

First Identification of *bla*_{NDM-16b}-Harboring IncX3 Plasmid in Pediatric *Klebsiella pneumoniae* Reveals Cross-Species Dissemination and Clinical Risk Patterns

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Abstract

Multidrug-resistant *Klebsiella pneumoniae* (MDR-KP) is a growing public health threat to children worldwide, but its genetic traits and clinical risk factors in pediatric patients are not well understood. To address this, we collected 39 MDR-KP isolates from 37 pediatric patients. We used whole-genome sequencing (WGS) to analyze their genomic features and examined statistical links between antimicrobial resistance (AMR) profiles and clinical data. Our results show that AMR patterns were significantly associated with bacterial genomic features (such as sequence type, number of resistance genes, and virulence gene count), patient factors (mode of delivery, gravidity, parity, age and admission weight), and clinical details (specimen type, admitting department and affected organ system). Specifically, ST11-type *K. pneumoniae* showed much higher resistance to sulfonamides (OR = 24.135, 95% CI: 1.656–351.801, $P = 0.020$). Notably, we detected the $bla_{NDM-16b}$ for the first time in a pediatric case, carried by a highly resistant strain (ETFK5283) on an IncX3 plasmid. Genetic analysis indicated this plasmid is closely related to one (pTMTA85711, AP024206) previously found in an *Escherichia coli* isolate from Tokyo, Japan. These findings highlight the urgent need for pediatric-focused genomic surveillance, tailored antibiotic use, and stronger infection control measures to manage high-risk MDR-KP in children.

1. Introduction

Infections caused by multidrug-resistant *Klebsiella pneumoniae* (MDR-KP) are a major global public health challenge. Limited treatment options often lead to high rates of illness and death [1]. The rise of MDR-KP is particularly concerning because these bacteria often produce extended-spectrum β -lactamases (ESBLs) and carbapenemases, leading to hospital outbreaks that are difficult to control [2, 3]. There is an urgent need to identify the risk factors for these infections, implement preventive measures, and improve clinical management, especially for immunocompromised patients [4].

This burden is especially heavy in pediatric care. Children—particularly newborns and infants—are more vulnerable due to their developing immune systems, fewer available drugs, and strict dosing requirements. MDR-KP is a leading cause of severe pneumonia, urinary tract infections, and bloodstream infections in hospitalized children [5]. Management is further complicated by physiological factors like immature organ function [6, 7]. Studies have reported a high prevalence and increasing trend of antimicrobial resistance in *K. pneumoniae* strains from pediatric patients [8]–[9], highlighting the need for genomic studies to understand how MDR-KP evolves and spreads in children.

MDR-KP cases in children are being reported worldwide. In Shanghai children's hospital, 16 bla_{NDM-5} -positive ST48 *K. pneumoniae* strains were found in neonatal and pediatric intensive care units (ICUs), most showing high resistance to β -lactam antibiotics [10]. In China, a hypervirulent strain carrying the *mcr-8.2* gene was reported in a pediatric sepsis case, demonstrating resistance to colistin [11]. Similarly, outbreak of carbapenem- and colistin-resistant strains have occurred in Indian NICUs [5]. In Ethiopia, a

high prevalence of *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} genes was found in isolates from newborns [7]. In the Gaza Strip, *K. pneumoniae* accounted for over 22% of ESBL-positive isolates in a pediatric hospital [12].

However, systematic studies on risk factors for antimicrobial resistance and mortality in pediatric MDR-KP infections remain limited. Some studies identified risk factors such as recent antibiotic use and cancer chemotherapy, but these were in adult populations [13]. While one study linked *bla*_{SHV} gene to higher newborn mortality, other predictors were not fully explored [7]. Other research in children has linked indwelling urinary catheters to mortality in Gram-negative bacteremia [14], and chemotherapy to increased risk of carbapenem-resistant *K. pneumoniae* bloodstream infections in children with hematologic cancers [15]. Conversely, factors like maternal anemia and early penicillin exposure were found to protect against MDR-KP colonization in newborns [16].

To address these knowledge gaps, we performed a detailed genomic and clinical analysis of MDR-KP isolates from pediatric patients. A flowchart summarizing the study design is provided in Supplemental Flowchart. We collected 39 MDR-KP isolates from pediatric infections to define their resistance profiles and genetic features. We also analyzed patient clinical data to identify factors influencing resistance. Significantly, we report what we believe is the first isolation of a *bla*_{NDM-16b}-carrying *K. pneumoniae* strain from a child, and we trace the origin of this rare resistance gene. Our findings provide crucial insights for shaping antibiotic stewardship, guiding therapy, and improving infection control in pediatric healthcare settings.

2. Materials and methods

2.1 Strain Isolation and Identification

From October 2018 to February 2024, we collected residual clinical specimens from pediatric inpatients at The First Affiliated Hospital of Zhengzhou University. These included sputum, urine, blood, anal swabs, and body fluids. *Klebsiella pneumoniae* isolates were obtained using standard culture methods to study the characteristics colonization and infection in this population.

This study was part of a multicenter project coordinated by the First Affiliated Hospital, Zhejiang University, which provided central ethical approval (FAHZU_Ethics_2023-0994-Quick). This approval covered sample collection and processing at all participating sites, including Zhengzhou. Written informed consent was waived by the ethics committee. Verbal consent was obtained from all legal guardians, as the study used only de-identified residual samples from routine tests, involved no additional procedures, and adhered to strict privacy protections. Consent was documented using the hospital's standardized admission form, which includes permission for the use of such samples in retrospective research.

Specimens were inoculated into brain heart infusion (BHI) broth and incubated at 37°C with shaking for 18–24 hours. The cultures were diluted with phosphate-buffered saline (PBS) and streaked onto

Columbia blood agar plates. After 24 hours of incubation at 37°C, morphologically distinct colonies were re-streaked to obtain pure isolates. Species identification was performed using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany). Confirmed *K. pneumoniae* isolates were stored in glycerol at -80°C for further analysis.

2.2 Antimicrobial Susceptibility Testing

We determined the minimum inhibitory concentrations (MICs) of 21 antimicrobial agents against *Klebsiella pneumoniae* using agar dilution and broth microdilution methods. The tested agents included: aztreonam, imipenem, meropenem, ceftriaxone, cefotaxime, ceftazidime, levofloxacin, ciprofloxacin, amikacin, gentamicin, piperacillin-tazobactam, fosfomycin, chloramphenicol, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, cefepime, ceftazidime-avibactam, tigecycline, polymyxin B, omadacycline, and eravacycline.

Susceptibility interpretations followed established standards: tigecycline and polymyxin B were interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (v15.0), while omadacycline and eravacycline were assessed according to the U.S. Food and Drug Administration (FDA) criteria (<https://www.fda.gov>). All other agents were evaluated based on CLSI M100-Ed35 guidelines (<https://clsi.org>). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as quality control strains.

2.3 Whole-Genome Sequencing (WGS) and Bioinformatics analysis

Genomic DNA was extracted using a commercial purification kit (QIAGEN, Hilden, Germany). Sequencing was performed on the Oxford Nanopore platform, and the resulting raw data were assembled into complete genome with Unicycler [17]. We used CheckM to assess assembly quality, retaining only those with $\leq 5\%$ contamination and $\geq 95\%$ completeness for downstream analysis.

Genome annotation was performed with Prokka [18]. The annotated genomes were then screened for several genetic determinants: antimicrobial resistance genes were identified using ResFinder (<https://cge.food.dtu.dk/services/ResFinder/>); virulence factors were queried against the Virulence Factor Database (VFDB); and insertion sequences (IS) and transposons were detected via ISFinder (<https://www-is.biotoul.fr/>). Multilocus sequence typing (MLST) was assigned based on the Institut Pasteur database (<http://bigsd.bweb.pasteur.fr/klebsiella/klebsiella.html>). Plasmid types were identified using both PlasmidFinder (<https://cge.food.dtu.dk/services/PlasmidFinder/>) [19, 20] and mlplasmids (<https://sarredondo.shinyapps.io/mlplasmids/>) [21]. Genetic contexts of specific plasmid genes were visualized with Easyfig [22], and multiple plasmid comparisons were generated using Proksee (<https://proksee.ca/>) [23].

2.4 Phylogenetic analysis

A maximum-likelihood phylogenetic tree was constructed with IQ-TREE based on core genes identified by Roary [24] to determine the phylogenetic relationships among the isolated *K. pneumoniae* strains from pediatric pneumonia cases. The tree was annotated and visualized using the Interactive Tree of Life (iTOL) platform (<https://itol.embl.de/>) [25].

2.5 Statistical analysis

We collected clinical data from the electronic medical record system of The First Affiliated Hospital of Zhengzhou University using patient hospitalization numbers. Data analysis and visualization were performed using IBM SPSS Statistics 25, R 4.4.0 and Origin 2024. We expressed quantitative data as mean ($\pm$ standard deviation, SD) for normally distributed variables, or as median with interquartile range (IQR) for non-normally distributed variables. Categorical data were presented as counts and percentages (n, %).

The primary outcomes were: (1) the total number of AMR phenotypes per *K. pneumoniae* strain (normality test: $P = 0.053$); (2) the resistance status of each strain to individual drug classes. All P values were two-sided and pertained only to the primary outcomes, with statistical significance set at $P < 0.05$.

For the analysis of the total number of AMR phenotypes per strain, we used t -tests or analysis of variance (ANOVA) for categorical variables, with post hoc pairwise comparisons via the Bonferroni method. For continuous variables, Pearson's correlation test was applied for normally distributed data, and Spearman's correlation test was used for non-normal (skewed) data. Variables with $P < 0.05$ in univariate linear regression were included in a generalized linear regression model.

For the analysis of resistance to each drug class, we employed the chi-square test or Fisher's exact test for categorical variables, followed by Bonferroni-corrected pairwise comparisons to identify intergroup differences. For continuous variables, the t -test was used for normally distributed data, and the Mann-Whitney U test for skewed data. Variables significant ($P < 0.05$) in univariate logistic regression were included in a multivariate logistic regression model, which was optimized through stepwise regression.

3. Results

3.1 Patient and Bacterial Isolate Characteristics

From 2018 to 2024, 39 *K. pneumoniae* isolates were collected from 39 clinical samples obtained from 37 pediatric patients at the First Affiliated Hospital of Zhengzhou University. These patients were from three departments: PICU (15/37, 40.6%), pediatric medicine (14/37, 37.8%), and pediatric surgery (8/37, 21.6%) (Fig. 1a). The median patient age was 2.0 years (IQR: 0.5–6.0) (Table S1), and the cohort included 24 males (64.9%) and 13 females (35.1%) (Fig. 1a).

The 39 specimens comprised sputum (15/39, 38.5%), anal swabs (11/39, 28.2%), urine (17.9%, 7/39), blood (5/39, 12.8%), and abdominal drainage fluid (1/39, 2.6%) (Fig. 1b). Notably, two *K. pneumoniae* isolates (ETFK5145 and ETFK5280) were obtained from sequential anal swabs of one male patient 20

days apart, and two others (ETFK3579 and ETFK3743) were isolated from blood and sputum specimens of a female patient 17 days apart.

Medical records were available for 28 patients, with 9 cases lost due to EMR migration. Among these 28 patients, primary diagnoses involved hematologic disorders (9/28, 32.1%), respiratory system diseases (6/28, 21.5%), urinary system disorders (4/28, 14.3%), and other conditions (9/28, 32.1%) (Fig. 1c). The median hospitalization duration was 18.5 days (IQR: 14.3–50.0), with a median of 6.5 diagnosed conditions per patient (IQR: 2.0–10.8) and a mean of 3.61 ($\pm$ 2.20) medication types administered. Twenty-seven patients were discharged or transferred, and one patient with isolate ETFK574 died.

3.2 Antimicrobial Susceptibility Profiles

Antimicrobial susceptibility testing (AST) confirmed that all 39 isolates were MDR-KP. As shown in Fig. 2, the isolates were resistant to all 20 tested antimicrobial agents except tigecycline. All 39 isolates were completely resistant to ceftriaxone, cefotaxime, and amoxicillin/clavulanate. The specific MIC values for each isolate are listed in Table S2.

Antibiotic usage histories were available for 27 patients (Table S3). Most bacterial strains (22/27, 81.5%) had been exposed to at least two antibiotics. Strain ETFK1306, which was exposed to the most antibiotics (up to nine), also showed a high number of resistance types (16/21).

These results reveal a serious antimicrobial resistance situation among pediatric *K. pneumoniae* isolates, emphasizing the difficulty in selecting effective treatment.

3.3 Molecular Typing and Phylogenetic Analysis

Whole-genome sequencing and MLST identified 24 distinct sequence types (STs) among the 39 *K. pneumoniae* strains. ST11 was the most prevalent (11/39, 28.2%) (Fig. 3, Table S2). All isolates carried fosfomycin and quinolone resistance genes, and exhibited diverse MDR profiles (Fig. 3).

ResFinder analysis showed that 66.7% (26/39) of isolates carried ESBL genes, with 25 strains harboring *bla*_{CTX-M-like} variants: *bla*_{CTX-M-65} (n = 7), *bla*_{CTX-M-3} (n = 5), *bla*_{CTX-M-14} (n = 4), *bla*_{CTX-M-15} (n = 4), *bla*_{CTX-M-27} (n = 3), and *bla*_{CTX-M-55} (n = 2). Additionally, 74.4% (29/39) of isolates were carbapenemase-positive, carrying *bla*_{KPC-2} (n = 16), *bla*_{NDM-1} (n = 9), *bla*_{NDM-5} (n = 2), *bla*_{NDM-15} (n = 1), *bla*_{NDM-16b} (n = 1), and *bla*_{OXA-484} (n = 1).

Notably, the ST17 strain ETFK5283 showed resistance to 18 out of 21 drug categories but carried relatively few resistance genes. Its resistome included the carbapenemase gene *bla*_{NDM-16b}, the acquired β -lactamase resistance gene *bla*_{SHV-11}, and resistance gene for sulfonamide (*su1*), aminoglycoside (*ant(3'')-IIa*), trimethoprim (*dfra15*), and fosfomycin (*fosA6*), as well as the efflux pump genes *oqxB* and *oqxA*.

Core genome-based phylogenetic analysis revealed a close relationship between ETFK798 and ETFK1306. These two strains, isolated from blood and sputum samples of different patients in the same PICU, shared identical resistance genes: *bla*_{KPC-2}, *bla*_{CTX-M-65}, *bla*_{TEM-1B}, *rmtB*, *oqxB* and *oqxA*. ETFK798 was obtained on April 27, 2019, while ETFK1306 was collected on June 16, 2019 from a patient hospitalized for 79 days. Although the hospitalization record for the ETFK798 patient was incomplete, epidemiological evidence indicated a high likelihood of spatiotemporal overlap between the two patients. These findings suggest clonal transmission of MDR strains with similar resistance determinants among hospitalized children.

3.4 Plasmid Analysis of NDM-16-producing *K. pneumoniae*

We selected the ST17 strain ETFK5283 for in-depth genomic analysis because it exhibited the highest number of resistance types (18/21) among all strains, despite carrying relatively few resistance genes. This strain was isolated from an anal swab of a 5-year-old female patient with respiratory failure.

The complete genome of ETFK5283 consists of one circular chromosome (4.88 Mb) and four plasmids (Fig. 4). The chromosome has an average G + C content of 57.68% and harbors 4,712 protein-coding genes, 88 tRNA genes, and 25 rRNA genes (Table S4). The four plasmids are: an IncFIB(K)-type plasmid (p5283-1, 205.0 kb), an IncX3-type plasmid (p5283-2, 45.1 kb), an IncR-type plasmid (p5283-3, 44.0 kb), and a Col440I-type plasmid (p5283-4, 9.4 kb).

The carbapenem resistance gene *bla*_{NDM-16b}, which encodes a metallo- β -lactamase capable of hydrolyzing nearly all β -lactam antibiotics (including penicillins, cephalosporins, and carbapenems), was located on the IncX3-type plasmid p5283-2 (Fig. 5a).

Only two circular plasmids carrying *bla*_{NDM-16b} have been previously reported in NCBI: the IncF-type plasmid pEC1188-NDM16 (MH213345) from an *E. coli* strain in China, and the IncX3-type plasmid pTMTA85711 (AP024206) from an *E. coli* strain in Japan. Plasmid p5283-2 showed 100% coverage and 100% identity with pTMTA85711, but only 10% coverage and 99.97% identity with pEC1188-NDM16. Comparative analysis confirmed that the genetic environment of *bla*_{NDM-16b} in p5283-2 and pTMTA85711 is identical, featuring the conserved structure *IS26-dsbD-trpF-ble-bla*_{NDM-16b}-*IS5* (Fig. 5b).

3.5 Analysis of AMR-Associated Factors

The drug resistance profiles of the 39 pediatric *K. pneumoniae* isolates varied significantly across multiple factors (Fig. 6a-g), including MLST, number of resistance genes, number of virulence genes, specimen type, hospital department, patient age and body weight, number of comorbidities, primary disease system, and maternal gravidity, parity, and delivery mode.

We analyzed factors associated with seven distinct outcomes (Table S5). ST11 type and hospital department were each significantly associated with four outcomes. The number of virulence genes was significant for three outcomes, while specimen type and number of resistance genes were each associated with two outcomes. All other factors were linked to only one outcome.

Pairwise comparisons revealed specific associations:

- For the number of resistance types (Fig. 6a), isolates from urine showed significantly more resistance types than those from rectal swabs (MD = 4.00, 95% CI: 0.25–7.75, $P = 0.031$). Isolates from patients with urinary system diseases also had more resistance types than those patients with other systems diseases (MD = 4.75, 95% CI: 0.06–9.44, $P = 0.046$).
- Hospital department was significantly associated with two outcomes:

For the number of resistance types (Fig. 6a), PICU isolates had more resistance types than those from Pediatric Surgery (MD = 3.94, 95% CI: 1.06–6.82, $P = 0.005$).

For sulfonamide resistance (Fig. 6g), PICU isolates differed significantly from Pediatric Medicine isolates ($P = 0.002$).

For outcomes with two or more significant factors, we built multivariate regression models using stepwise selection from univariate-significant variables (Tables S6 and S7). Both Tables 1 and 2 show that ST11 type isolates consistently had higher resistance than non-ST11 types.

Hospital department also significantly influenced resistance: PICU isolates showed higher resistance in both the number of resistance types and aminoglycoside resistance outcomes (Fig. 6a, c).

Using sputum isolates as reference, rectal swab and urine isolates had significantly higher resistance levels for the number of resistance types, while abdominal drainage fluid isolates had lower resistance (Fig. 6a).

For sulfonamide resistance, two multivariate logistic regression models were constructed using (1) sequence type (ST) and number of resistance genes; (2) number of virulence genes and number of resistance genes. Both models confirmed that more resistance genes significantly increased sulfonamide resistance, whereas more virulence genes decreased it (Fig. 6g).

Table 1
Generalized Linear Model Analysis of Antimicrobial Resistance Type Counts in *K. pneumoniae* Isolates

Variable ¹	<i>b</i>	SE	95% CI	Wald χ^2	<i>P</i>
Anal swab	4.393	0.9431	[2.545, 6.242]	21.701	< 0.001
Urine	5.567	1.7656	[2.107, 9.028]	9.943	0.002
Abdominal drainage fluid	-4.667	2.2011	[-8.981, -0.353]	4.495	0.034
ST11	4.667	0.8368	[3.029, 6.305]	31.174	< 0.001
Diseases of urinary system	-11.484	2.3856	[-16.160, -6.808]	23.174	< 0.001
Pediatric medicine	-1.944	0.9563	[-3.819, -0.070]	4.134	0.042

1: Sputum specimens were used as the reference group. The pediatric intensive care unit (PICU) served as the reference for discharge departments. MLST comparisons were made against non-ST11 sequence types. Disease classifications were referenced against conditions affecting other organ systems.

Table 2

Multivariate logistic regression models evaluating resistance to individual antimicrobial classes.

Drug	Variable	<i>b</i>	SE	Wald χ^2	<i>P</i>	OR	95% CI
Carbapenems	Pediatric surgery ¹	-3.481	1.333	6.820	0.009	0.031	[0.002, 0.420]
Aminoglycosides	Pediatric surgery ²	-3.509	1.667	4.430	0.035	0.030	[0.001, 0.785]
Fosfomycin	MLST ^a	3.880	1.690	5.270	0.022	48.250	[1.760, 1319.470]
Sulfonamides ^b	MLST ^a	3.184	1.367	5.423	0.020	24.135	[1.656, 351.801]
Sulfonamides ^b	Drug resistance gene	0.829	0.418	3.933	0.047	2.291	[1.010, 5.198]
Sulfonamides ^c	Drug resistance gene	1.339	0.634	4.465	0.035	3.815	[1.102, 13.210]
Sulfonamides ^c	Virulence gene	-0.346	0.171	4.113	0.043	0.707	[0.506, 0.988]

1: The control group is pediatric medicine.

2: The control group is PICU.

a: The control group was non-ST11 type.

b: A multivariate logistic regression model was constructed incorporating ST11-type classification and the number of resistance genes.

c: A multivariate logistic regression model was developed incorporating the number of resistance genes and the number of virulence genes.

4. Discussion

This study provides an integrated genomic and clinical overview of MDR-KP in pediatric patients. We observed notable heterogeneity in AMR profiles across clinical departments, specimen types, and host-

related factors such age, delivery mode, and underlying disease. The higher resistance diversity in PICU isolates compared to other wards likely reflects stronger antibiotic selection pressure and higher patient acuity, consistent with previous reports of intensive care units as hotspots for resistant selection.

The cohort consisted of 37 pediatric patients, mainly from the PICU (40.6%) and pediatric medicine (37.8%). ESBL-producing *K. pneumoniae* (ESBL-KP) has been detected in community-dwelling children [26], indicating substantial strain transmissibility. We observed significant inter-departmental variation in resistance diversity ($F = 5.90$, $P = 0.006$), and in resistance to carbapenems ($\chi^2 = 6.27$, $P = 0.032$), aminoglycosides ($\chi^2 = 6.85$, $P = 0.035$) and sulfonamides ($\chi^2 = 11.13$, $P = 0.003$). In a generalized linear model (GLM) with PICU as reference, pediatric medicine patient showed lower antimicrobial resistance ($b = -1.944$, 95% CI: -3.819 – -0.070 , $P = 0.042$), reflecting the PICU's elevated antibiotic pressure [27].

We analyzed five specimen types, with sputum having the highest isolation rate (15/39, 38.5%). This aligns with a report by Ma et al. on pediatric CRKP isolates in Henan, China, where sputum was also predominant (63/108, 58.33%) [28]. A Ghanaian study further detected ESBL-KP in 40.9% (178/435) of pediatric fecal samples [29], indicating that MDR-KP can colonize multiple body systems in children. We found significant variation in resistance diversity across specimen types ($F = 3.21$, $P = 0.035$) and in sulfonamide resistance by specimen source ($\chi^2 = 8.79$, $P = 0.038$). These differences may stem from anatomical variations in local selection pressures and immune surveillance. The high sputum isolation rate may reflect prolonged respiratory exposure to pathogens. Using sputum as reference in GLM, urinary isolates showed higher resistance ($b = 5.567$, 95% CI: 2.107 – 9.028 , $P = 0.002$). This aligns with multicenter data from Poland, where urinary sources predominated in pediatric *K. pneumoniae* infections [30], underscoring the need for enhanced urinary pathogen surveillance.

Resistance diversity also differed significantly by disease system ($F = 3.24$, $P = 0.039$). In a GLM with other system diseases as reference, urinary system disease patients had lower AMR in *K. pneumoniae* isolates ($b = -11.484$, 95%CI: -16.160 – -6.808 , $P < 0.001$). These findings highlight how resistance profiles vary by underlying diseases, supporting the need for personalized antimicrobial therapy. No significant AMR differences were found between hematological diseases and other system. However, MDR-KP from pediatric bloodstream infections has been widely reported. For example, Luo et al. isolated an ST11-KL64 hv-CRKP strain from a pediatric hematopoietic stem cell transplant recipient, showing resistance to carbapenems, tigecycline, and colistin [31]. MDR-KP also accounted for 15.1% of pathogens in neonates with sepsis in an Ethiopian tertiary hospital [32].

Among the 39 isolates, ST11 was the most prevalent sequence type (11/39, 28.2%). GLM revealed ST11 strains had significantly higher antimicrobial resistance than non-ST11 strains ($b = 4.667$, 95% CI: 3.029 – 6.305 , $P < 0.001$). Multivariable logistic regression identified ST11 as an independent risk factor for sulfonamide resistance (OR = 24.135 , 95% CI: 1.656 – 351.801 , $P = 0.020$). In a cohort of 460 pediatric MDR-KP isolates analyzed by Sandip Patil et al., ST20 was most common (18.5%), followed by ST16 (14.8%), ST1162 (11.1%), ST111 (9.8%), ST147 (8.3%), and ST2407 (6.5%) [8]. These findings show MDR-

KP is distributed across diverse sequence types in children, underscoring the need for continuous surveillance and comparative analysis.

MDR-KP isolates from pediatric populations show the increasing importance of *bla*_{NDM} gene carriage. An analysis of 111 NDM-KP isolates from Chinese children's hospitals found *bla*_{NDM-5} (70/111, 63.1%) and *bla*_{NDM-1} (40/111, 36.0%) were the main genotypes, with IncX3 plasmids and diverse STs aiding dissemination [33]. Another study of 198 CRKP isolates from a Shanghai Children's Hospital (2013–2021) identified *bla*_{NDM} as the dominant carbapenemase gene (48.5%) [34]. Internationally, *bla*_{NDM-1} remains the most common variant in pediatric *K. pneumoniae* [35–37]. In isolate ETFK5283, we identified the rare *bla*_{NDM-16b} gene on an IncX3 plasmid (p5283-2). Only two other circular plasmids carrying this gene have been reported in NCBI: pEC1188-NDM16 (from *E. coli* EC1188 in China) and pTMTA85711 (from *E. coli* TA8571 in Japan). Comparative analysis showed high similarity between p5283-2 and pTMTA85711. The latter, from a 2018 urine sample of a hospitalized patient in Tokyo, showed high β -lactam resistance and was the first report of *bla*_{NDM-16b} on a transferable IncX3 plasmid [38]. Both p5283-2 and pTMTA85711 carry *bla*_{NDM-16b} within a conserved structure flanked by *IS5* and *IS26*, suggesting a possible evolutionary link between *K. pneumoniae* ETFK5283 and *E. coli* TA8571. Hui Zuo et al. proposed that *bla*_{NDM-16b} may be a mutant derived from *bla*_{NDM-5} via single-nucleotide change [39]. Given the clinical prevalence of *bla*_{NDM-5}, it is important to monitor its potential mutation to *bla*_{NDM-16b} and subsequent spread.

A higher virulence gene count was associated with lower sulfonamide resistance (OR = 0.707, 95% CI: 0.506–0.988, *P* = 0.043). These align with emerging mechanistic insights. Suyeon Park et al. reported that tigecycline resistance in hvKP correlated with reduced virulence and capsular polysaccharide production, possibly due to downregulation of the OmpK35 porin [40]. However, the relationship between virulence and resistance is complex; for instance, hvKP strains carrying IncFIK34 plasmids have acquired carbapenem resistance without losing virulence [41]. The rising prevalence of multidrug-resistant hvKP strains [42, 43] highlights the urgent need for enhanced surveillance and new therapeutic strategies.

Parity, gravidity, delivery mode, patient age and admission weight did not show significant independent effects on AMR in regression analyses, possibly due to limited sample size. John et al. identified antibiotic exposure, prolonged hospitalization, mechanical ventilation, low birth weight, and prematurity as key risk factors for resistant Gram-negative infections in infants [44]. Further studies with more comprehensive clinical data are needed to clarify the determinants of AMR in pediatric *K. pneumoniae*.

The WHO Research Agenda on AMR in Human Health has identified 40 research priorities for 2030, emphasizing antibiotic use and outcomes in neonates and children [45]. As a priority pathogen [46], *K. pneumoniae* poses a major disease burden in this vulnerable group. Household interventions, such as health education promoting hand hygiene and mask-wearing in crowded settings, can help prevent infections [47, 48]. Vaccination is a cost-effective strategy for establishing herd immunity and could

reduce antibiotic consumption by 22%, alleviating the economic burden of resistant infections [49, 50]. Preclinical studies show promise for several *K. pneumoniae* vaccine candidates and alternative therapeutics [51, 52]. Future licensure of *K. pneumoniae* vaccines would significantly support the goals of the Immunization Agenda 2030 [53].

Our study has several limitations. First, the single-center design and relatively small sample size (39 isolates from 37 patients) may affect generalizability. However, this is the first report of *bla*_{NDM-16b} in a pediatric population, and the detailed genomic characterization provides a valuable foundation for larger multi-center studies. Second, the lack of longitudinal sampling or follow-up limited our ability to track transmission or clinical outcomes over time—a common constraint in retrospective studies. Future prospective studies with serial sampling could clarify the evolution and spread of MDR-KP. Third, clinical correlation analyses were affected by incomplete data: antibiotic exposure history was available for only 27 isolates, and clinical data for nine cases were lost due to system migration. This incompleteness, inherent in retrospective data, may have reduced statistical power, but the available data still robustly support our main genomic and resistance conclusions. Finally, while genomic analyses suggest a possible evolutionary link between ETK5283 and *E. coli* TA85711 via horizontal gene transfer, this was not experimentally confirmed. We have used cautious language (e.g., “suggests,” “it is plausible”) and avoided causal claims. Further studies, such as plasmid conjugation assays, could test this hypothesis.

In light of these limitations, we emphasize that our study provides important initial insights into the genomic context and potential dissemination mechanisms of *bla*_{NDM-16b} in pediatric MDR-KP. Future work incorporating multi-institutional cohorts, prospective follow-up, and functional genomics will be essential to validate and extend these findings.

5. Conclusion

In conclusion, this study offers a comprehensive genomic and clinical characterization of MDR-KP in pediatric patients, revealing significant associations between AMR phenotypes and microbiological and host-related factors. Resistance diversity was influenced by specimen type, underlying disease, hospital department, and patient characteristics such as age, admission weight, delivery mode, gravidity, and parity. ST11 emerged as a high-risk clone independently linked to sulfonamides and Fosfomycin resistance. We report the first pediatric *K. pneumoniae* isolate harboring the *bla*_{NDM-16b} on a mobile IncX3 plasmid, showing high similarity to a plasmid from a Japanese *E. coli* strain. This finding reveals a new pediatric reservoir for *bla*_{NDM-16b} and suggests possible interspecies and cross-regional transmission mediated by *IS26*-associated mobile elements. Our results underscore the importance of incorporating pediatric genomic surveillance into national AMR control programs and highlight the need for strengthened infection prevention and precision antimicrobial stewardship targeting high-risk clones.

Abbreviations

Abbreviation	Full text
MDR-KP	Multidrug-resistant <i>Klebsiella pneumoniae</i>
WGS	Whole-genome sequencing
AMR	Antimicrobial Resistance
ESBL	Extended-spectrum β -lactamase
ICU	Intensive Care Unit
NICU	Neonatal Intensive Care Unit
BHI	Brain Heart Infusion
MALDI-TOF/MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MIC	Minimum Inhibitory Concentration
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
IS	Insertion Sequences

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee (IIT Review Subcommittee) of the First Affiliated Hospital, Zhejiang University School of Medicine (Acceptance: IIT20230479B; Approval: FAHZU-2023-0994-Quick), and strictly adhered to the principles of the Declaration of Helsinki.

This study utilized exclusively de-identified, publicly available genomic data from:

(a) BIGSdb-Pasteur (<https://bigsdb.pasteur.fr/>) (data policy: All data are freely available for research; original ethical approvals were obtained by data submitters). (b) NCBI GenBank (under NIH Policy: Publicly available data with no personal identifiers are exempt from IRB review). Per international guidelines (CIOMS/WHO) and institutional review board determination, secondary computational analysis of such pre-existing anonymized data is exempt from ethics approval.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

WM conceived the study and provided research samples and ideas. ZL performed data analysis and wrote the manuscript. JS performed the experiments and collected the data. TY participated in statistical analysis and data interpretation. CG contributed to study design and methodology development. YL performed sample preparation and processing. YZ contributed to figure preparation and data visualization. XY participated in literature search and manuscript revision. KW contributed to experimental execution and quality control. CW participated in data collection and bioinformatics analysis. HX provided technical guidance and supervision. XJ contributed to research coordination and project management. BZ acquired funding and critically reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Not applicable

Data Availability

The datasets generated during this study are available in the Zenodo repository (<https://zenodo.org/records/16612711>), including raw sequencing data, analytical datasets for statistical results and figures. All data supporting this study are publicly available in BIGSdb-Pasteur database (<https://bigsdb.pasteur.fr/>) and NCBI GenBank (CP185387–CP185391; <https://www.ncbi.nlm.nih.gov/genbank/>).

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Figures

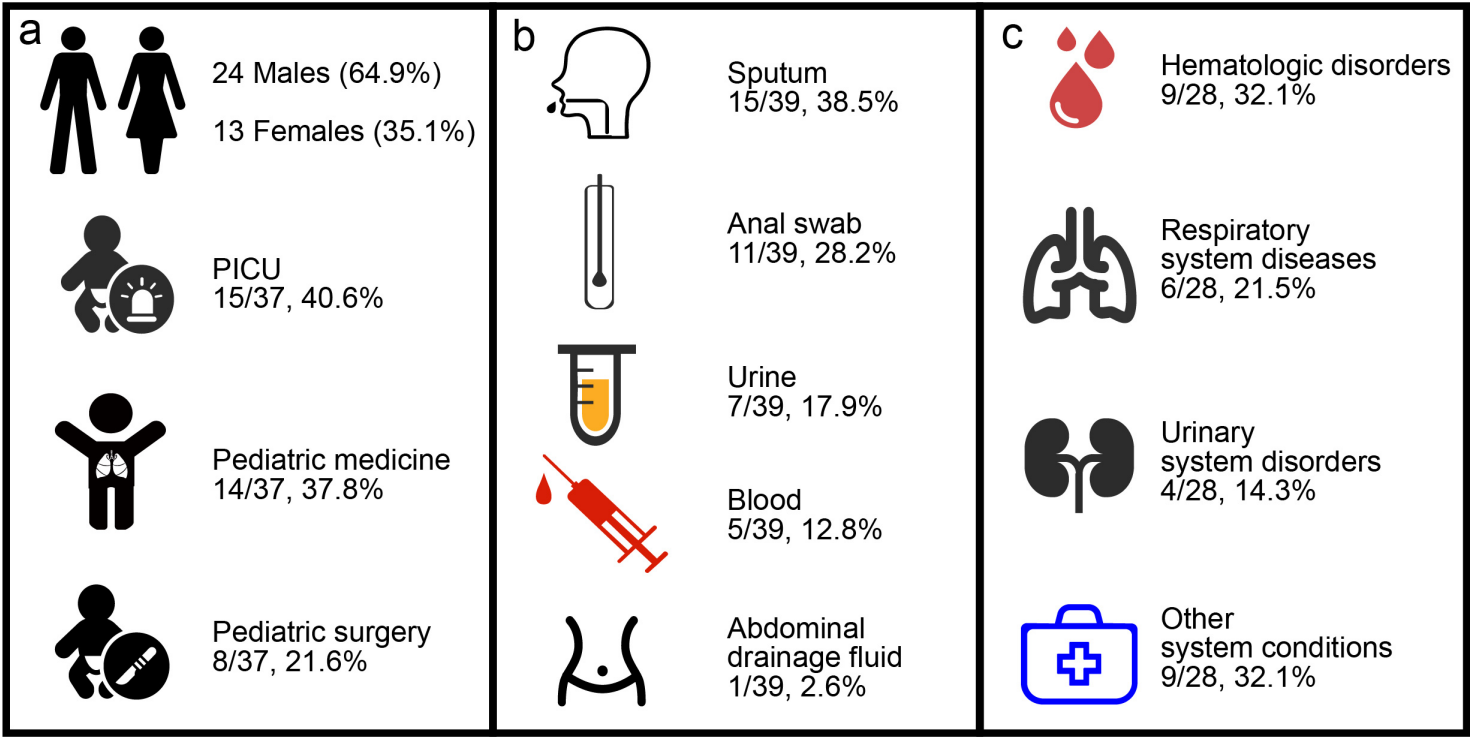


Figure 1

Demographic and clinical characteristics of study participants. (a) illustrates gender distribution and department allocation of pediatric patients; (b) demonstrates the proportion of different specimen types; (c) presents disease classification based on primary diagnoses.

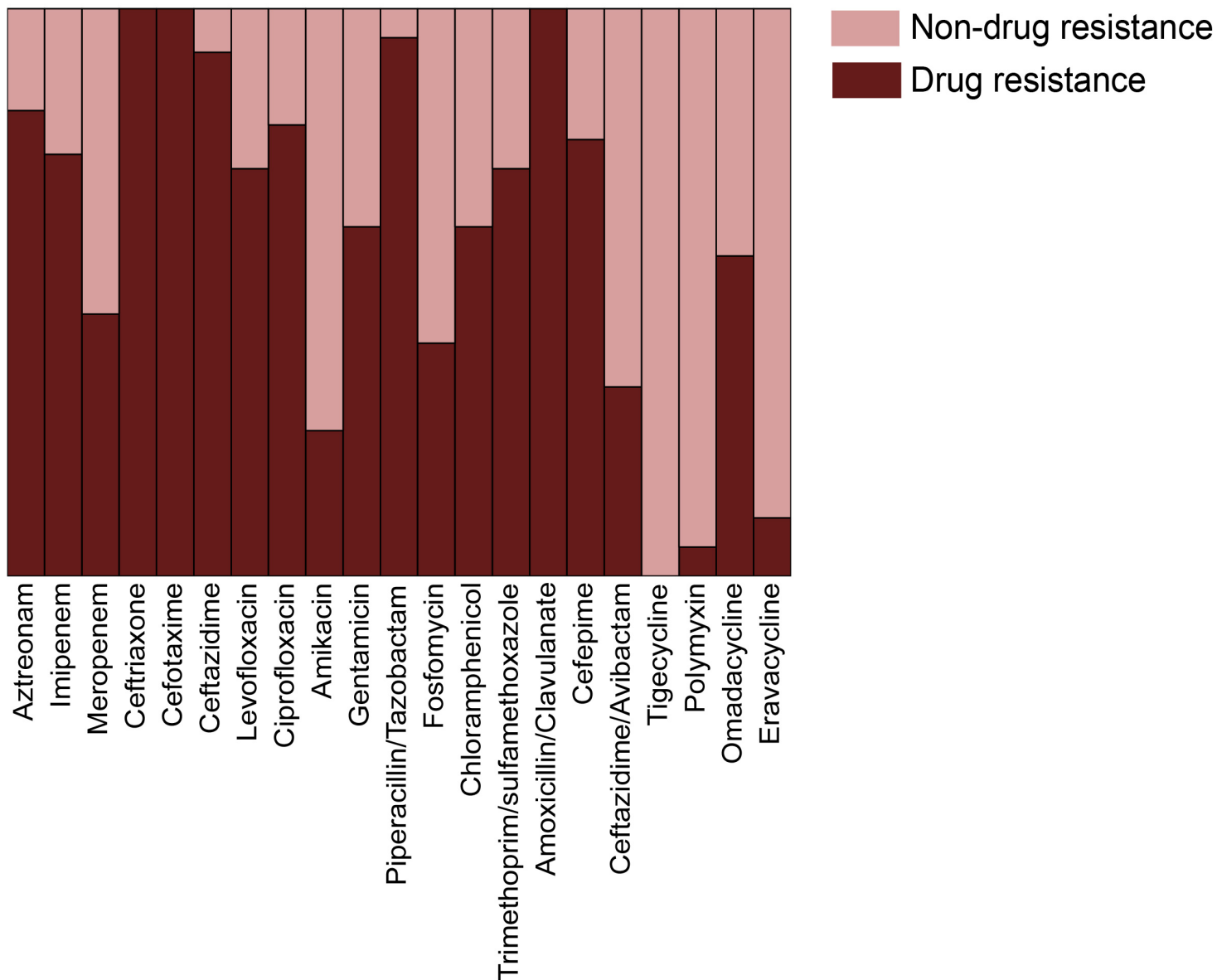


Figure 2

Stacked bar chart demonstrating the antimicrobial resistance profiles of 39 *K. pneumoniae* isolates. Two distinct color blocks are used to represent the number of resistant and non-resistant isolates for each antimicrobial agent respectively.

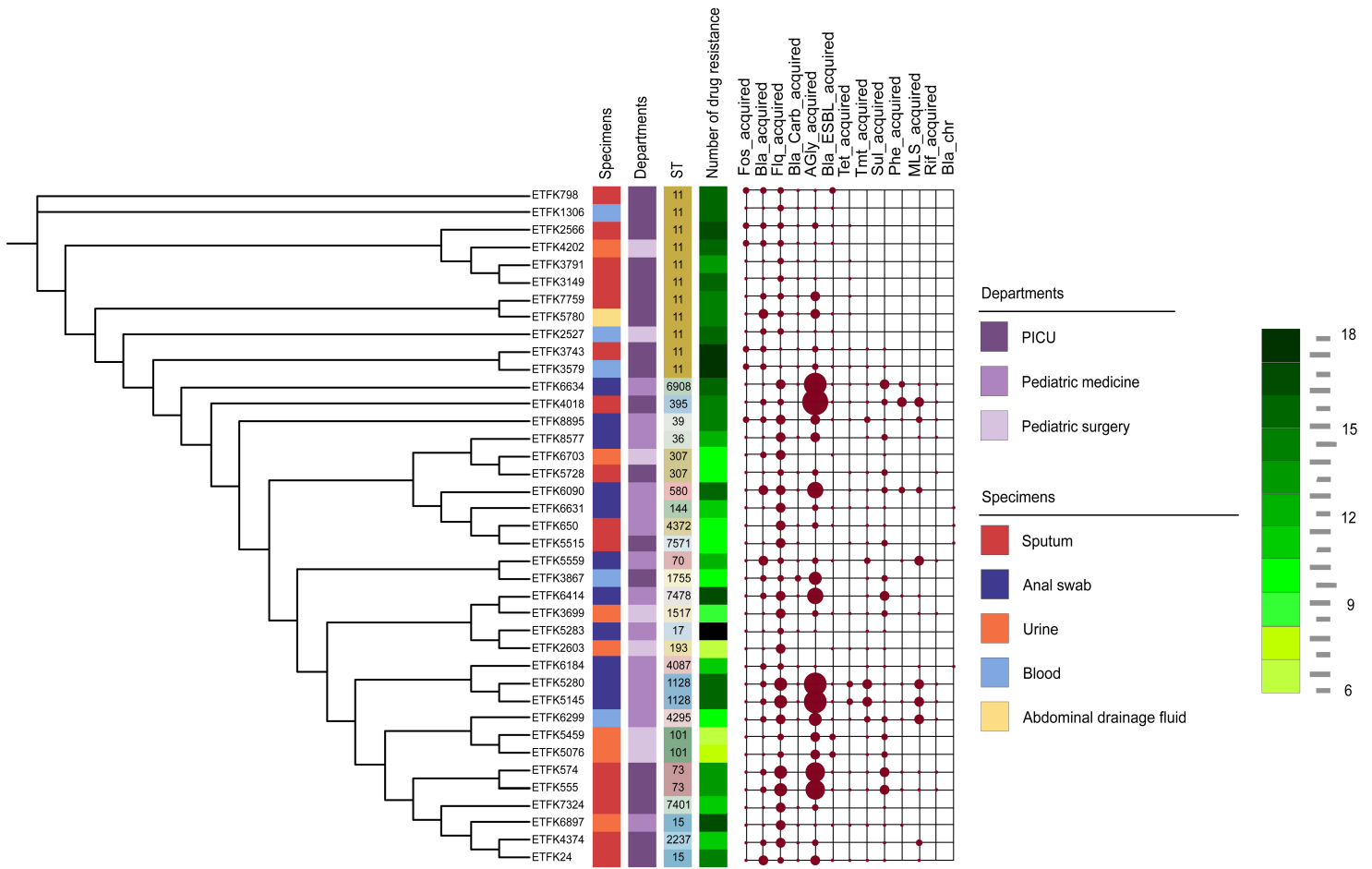


Figure 3

Phylogenetic analysis. The maximum likelihood tree illustrates strain characteristics categorized by clinical specimen type, isolation department, ST, antimicrobial resistance category count (color intensity correlates with the number of resistance categories), and resistance gene profiles (dot size corresponds to the quantity of detected resistance genes).

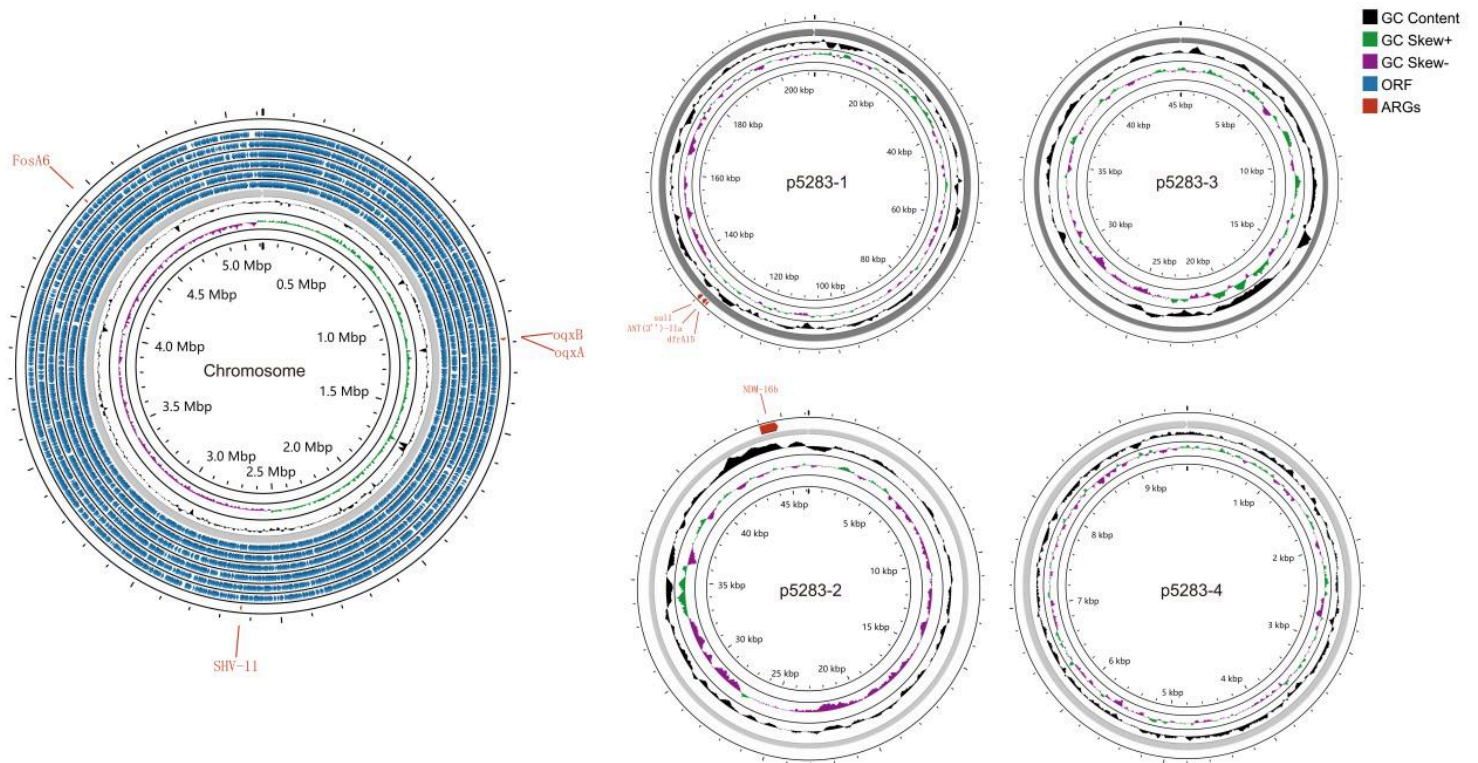
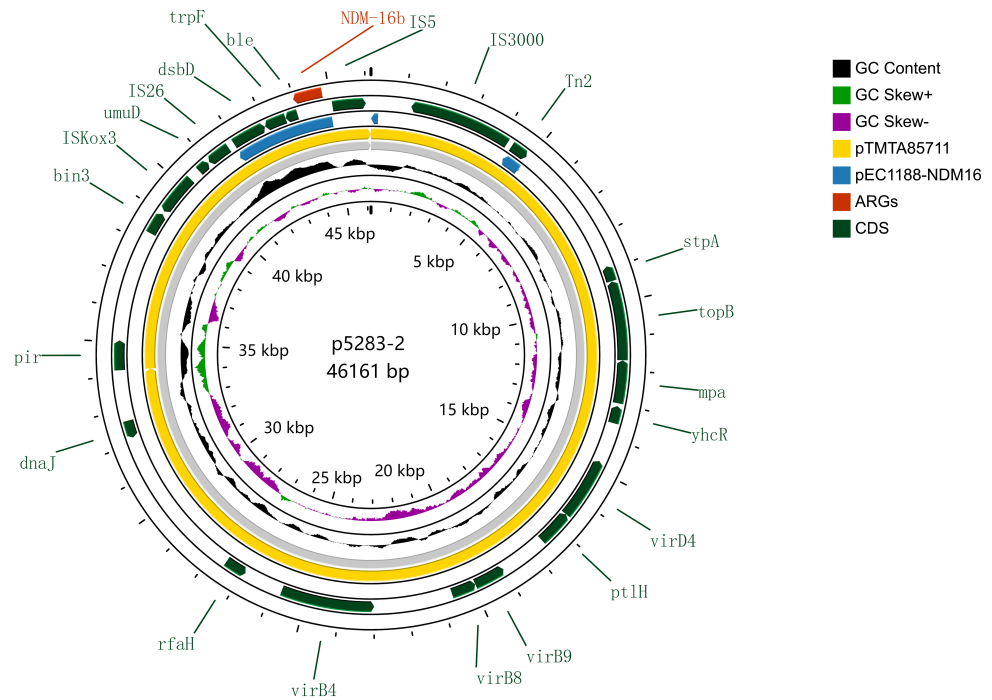


Figure 4

Genomic characterization of *K. pneumoniae* ETFK5283. Guanine-Cytosine Content (GC Content): Represents the ratio of guanine (G) and cytosine (C) content in the genome, and the depth or high or low indicates the change of GC content. Guanine-Cytosine Skew Positive (GC Skew+) and Guanine-Cytosine Skew Negative (GC Skew-): The amount of guanine (G) and cytosine (C) differed, with positive indicating a greater amount of C relative to G and negative reversed. Open Reading Frame (ORF): A continuous nucleotide sequence in a genome capable of encoding a protein. Antibiotic Resistance Genes (ARGs): Genes in the genome that encode proteins that are resistant to antibiotics.

a



b

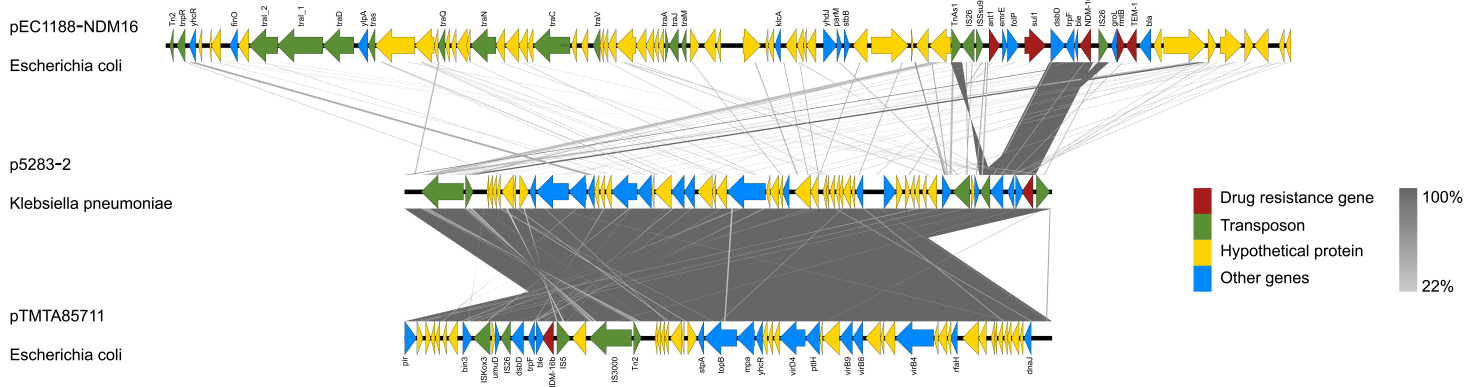


Figure 5

Comparative analysis of the genetic context of plasmid p5283-2.

(a) Alignment of plasmid p5283-2 with two *bla*_{NDM-16b}-carrying plasmids. The innermost ring represents the backbone of p5283-2, with plasmid pTMTA85711 and plasmid pEC1188-NDM16 displayed moving outward. Coding sequences (CDS) denote protein-encoding DNA regions. **(b)** Linear comparison of the genetic architectures of p5283-2, pTMTA85711, and pEC1188-NDM16. Homologous regions are shaded in gray. Resistance genes, transposases, hypothetical proteins, and other functional genes are annotated by arrows colored red, green, yellow, and blue, respectively.

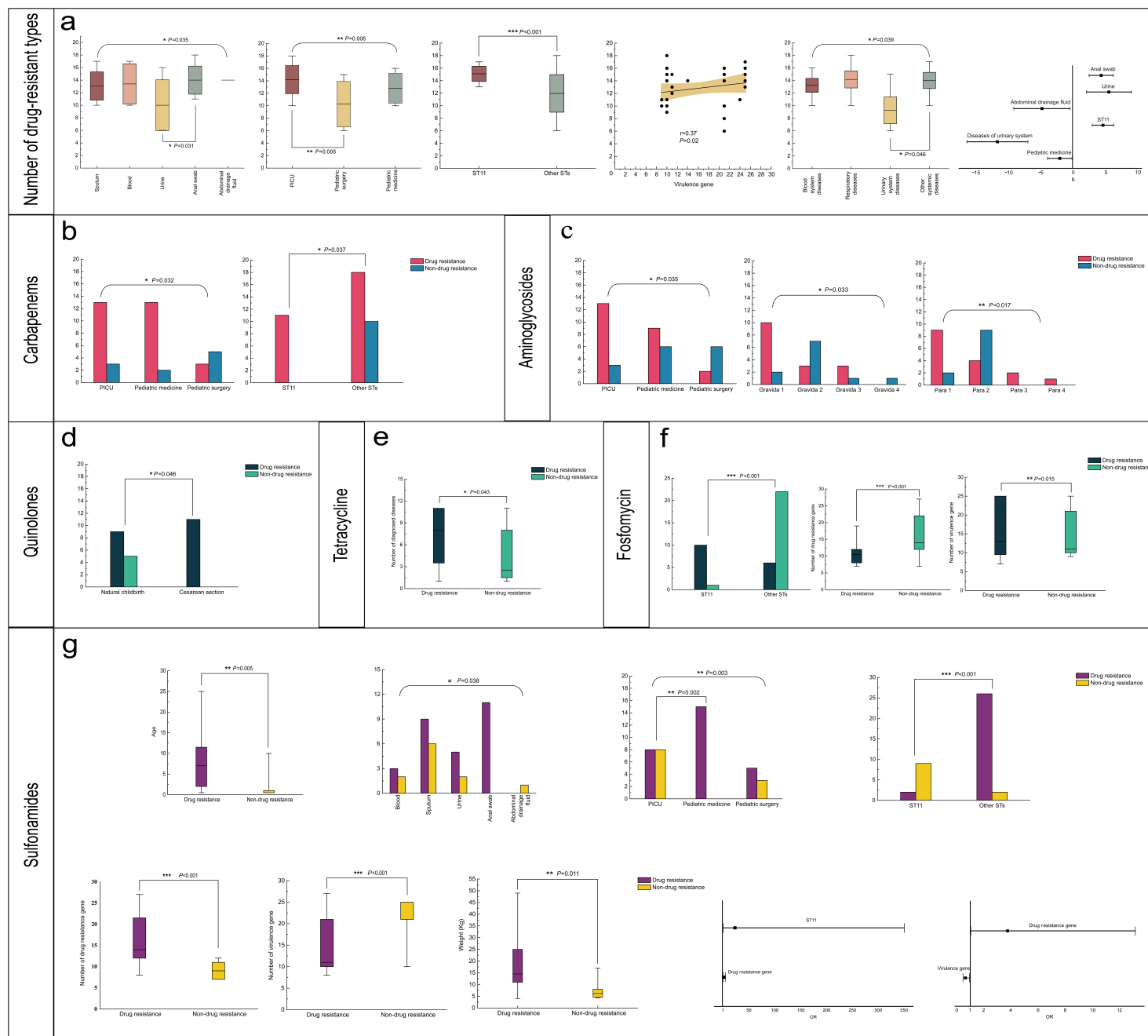


Figure 6

Differential Analysis of Antimicrobial Resistance in *K. pneumoniae* Isolates from Pediatric Patients

The whiskers of boxplots denote the maximum and minimum values, while the boxes represent the standard deviation range.

(a) The horizontal line in the box indicates the mean value. Analysis perspectives from left to right include specimen types, clinical departments, ST11 type status, virulence gene counts, disease system classification based on primary diagnosis, and a forest plot derived from generalized linear regression model results, with the number of antimicrobial resistance types as the outcome variable.

(b) Carbapenem resistance status as the outcome variable; analyzed variables from left to right include clinical departments and ST11 type status.

- (c) Aminoglycoside resistance status as the outcome variable; analyzed variables from left to right include clinical departments, parity, and gravidity.
- (d) Quinolone resistance status as the outcome variable; the analytical variable is birth delivery mode.
- (e) The horizontal line in the box indicates the median value. Tetracycline resistance status as the outcome variable; the analytical variable is the number of concurrent diagnoses.
- (f) The horizontal line in the box indicates the median value. Fosfomycin resistance status as the outcome variable; analyzed variables from left to right include ST11 type status, resistance gene counts, and virulence gene counts.
- (g) Sulfonamide resistance status as the outcome variable; analysis variables from left to right and top to bottom include patient age, specimen types, clinical departments, ST11 type status, resistance gene counts, virulence gene counts, admission body weight, and a forest plot derived from multivariable logistic regression results.

Supplementary Files

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