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Clinical and genomic characterization of *Klebsiella pneumoniae* infections in Dhaka, Bangladesh

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Abstract

Background: *Klebsiella pneumoniae* (Kpn), a WHO priority pathogen with high rates of antimicrobial resistance (AMR), has emerged as a leading cause of hospital acquired pneumonia and neonatal sepsis. *Objective:* We aimed to define the clinical characteristics of a cohort of patients with Kpn infection in Dhaka, Bangladesh and to perform phenotypic and genetic characterization of the associated isolates.

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Author contributions

JBH, RCL, FQ designed the study. ZK, JBH, RCL and FQ developed the study protocol and obtained ethical approval. SMS and RR supervised the clinical site; NNT, MN, KA and UK were involved in case enrollment and data collection. SK, ZK and ABS conducted the laboratory work. ZK, SAM, SS, TH and SK analyzed the data. ZK, SK and SS interpreted the results. ZK and SS prepared the draft manuscript. All the authors reviewed and finalized the manuscript and consented for publication.

Declaration of competing interest

SET, JBH and RCL have received royalties from UpToDate. All other authors: no reported conflicts of interest.

Ethical approval

Approval from the National Research Ethics Committee (NREC) of Bangladesh Medical Research Council (BMRC) (Registration number 43617082021; Date of approval: 23 January 2022) was obtained for the study. All procedures were performed in compliance with the NREC.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.12.016.

Methods: We retrospectively extracted clinical data about patients at Dhaka Medical College Hospital from whom *Klebsiella spp* was isolated from a clinical specimen collected between February and September 2022. We used standard microbiologic techniques to evaluate AMR and whole-genome sequencing (WGS) to assess dominant lineages, common capsular (K) and O-polysaccharide (O) antigen types, and AMR and virulence genes.

Results: Ninety-eight patients were included, with diagnoses of pneumonia (38/98, 39 %), wound infection (29/98, 31 %), urinary tract infection (29/98, 31 %) and bacteremia (2/98, 2 %). We tested isolates for susceptibility to eight classes of antibiotics. Of the 98 isolates, 41 % were multidrug resistant (MDR), 15 % were extensively drug resistant (XDR), and 16 % were pan-drug resistant (PDR). Three isolates (3 %) were resistant to polymyxin B. Outcome data were available for 46 patients; 4 patients (8 %) died from infections caused by PDR ($n = 2$), XDR ($n = 1$), and MDR isolates ($n = 1$). WGS revealed a high degree of genomic diversity, with multiple sequence types (STs), O-types and K-types represented; ST16:K81:OL101 and ST43:K30:O1 were the most prevalent.

Conclusion: Our findings suggest alarming levels of AMR among Kpn isolates in Bangladesh and a critical need for improved treatment modalities and vaccine development.

Keywords

Klebsiella pneumoniae ; Antimicrobial resistance; Bacterial virulence; Sequence types

1. Introduction

Antimicrobial resistance (AMR) in Gram-negative bacteria is an urgent health threat, due to the emergence of strains that are resistant to all or most available antimicrobials [1,2]. This phenomenon relates to inappropriate use of antibiotics in clinical and community settings, and is often facilitated by the horizontal transfer of resistance genes between bacteria [3]. *Klebsiella pneumoniae* (Kpn) is amongst the leading causes of hospital-acquired infections (HAI) globally, including urinary tract infections (UTI), pneumonia, wound infections, bacteraemia leading to sepsis, and other invasive infections [4]. Infections caused by multidrug resistant (MDR) Kpn are increasingly reported in hospitalized patients in Bangladesh and are an important cause of morbidity and mortality [5].

Several circulating clones of Kpn are responsible for up to 8 % of all nosocomial bacterial infections affecting high-risk patients. These dominant lineages show varying resistance to late generation cephalosporins and carbapenems, and include sequence type (ST) 258, which has been isolated in the US and Europe [6], and ST11, which has been detected in Southeast Asia [7]. No data are available regarding currently circulating Kpn clones in Bangladesh.

Vaccines represent one potential solution for the increasing threat of AMR Kpn strains. However, the high diversity of surface antigens poses a challenge for vaccine design. The O-specific polysaccharide (O-antigen) is diverse, having 9 different types, and there are 79 distinct capsular polysaccharides (K-antigen) [8]. For this reason, an understanding of the features of invasive Kpn infection, including the diversity of O-antigen and K-antigens, is essential [9].

Here, we describe the characteristics of Kpn and other closely related *Klebsiella* spp. infections in a tertiary hospital in Bangladesh, focusing on clinical and bacterial characteristics and applying a combination of phenotypic characterization and whole genome sequencing (WGS). Our study findings highlight the importance of surveillance and the need for appropriate antimicrobial treatment in high-burden settings such as Bangladesh.

2. Methods

2.1. Study population and clinical data collection

Cases (n = 98) were included in this study if a *Klebsiella* spp. was isolated from a sputum, urine, wound swab, endotracheal aspirate, blood or catheter tip specimen in the laboratory of Dhaka Medical College Hospital (DMCH) in Dhaka, Bangladesh, between February and September 2022. Available demographic, clinical and laboratory data were extracted from the hospital medical record using a standardized data entry tool. Infections were classified as hospital-acquired if they were identified in a hospitalized patient at least 48 h after admission [10,11]. Due to missing information in the hospital registry, clinical outcome data (i.e. clinical recovery or death) were available for only a subset of cases (63/98). Informed written consent was not obtained for this study as there was no direct data collection from human subjects.

2.2. Characterization of Kpn strains and AMR testing

Bacterial isolates were identified in the DMCH microbiology laboratory using conventional phenotypic microbiological methods according to institutional standard operating procedures and were reconfirmed by biochemical testing at the laboratory of the institute for developing Science and Health initiatives (ideSHi) in Dhaka, Bangladesh. Antimicrobial susceptibility for 14 antibiotics was ascertained by the Kirby-Bauer disk diffusion method and interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria. Minimum Inhibitory Concentrations (MICs) (range: 0.50–16 mg/L) were determined for colistin using a commercial VITEK 2 AST system and broth microdilution.

For selected bacterial isolates that yielded discordant biochemical test results, identification and susceptibility were confirmed using the VITEK 2 identification and susceptibility cards (VITEK® 2 Compact, bioMérieux, Inc., USA). Strains were classified as multidrug resistant (MDR), extensively drug-resistant (XDR) or pan-drug resistant (PDR) based on the antimicrobial susceptibility testing profile for 14 tested antimicrobials (not including colistin). MDR was defined as resistance to at least one agent in three or more antimicrobial categories, XDR was defined as resistance to at least one agent in all but two or fewer antimicrobial categories, and PDR was defined as resistance to all agents in all antimicrobial categories [12].

2.3. Isolate DNA extraction, library preparation, and sequencing

Genomic DNA from confirmed isolates was extracted using a QIAamp DNA Mini Kit (Qiagen, Germantown, MD) and quantitated using a Qubit 4 Fluorometer (ThermoFisher Scientific, Waltham, MA). WGS was conducted at the Vanderbilt University Medical Center Vantage core facility (Nashville, TN). Library prep was done with the Twist Biosciences

(San Francisco, CA) NGS Library Prep Kit and 150-base paired-end reads were sequenced on an Illumina NovaSeq60 0 0 (San Diego, CA). An average of 13.8 million reads was generated per isolate.

2.4. Whole genome sequencing data analysis

We used Trimmomatic [13] and FastQC [14] for read trimming and quality control, respectively. Burrow-Wheeler Aligner (BWA) [15] was used for read alignment to reference strain NTUH-K2044, and Pilon [16] was used to call variants and generate variant-calling files [17]. Recombination removal was performed using Gubbins version 2.3.1 [18]. A midpoint-rooted phylogenetic tree of the 86 Kpn isolates, as confirmed bioinformatically using Kleborate [19], was generated using RAxML [20] and visualized using R package ggtree and ggtreeextra [21,22]. Genome assemblies were generated using SPAdes [23], and these assemblies were used to determine *Klebsiella*-specific characteristics using the Kleborate tool. AMRFinderPlus [24] and PlasmidFinder [25] were run on assemblies to identify AMR and virulence determinants.

2.5. Statistical analysis

Statistical comparisons between proportions were computed using Pearson's Chi-square test. R version 4.2.1 was used for statistics and data visualization. Statistical significance was defined as a *P*-value ≤0.05.

3. Results

3.1. Clinical characteristics of Klebsiella spp. cases

We identified 98 patients with *Klebsiella* spp. cultured from clinical specimens at DMCH between February and September 2022. The demographic and clinical characteristics of the 98 patients are shown in Table 1. Among the patients, 56 (57 %) were male and 42 (43 %) were female. Most patients were adults (78/98, 80 %), and patients ranged in age from 14 days to 80 years, with a median age of 36 years. Most of the patients had pneumonia (38 %), followed by wound/tissue infections (30 %) and urinary tract infections (30 %). For the subset of patients for whom the hospital admission date was available, we ascertained that 39/63 (62 %) were HAI and the remaining 24/63 (38 %) were community-acquired infections (CAI).

3.2. AMR patterns of Klebsiella spp. isolates

Fig. 1A shows the proportion of *Klebsiella* spp. isolates susceptible to each of the 14 tested antibiotics. More than half of the study isolates were resistant to the following antibiotics: ceftriaxone, ceftazidime, cefepime, ciprofloxacin, levofloxacin, cotrimoxazole, and doxycycline. Overall, 40/98 (41 %) isolates were MDR, 15/98 (15 %) were XDR, and 16/98 (16 %) were PDR. On broth microdilution testing, 3/98 (3 %) isolates demonstrated a colistin MIC > 4 μg/ml. Resistant isolates were distributed across different infection sources (Fig. 1B). XDR and PDR isolates were more common among HAI than among CAI (Fig. 1C). Significant differences between HAI and CAI were noted for XDR (P value 0.0 0 03), PDR (P value 0.001) as well as non-MDR (P value < 0.001) by Fisher's exact test.

We obtained clinical outcome data for a subset (47 %, 46/98) of patients. Of these, 10/46 (22 %) were hospitalized for a duration of more than 30 days, and 4/46 patients (8 %) died as a complication of pneumonia ($n = 3$) or urinary tract infection ($n = 1$). Of the four patients who died, two had infections caused by PDR isolates, one had infection caused by an XDR isolate, and one had infection caused by an MDR isolate.

3.3. Genomic diversity of clinical isolates

We performed WGS on the *Klebsiella* isolates. Eighty-six were identified by WGS as Kpn, while the remainders were identified as *Klebsiella quasipneumoniae* subsp. *similipneumoniae* (8 isolates), *Klebsiella variicola* (3 isolates) and *Klebsiella aerogenes* (1 isolate). The GenBank accession IDs along with the metadata for all the sequenced isolates are in Supplementary File 1. The 86 Kpn isolates comprised 42 sequence types (STs), revealing considerable diversity, which was confirmed by results from phylogenetic reconstruction (Fig. 2). Despite overall high diversity, some STs were represented by more than one isolate, including ST11 (9 [11 %]), ST15 (4 [5 %]), ST16 (9 [11 %]), ST23 (6 [7 %]), ST29 (3 [4 %]), ST43 (4 [5 %]), ST45 (3 [4 %]), ST48 (5 [6 %]), and ST147 (4 [5 %]). These overrepresented STs were distributed across multiple hospital wards, without evidence of epidemiologic linkage. A pairwise comparison of isolates found that no two isolates were fewer than 25 SNPs apart, suggesting the isolates were not clonal.

3.4. O and K types

We next investigated the O- and K-antigen types of the 86 Kpn isolates. We found that the O1 type predominated, comprising 40/86 (47 %) of the isolates (Fig. 2). The O1 type also predominated across different specimen types (11/37, 30 % of sputum/tracheal aspirates; 11/29, 40 % of urine; and 18/29, 62 % of wound swabs). The distribution of K antigen types was also very broad. Among the 37 K-antigen types identified, we found K81 to be most abundant (9/86, 10 %), followed by K24 (8/86, 9 %), and K1 (6/86, 7 %); five K types- K2, K62, K64, K30 and KL112 -had the same frequency distribution (4/86, 5 %). The remaining 43/86 (50 %) isolates were other K types.

3.5. AMR and virulence genes in Kpn

We found that the presence of AMR genes was consistent with the phenotypic AMR profile of the study isolates. A total of 78 AMR genes were distributed among the 86 isolates. All of the Kpn isolates had one or more chromosomally-encoded *bla* gene (Supplemental File 1) and 54/86 (63 %) had additional antimicrobial resistance genes (ARGs) including $bla_{\text{OXA-1}}$, $bla_{\text{OXA-9}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{DHA-1}}$ and $bla_{\text{LAP-2}}$, which confer resistance to beta-lactams. Sixty-one isolates (71 %) carried $bla_{\text{CTX–M-15}}$ and were suspected extended spectrum beta lactamase (ESBL) producers. Strikingly, 37/86 (43 %) of the Kpn isolates harbored at least one carbapenemase gene (Fig. 3A), with $bla_{\text{OXA-181}}$ being the most frequent (24/86, 28 %) either alone or in combination with $bla_{\text{NDM-1}}$ or $bla_{\text{NDM-5}}$. The distribution of ST, O and K-types across isolates carrying one or more of these AMR genes along with their genomic virulence and resistance characteristics is outlined in Supplementary File 2. We observed concordance between carbapenem resistance phenotypes and carbapenemase carriage in all but 6/86 (7 %) isolates; all six of these isolates without a carbapenemase carried the beta-lactamase $bla_{\text{CTX–M-15}}$, and two of the isolates had truncations in porin OmpK35,

which likely reduced carbapenem uptake. In contrast, one isolate with both *bla*$_{\text{OXA-181}}$ and *bla*$_{\text{NDM-5}}$ did not show phenotypic resistance to any of the three carbapenems tested. We hypothesize that this discordance may have arisen from the combination of *bla*$_{\text{SHV}}$ and OmpK mutations, but we did not formally assess this possibility.

Kpn isolates typically are either MDR or hypervirulent, and we sought to understand whether there was a convergence of resistance and virulence gene presence. To do so, we used Kleborate to determine the number of resistance and virulence genes, respectively, in each isolate. We found that four isolates with some of the highest resistance and virulence scores belonged to ST43 (Fig. 3B). These four isolates were ESBL and carbapenemase producers, as evidenced by carriage of *bla*$_{\text{CTX–M-15}}$ (4/4[100 %]), *bla*$_{\text{TEM-1D}}$ (2/4[50 %]), *bla*$_{\text{OXA-9}}$ (2/4[50 %]), *bla*$_{\text{SHV-11}}$ (2/4[50 %]) and *bla*$_{\text{OXA-181}}$ (4/4[100 %]). Moreover, these four ST43 all belonged to the K30 and O1 antigen types. They had high virulence scores due to the presence of *rmpA2, ybt, iro* and *iuc* genes, which together form the genetic criteria defining hypervirulence [1,26], and high resistance scores, due to the presence of carbapenemase and colistin resistance genes. In contrast to ST43, we found six hypervirulent ST23:K1:O1 isolates encoding *iuc1, iro1, rmp1, rmpA2* and *wzi* virulence genes with few resistance markers. The isolates with the greatest number of resistance genes were from the lineage ST16:K81:OL101. The lineage ST11:K24:O2a was of particular concern as these isolates were carbapenem-resistant, and two of them contained siderophore encoding genes (Yersiniabactin, Aerobactin and Salmochelin) and genes responsible for mucoviscosity (*rmpA, rmpA2*) and colibactin (*pks* cluster).

We also investigated the resistome and virulome of the most predominant resistant molecular combinations and the most predominant virulent molecular combinations (Supplementary Fig. 4A). ST16:K81:OL101 had the highest number of resistance genes. Among the three isolates with resistance to colistin, one belonged to ST16:K81:OL101 and two belonged to ST11:K24:O2a. ST43:K30:O1 was the type with the highest virulence scores (Supplementary Fig. 4B).

We used PlasmidFinder to assess plasmid variability in the 86 Kpn isolates (Supplementary File 3). Of the 28 identified plasmid types, IncFIB(K) (plasmid incompatibility groups) was the most frequent plasmid (70 %), followed by another resistance plasmid Col4401 (49 %). Plasmids such as IncFII, IncR, IncFII(K) and IncHI1B, which are known to carry carbapenemases [27], were also found in 34 (IncFII, IncR), 32 (IncFII(K)) and 27 (IncHI1B) Kpn isolates. Only one KPN isolate (KPN-01–061) was devoid of any known type of plasmid by PlasmidFinder.

4. Discussion

In this study, we investigated clinical isolates from a series of patients diagnosed with invasive infections by *Klebsiella* spp. in the largest tertiary level hospital in Bangladesh during an eight-month period in 2022. We found high rates of AMR among the *Klebsiella* isolates, including to the first-line antibiotic (ceftriaxone) administered to hospitalized patients in this setting. Over one-third of isolates were resistant to carbapenems, driven by frequent carriage of OXA-181 and NDM-5. We also identified a diversity of O antigens,

K antigens, and STs among the clinical isolates, which is relevant for future vaccine development for *Klebsiella*.

In our study, specimens from pneumonia patients were the most common source of the study isolates. This is similar to recent findings from Pakistan, where respiratory samples were the most common source of resistant *Klebsiella* infection [28]. Similar observations regarding the specimen source have also been reported in Singapore and India [29,30]. Bacteremia with Kpn was uncommon in our study population. Low use of blood culture in Bangladesh, or the possibility of antibiotic administration prior to sample collection, may have contributed to this observation [31,32].

More than half of the clinical isolates in this study were resistant to seven antibiotics tested, and 70 % of the isolates were ESBL-producing. The high rate of isolates harboring carbapenemase genes- $bla_{\text{NDM-5}}$ (35 %), $bla_{\text{OXA-181}}$ (38 %) – is consistent with the concerning AMR rates prevailing in Bangladesh and other Southeast Asian countries [28,33]. A recent study in Dhaka found *Kpn* to be a leading cause of sepsis in children hospitalized with pneumonia; 4 of 11 children had infections with *Kpn* that were resistant to all first- and second-line antibiotics, and all of these children died [34].

The clinical isolates in our study had a heterogenous distribution of 37 K types and 7 O types. We also identified a marked diversity of sequence types, but there were several high-risk MDR clones belonging to the ST43:K30:O1 serotype. This contrasts with findings from other parts of the world, where the hospital-adapted high-risk ST258 lineage is common [35,36].

Our study has limitations. First, we were unable to collect detailed follow up information for all 98 cases, and hence our conclusions about mortality are limited. Additionally, our study isolates were obtained from a single hospital setting (although it is the largest tertiary care hospital in Bangladesh), which may not be representative of other clinical settings in Bangladesh. We assessed virulence factors based on WGS, but we were unable to perform phenotypic tests of virulence, such as assessing hypermu-coviscosity or siderophore activity. We noted a few discrepancies between phenotypic and genotypic carbapenem resistance profiles, which suggests a need for further understanding of the drug resistance mechanism in these selected isolates and the importance of parallel phenotypic antimicrobial susceptibility testing and WGS analysis in an area of high burden [37].

In conclusion, our study provides insights regarding the current epidemiology of Kpn infection in Bangladesh. High-level AMR is common in this organism in a hospital setting in Dhaka and includes resistance to broad-spectrum antimicrobials of last resort. These findings validate the need for improved surveillance systems for AMR Kpn in Bangladesh and vaccine development for control of this rapidly emerging AMR pathogen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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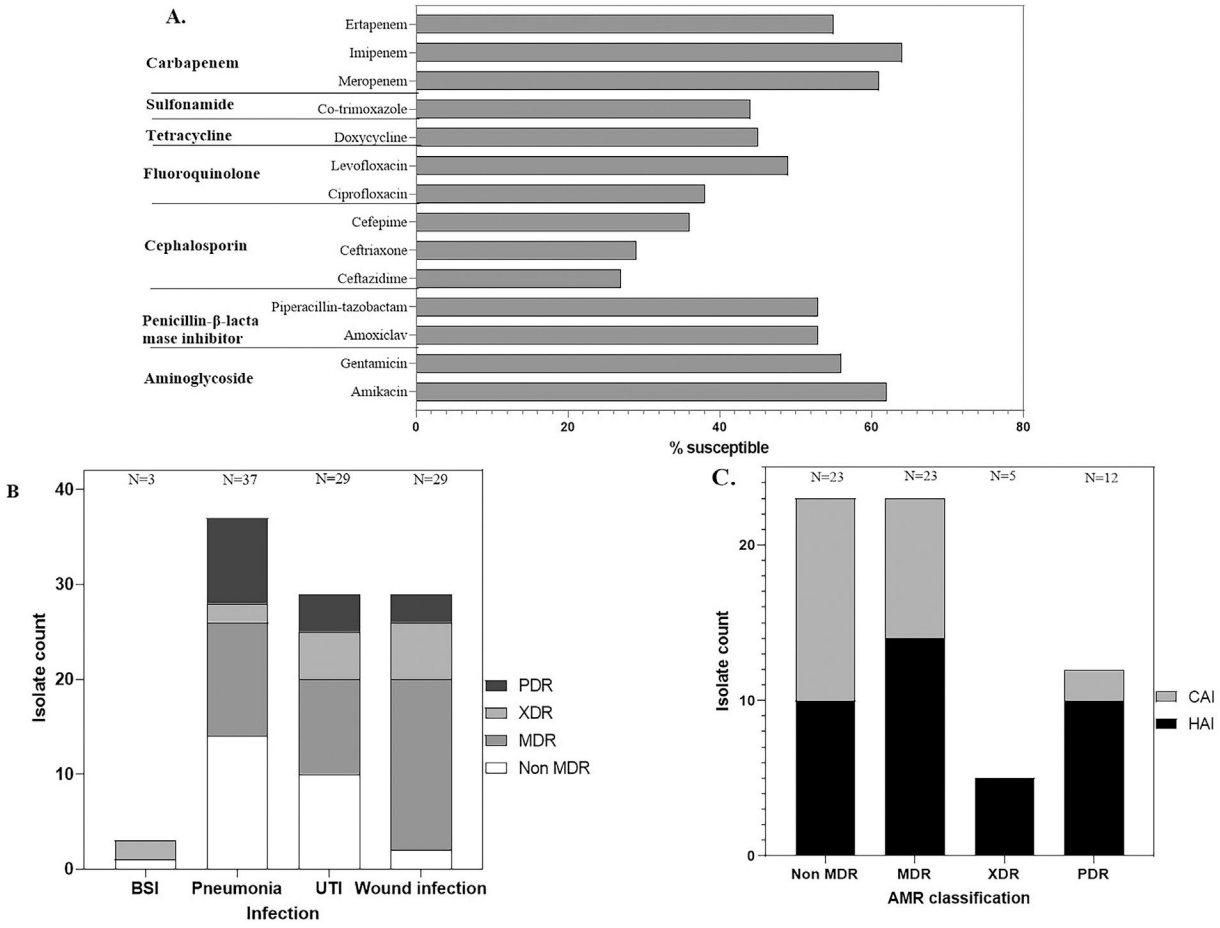

Fig. 1. AMR patterns of *Klebsiella* isolates.

A. Antimicrobial susceptibility testing results by disk diffusion for the 16 tested antibiotics**.** **B.** Number of non-MDR, MDR, XDR, and PDR isolates by isolate source. **C.** Number of HAI and CAI, indicated by non-MDR, MDR, XDR, and PDR classification.

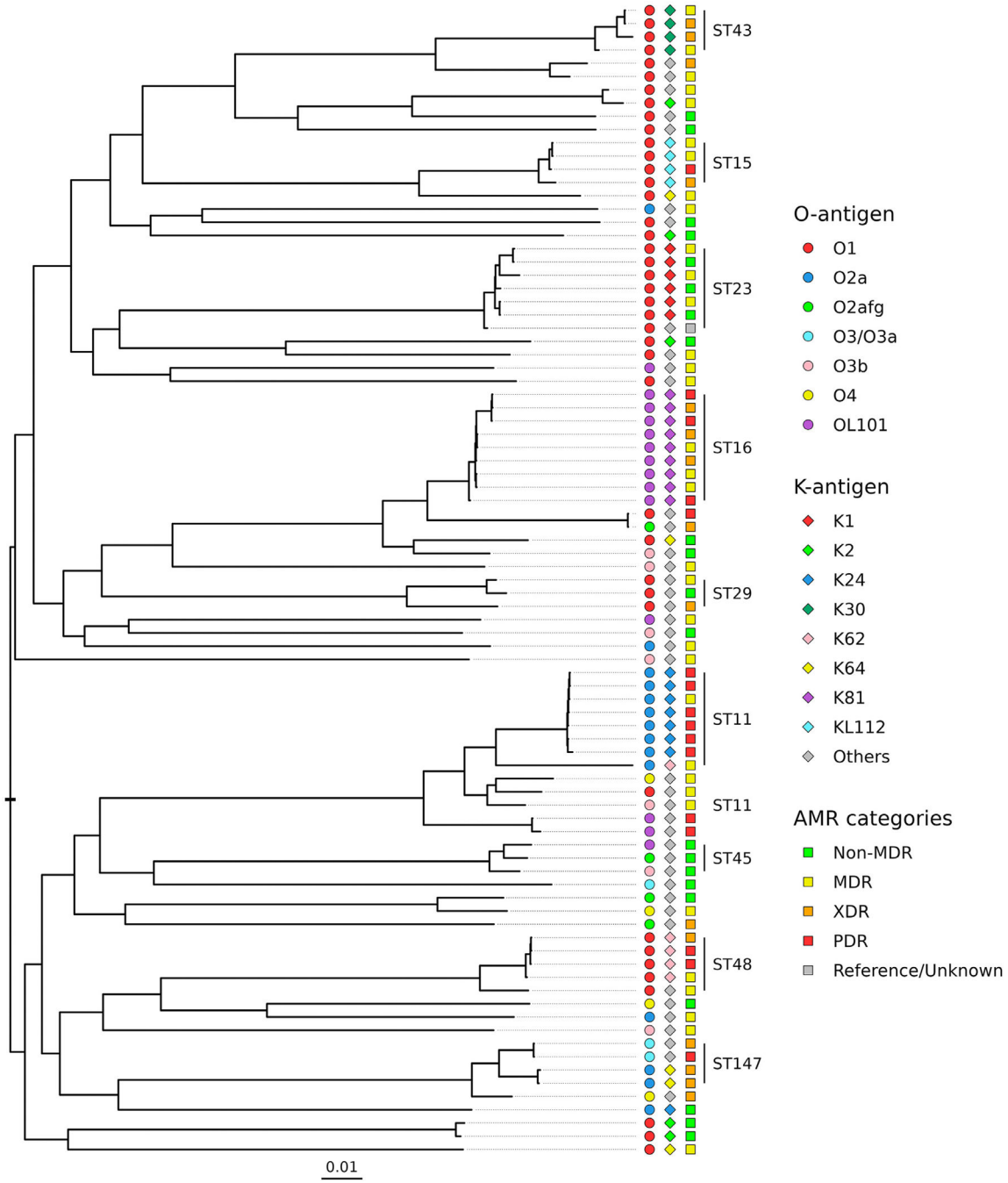

Fig. 2. Phylogenetic tree representing the 86 Kpn isolates.

Midpoint-rooted phylogenetic tree of 86 Kpn isolates with O- and K- antigen types and phenotypic AMR. O and K antigen types are indicated by circles and diamonds, respectively. The phenotypic AMR categories of the respective isolates are indicated by squares in the outermost column.

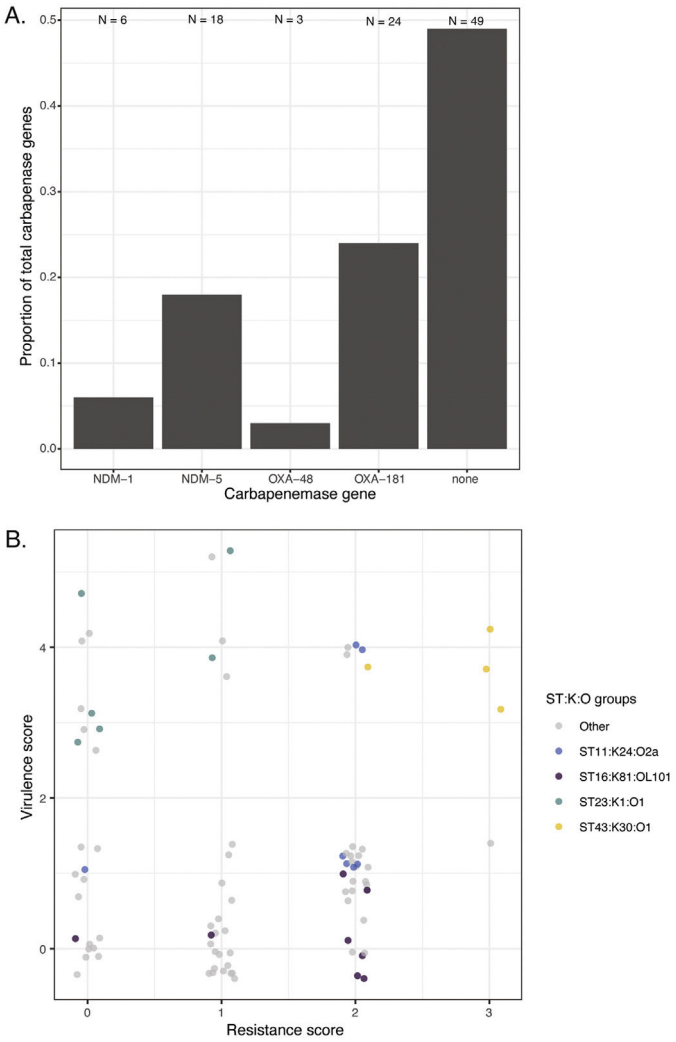

Fig. 3. Resistance and virulence characteristics of Kpn isolates.

A. Proportion of total carbapenemase genes found across all isolates. **B.** Comparison of the Kleborate resistance (x-axis) and virulence (y-axis) scores, with four ST:K:O combinations highlighted. Resistance and virulence scores are integer values, but jitter was used in the plot to distinguish isolates.

Table 1

Demographic and clinical characteristics of *Klebsiella spp* cases (N = 98[*])

Characteristic	
Age (median, range)	36 years (14 days – 80 years)
Gender	
Female	42 (43 %)
Male	56 (57 %)
Source of isolate	
Sputum or tracheal aspirate	37 (38 %)
Wound swab	29 (30 %)
Urine	29 (30 %)
Blood	3 (3 %)
Source of infection [*]	
Hospital-acquired	39/63 (62 %)
Community-acquired	24/63 (38 %)

[*]Data regarding source of infection was available for 63 of the 98 study subjects.