ST25 [$n$ = 1]). The single hvKp strain belonged to ST2096, a single-locus variant of ST14 (Table 2). It was defined as hypervirulent based on the presence of *iuc*, *ybt*, and hypermucoviscosity on culture (string test positive). It also contained the enterobactins, *fepG* and *entS* (26). The plasmid in this isolate also carried β-lactamases and carbapenemases ($bla_{\text{OXA-1}}$ and $bla_{\text{OXA-48}}$). Consequently,

Table 2. The distribution of AMR and virulence determinants in sequenced organisms

| Organism ID | ST | Yersiniabactin | YbST | K type | Aerobactin/ Enterobactin | Mutation in *mgrB* gene | *bla*$_{CTX-M-1}$ | *bla*$_{NDM-1}$ | *bla*$_{OXA-48}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ST14 | *ybt 14; ICEKp5* | 140 | KL2 | *fepG, entS* | disrupted by IS110 element at 129th bp | + | + | - |
| 2 | ST14 | *ybt14; ICEKp5* | 140 | KL2 | *fepG, entS* | nil | + | + | - |
| 13 | ST14 | - | 0 | KL2 | *fepG, entS* | disruption by ISKpn26 element at 74th bp | + | + | - |
| 20 | ST14 | - | 0 | KL2 | *fepG, entS* | nil | + | + | - |
| 21 | ST14 | *ybt 14; ICEKp5* | 140 | KL2 | *fepG, entS* | non-synonymous mutation at 82th bp (T→G, Cys28Gly) | + | + | - |
| 24 | ST14 | - | 0 | KL2 | *fepG, entS* | nil | + | + | - |
| 25 | ST14 | - | 0 | KL2 | *fepG, entS* | nil | + | + | - |
| 26 | ST14 | *ybt 14; ICEKp5* | 140 | KL2 | *fepG, entS* | nil | + | + | - |
| 28 | ST14 | - | 0 | KL2 | *fepG, entS* | nil | + | + | - |
| 7 | ST147 | *ybt 9; ICEKp3* | 183 | KL64 | *fepG, entS* | lack of *mgrB* | + | + | - |
| 9 | ST147 | *ybt 9; ICEKp3* | 183 | KL64 | *fepG, entS* | disrupted by IS110 element at 74th bp | + | + | - |
| 16 | ST147 | - | 0 | KL10 | *fepG, entS* | nil | + | + | - |
| 17 | ST147 | - | 0 | KL10 | *fepG, entS* | nil | + | + | - |
| 27 | ST147 | - | 0 | KL10 | *fepG, entS* | nil | + | - | - |
| 19 | ST231 | *ybt 14; ICEKp5* | 145 | KL51 | *fepG, entS* | disrupted by IS110 element at 116th bp | + | + | + |
| 30 | ST231 | *ybt 14; ICEKp5* | 145 | KL51 | *fepG, entS* | disrupted by IS110 element at 117th bp | + | + | + |
| 14 | ST2096 | *ybt 14; ICEKp5* | 140 | KL64 | *fepG, entS, iucABCD* | nil | + | - | + |
| 15 | ST25 | *ybt 14; ICEKp5* | 149 | KL2 | *fepG, entS* | nil | + | + | - |

ST, sequence type.

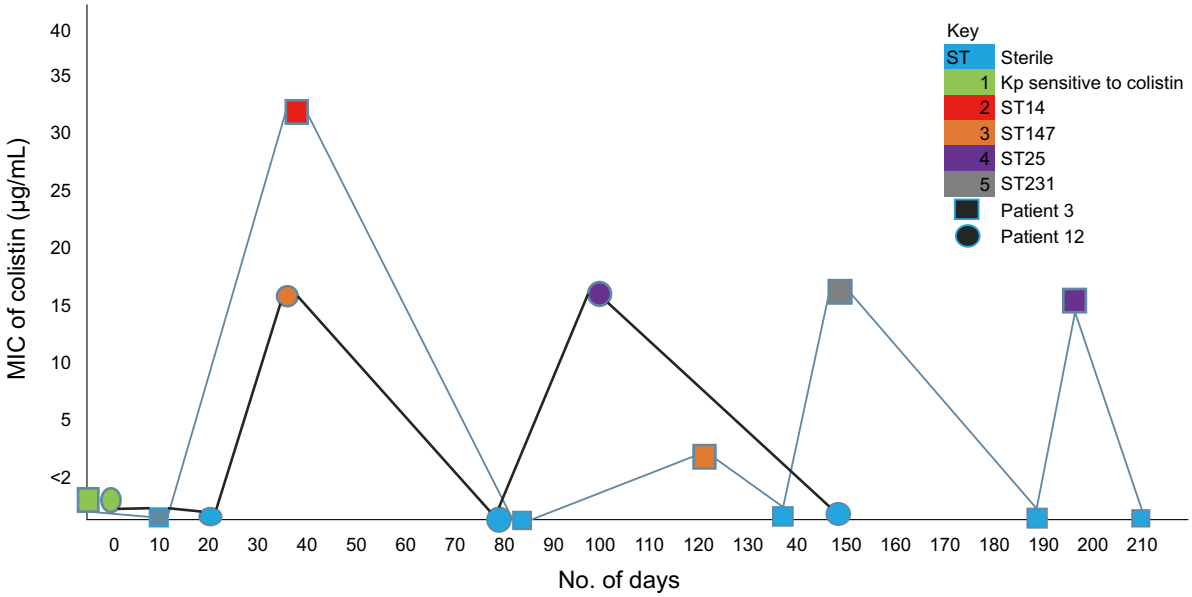


Fig. 3. (Color online) Patients with recurrent UTI due to colistin resistant *K. pneumoniae*. A line diagram of multiple *K. pneumoniae* STs isolated from 2 patients showing their colistin MIC on x-axis and no. of days on y-axis. Day 0 is day of 1st culture positivity.

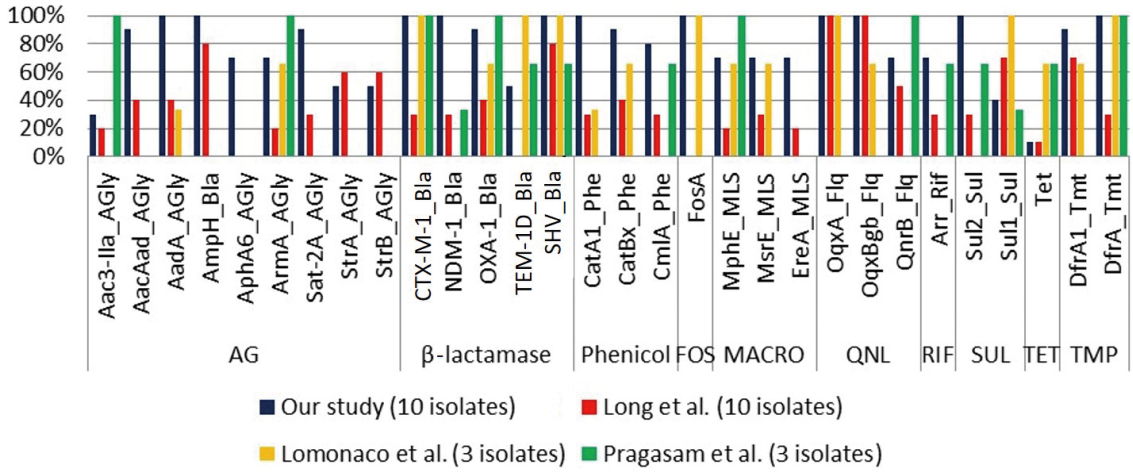


Fig. 4. (Color online) Resistance profile of ST14 isolates described in different studies compared with our isolates. AG, aminoglycosides; FOS, Fosfomycin; MACRO, macrolides; QNL, quinolone; RIF, rifampicin; SUL, sulphonamide; TET, tetracycline; TMP, trimethoprim.

we determined that the ST2096 isolate was a convergent XDR-hvKp variant. Six of the 10 isolates possessing *ybt* were also colistin resistant, with corresponding mutations in *mgrB* in 5 of the 10 isolates and deletions in one isolate (Table 2). All isolates with the *ybt* locus also possessed β-lactamases $bla_{SHV}$, $bla_{CTX\text{-}M\text{-}1}$; 8 possessed $bla_{TEM\text{-}1D}$; 3 were carbapenemase ($bla_{OXA\text{-}48}$) producers, and 9 were metallo-β lactamase ($bla_{NDM\text{-}1}$) producers (Fig. 1, Table 2). Additionally, all isolates contained the *fepG* (iron enterobactin) and *entS* (enterobactin) siderophores, which also contribute to virulence (6). No other hypermucoviscous variants, or isolates containing salmochelin or colibactin were identified. The most common K-type among these isolates was K2 ($n$ = 10), followed by K10, K51, and K64 (Fig. 1, Table 2).

## DISCUSSION

*Klebsiella pneumoniae* is the second most common organism associated with UTIs after *E. coli*, accounting for approximately 20% of the cases at our center (unpublished data). Crucially, the prevalence of MDR *K. pneumoniae* is 6 times higher than that of *E. coli* and UTIs caused by MDR *K. pneumoniae* are associated with increased morbidity (8). Colistin resistance is also emerging in India (27,28), and we identified organisms that were not susceptible to colistin. Colistin resistance in *K. pneumoniae* is commonly associated with the modification of lipid A, mutations in efflux pumps, and plasmid-mediated *mcr* genes (25). We did not detect any *mcr* genes or evidence of mutations in PmrA/PmrB, PhoP/PhoQ, *ram*A, or *ccr*B. We detected mutations in

*mgrB*, which encodes a small transmembrane protein that mediates negative feedback through the PhoQ/PhoP system (29). Several studies have shown that mutations in *mgrB* (present in 40% of the cases in this study) lead to colistin resistance (11). These mutations are thought to occur due to the selective pressure of colistin usage, colonization, increased transmissibility, and prolonged survival of these isolates. Other mechanisms, such as the accumulation of capsular polysaccharides or efflux pumps, may also contribute to colistin resistance in *K. pneumoniae* (30).

ST14 was identified as the predominant ST in this study. This is in accordance with a study by Long et al. (31), who found that ST14 is common in India. A study conducted in Vellore in southern India detected ST231, ST14, and ST147 (30), which is similar to the distribution of STs observed in this study. By combining data from comparative analyses of ST14 isolates from the aforementioned study in Vellore (30), the USA (31), and Pakistan (32), and our data, we observed that Indian isolates possessed more AMR genes than the organisms from the other countries (Fig. 4). Additionally, the ST14 isolates from India clustered into a single clade, suggesting a common origin, which is concerning given their capacity to cause UTIs and bloodstream infections.

The majority of isolates possessed enterobactin (*entS*) and half had siderophores such as yersinibactin (*ybt*), both of which contribute to virulence (7). The iron-scavenging ability of enterobactin can be limited by the binding of host lipocalin-2 to a ferric enterobactin. The presence of *ybt* aids in overcoming this defense mechanism and facilitates bacterial invasion (33,34). All isolates possessing *ybt* were ESBL or carbapenemase producers. The *ybt* locus is mobilized by a self-transmissible integrative conjugative element, which can also carry additional virulence determinants such as *iro* and *clb* (33). We identified a single ST2096 *K. pneumoniae* strain harboring aerobactin (*iucABCD*), *ybt* and $bla_{\text{OXA-1}}$ and $bla_{\text{OXA-48}}$, that was defined as a convergent XDR-hvKp variant (Table 2). This organism was isolated from a patient with recurrent UTIs and renal failure associated with urinary tract abnormalities. The patient was treated with aminoglycosides, cephalosporins, cotrimoxazole, and colistin during his 23 days of hospitalization. Despite antimicrobial therapy, his recovery was prolonged, and bacterial cultures remained positive 10 days after corrective surgery. The presence of convergent hvKp has been reported in various parts of India (28), and Wyres et al. (7) described Indian hvKp as belonging to ST2096 and ST231. Generally, hvKp is confined to community transmission, but we speculate that the virulence plasmid can integrate into classical hospital XDR-Kp and trigger major outbreaks.

The main concern regarding highly resistant and hvKp-inducing UTIs is that they are associated with high morbidity. Many patients, including those with non-hvKp infections, exhibit clinical evidence of urosepsis. We observed that several patients admitted to a single ward were infected with the same ST, indicating that these infections may have been acquired from a common source. Concurrently, some patients had recurrent infections with multiple STs circulating in a specific ward, providing further evidence of hospital transmission. As shown in Fig. 2, a very low genetic distance was observed between some isolates (e.g., 7ST147 and 9ST147, one SNP; 2ST14 and 26ST14, 2 SNPs; 20ST14 and 24 ST14, 3 SNPs; and 30ST231 and 19ST231, 3 SNPs). These appear to have been closely related and suggest nosocomial transmission.

Patients with recurrent UTIs often have prolonged hospital stays and are exposed to multiple antimicrobials, including carbapenems and colistin (8). MDR *K. pneumoniae* that circulates in hospitals is a major concern, as resistance genes are principally plasmid-mediated, and their ability to acquire further resistance genes gives them the potential to become XDR and PDR. This scenario could lead to more serious outbreaks associated with higher morbidity and mortality.

As the majority of the colistin-resistant isolates in this study were ESBL and NDM-1 producers, there are limited options for treating XDR infections (7). Therefore, new approaches are required to prevent and treat infections caused by these organisms. Formulating appropriate antimicrobial and infection control policies and compliance with hand hygiene, barrier nursing, and antimicrobial stewardship may help slow the emergence of these highly resistant pathogens. Periodic surveillance of hospital wards to assess circulating organisms and their antimicrobial susceptibility profiles should support the choice of empirical therapy.

In conclusion, this study provides new insights into the genes that facilitate AMR and virulence of *K. pneumoniae* associated with UTIs in India. The results suggest that convergent XDR-hvKp is present in our healthcare facility. We hypothesise that such organisms have outbreak potential, warranting more effective antimicrobial stewardship and improved infection control strategies in India.

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**Conflict of interest** None to declare.

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