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ORIGINAL RESEARCH

Hospital-Based Surveillance of *Klebsiella pneumoniae* and Other *Klebsiella* species in Southern Saudi Arabia (2012–2024): Escalating Carbapenem and DTR Resistance with Species-Specific Phenotypes and Genotypes

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Background: *Klebsiella* species, particularly *Klebsiella pneumoniae*, are major healthcare-associated infections showing increasing resistance to carbapenems and other antibiotics. In Saudi Arabia, rising antimicrobial resistance (AMR) and the emergence of difficult-to-treat resistance (DTR) phenotypes pose significant clinical challenges.

Methods: A 12-year retrospective surveillance study (2012–2024) of 9790 non-duplicate *Klebsiella* isolates from Aseer Central Hospital in southern Saudi Arabia. Species-level identification, antimicrobial susceptibility testing for each isolate was done. Molecular characterization of carbapenemase genes for selected isolates were performed. Temporal resistance trends were assessed using logistic regression.

Results: *K. pneumoniae* accounted for 93.0% of isolates, with *non-pneumoniae* species showing distinct resistance profiles. Carbapenem resistance surged from 12.4% in 2012 to 69.3% in 2021, while DTR prevalence rose to 50.7%. ICU and inpatient settings exhibited the highest resistance rates. Molecular testing revealed widespread bla_OXA-48-like (35.7%), bla_NDM (17.9%), and emerging bla_KPC (14.3%) genes, with 25% of isolates co-harboring multiple carbapenemases. Progression from colonization to bloodstream infection occurred in 1.7% of *K. pneumoniae* cases, primarily from respiratory and urinary sources.

Conclusion: This study highlights escalating carbapenem and DTR resistance among *Klebsiella* species in southern Saudi Arabia, driven by species-specific mechanisms and widespread carbapenemase gene dissemination. Findings underscore the need for species-level diagnostics, expanded molecular surveillance, and integrated stewardship strategies to curb the spread of multidrug-resistant *Klebsiella*.

Keywords: *Klebsiella pneumoniae*, carbapenem-resistant enterobacterales, difficult-to-treat resistance, antimicrobial resistance surveillance, carbapenemase genes, Saudi Arabia

Introduction

Klebsiella species, especially *K. pneumoniae*, are major causes of both healthcare-associated and community-onset infections, including bloodstream infections, pneumonia, urinary tract infections, and surgical site infections.[1,2] Their success stems from intrinsic multidrug resistance and a strong ability to acquire additional resistance via plasmids and

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transposons,[1,3] including the combined effects of β-lactamase production, efflux pump overexpression, and reduced outer membrane permeability, which collectively drive multidrug-, extensively drug-, and pandrug-resistant phenotypes.[4] This has driven the global rise of MDR *Klebsiella*, particularly strains producing extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases.[1,3,5]

The spread of carbapenem-resistant *K. pneumoniae* (CRKP) has worsened clinical outcomes, limiting treatment options and increasing mortality, hospital stays, and costs.[5,6] Recognizing this threat, the WHO designated CRKP as a Priority 1: Critical pathogen.[1] These strains often carry *bla_OXA-48, bla_NDM*, and *bla_KPC* genes on mobile genetic elements, promoting horizontal transfer within Enterobacterales.[6,7]

In the GCC region, including Saudi Arabia, surveillance shows a rising burden of carbapenem-resistant *K. pneumoniae*. A molecular study from southern Saudi Arabia found *bla_OXA-48-like* in 62.5%, *bla_NDM-1* in 25%, and *bla_KPC* in 4.2% of isolates, with 12.5% co-harbouring *bla_OXA-48-like* + *bla_NDM-1*, and 2.1% carrying all three genes, indicating extensive plasmid-mediated spread.[8] In the Aseer region, ertapenem resistance rose from 9.7% in 2013 to 35.8% in 2022, alongside increased resistance to meropenem, ciprofloxacin, and aminoglycosides.[9]

Beyond regional prevalence estimates, understanding species-specific resistance mechanisms and longitudinal resistance dynamics is essential for accurate interpretation of surveillance data and effective antimicrobial stewardship. Nevertheless, most published studies from Saudi Arabia and the Gulf region remain cross-sectional, focus predominantly on *Klebsiella pneumoniae* without species-level differentiation, and lack longitudinal modeling to assess temporal resistance dynamics across inpatient and outpatient settings. Consequently, the evolution of carbapenem resistance and DTR phenotypes over time remains incompletely characterized, particularly in southern Saudi Arabia.[8–10] At King Fahad Medical City, intensive care unit (ICU) admission was required in 38.6% of *K. pneumoniae* bloodstream infection cases, with significantly higher mortality observed in carbapenem-resistant infections (57.9% vs 25.6%, $p = 0.001$).[10]

The emergence of the DTR phenotype, defined as resistance to all β-lactams (including carbapenems) and fluoroquinolones, represents a growing concern. First described by Kadri et al in a US multicenter study, DTR is linked to therapeutic failure and reliance on toxic agents like colistin and tigecycline.[11,12] Kadri's study showed worse outcomes in patients with DTR Gram-negative bacteremia.[11] While routine detection in the region is limited, ICU phenotypic trends in Saudi Arabia reflect its rising prevalence.[8,9,12] In Saudi hospital surveillance, DTR is not routinely reported as a distinct resistance category; however, it can be reliably inferred using phenotypic susceptibility data. Several Saudi studies have documented *Klebsiella pneumoniae* isolates with concurrent non-susceptibility to carbapenems, extended-spectrum β-lactams, and fluoroquinolones phenotypic resistance profiles that fulfill the DTR definition proposed by Kadri et al and are most frequently observed in ICU-associated infections.[8–12]

Species-level surveillance of *Klebsiella* infections remains limited in the region, despite the clear clinical relevance of non-*pneumoniae* species such as *K. oxytoca* and *K. aerogenes*. Most regional epidemiologic reports aggregate *Klebsiella* spp. or focus exclusively on *K. pneumoniae*, an approach that obscures fundamental biological and clinical differences among species with distinct intrinsic resistance mechanisms and antimicrobial susceptibility profiles. Notably, *K. aerogenes* harbours an inducible chromosomal AmpC β-lactamase, which can undergo derepression and confer resistance to multiple β-lactams, including third-generation cephalosporins, even in the absence of ESBL or carbapenemase production.[3,13] As highlighted by Jacoby and the IDSA, AmpC derepression in *K. aerogenes* contributes to broad β-lactam resistance with direct implications for empirical therapy selection and antimicrobial stewardship.[3,13] Consequently, empirical regimens appropriate for *K. pneumoniae* may be ineffective against non-*pneumoniae* species, particularly *K. aerogenes*. From an epidemiologic and infection control perspective, failure to distinguish *Klebsiella* species can mask species-specific resistance amplification, misrepresent local resistance burdens, and obscure clinically meaningful resistance phenotypes, including carbapenem and difficult-to-treat resistance patterns. Accordingly, incorporating species-level differentiation and comparative analysis of *K. pneumoniae* and non-*pneumoniae Klebsiella* species is essential not to redefine known resistance mechanisms, but to accurately characterize species-specific resistance trajectories, inform empirical therapy and stewardship strategies, and align regional surveillance outputs with clinically actionable infection control and treatment priorities in Saudi Arabia and the GCC.

The spread of resistant *Klebsiella* strains between hospitals and the community is increasingly evident, with multiple studies reporting ESBL- and carbapenemase-producing isolates in outpatient settings, indicating spillover of hospital

clones.[9,14] This blurs traditional boundaries and calls for integrated surveillance across care sectors. Despite expanding evidence, key knowledge gaps remain in Saudi Arabia: few studies use time-series models, most are cross-sectional, and data stratified by clinical setting or infection source are limited. Current molecular data, though based on small isolate subsets, contribute to an evolving understanding of resistance genes and strain circulation patterns. Regional molecular investigations have demonstrated that carbapenemase-encoding genes are present across both Enterobacterales and non-Enterobacterales Gram-negative pathogens in nosocomial settings, highlighting the role of shared hospital resistance reservoirs and facilitating the dissemination of carbapenem resistance.[7]

To address these critical epidemiological and surveillance gaps, the present study retrospectively analyzed *Klebsiella* spp. isolates collected over 12 years (2012–2024) at a tertiary care hospital in southern Saudi Arabia. The study aimed to (1) compare antimicrobial resistance profiles between *Klebsiella* pneumoniae and *non-pneumoniae Klebsiella* species, (2) assess trends in antimicrobial resistance using logistic regression modeling, with a particular focus on carbapenem-resistant and DTR phenotypes, (3) evaluate species-specific resistance patterns across different clinical settings, including inpatient, outpatient, and intensive care units (ICUs), and (4) characterize the molecular distribution of carbapenemase genes among phenotypically resistant isolates.

This study provides one of the most comprehensive longitudinal analyses of *Klebsiella* resistance in Saudi Arabia to date, offering insights critical for empirical therapy, stewardship strategies, and infection control policy.

Materials and Methods

Study Design, Setting, and Data Curation

This was a retrospective longitudinal observational study based on laboratory surveillance data collected at Aseer Central Hospital, a tertiary care center in southern Saudi Arabia. Isolates were obtained from various clinical specimens including blood, respiratory, and wound samples as part of routine diagnostic and therapeutic procedures. No samples were collected specifically for research purposes. The study included all *Klebsiella* isolates obtained from patients aged ≥12 years between January 2012 and September 2024. Pediatric cases (<12 years) were excluded to maintain demographic and clinical consistency, as younger children represent a distinct population with different host factors, healthcare exposure patterns, and antimicrobial prescribing practices that could confound longitudinal resistance trend analysis. Restricting the cohort to patients aged ≥12 years allowed for a more homogeneous epidemiologic assessment aligned with routine hospital surveillance and antimicrobial stewardship practices. Duplicate isolates, defined as repeat isolation of the same species from the same site and episode, were removed; only the first isolate per specimen type per patient was analyzed. Data was extracted from the hospital's LIS and curated in Microsoft Excel. The dataset comprised *Klebsiella* isolates recovered from routine clinical specimens submitted for diagnostic purposes. Consistent with laboratory-based surveillance practice, isolates were not adjudicated as representing colonization versus infection at the patient level. All isolates were analyzed collectively to characterize species distribution and antimicrobial resistance patterns across clinical submissions rather than infection episodes.

For surveillance classification, isolates were categorized as community-associated or hospital-associated based on the timing of specimen collection relative to hospital admission, in line with standard epidemiological definitions. Isolates obtained on the day of admission or within the first 48 hours of hospitalization were classified as community associated. Isolates recovered more than 48 hours after hospital admission were classified as hospital associated. Isolates obtained following hospital discharge were classified as community-associated unless collected during a documented readmission episode, in which case the timing criteria were reapplied.

Antimicrobial agents standardized for reporting purposes, and antimicrobial susceptibility interpretations retained as recorded in the laboratory information system (LIS) at the time of testing, in accordance with the CLSI guidelines in effect during each study year. Historical susceptibility results were not reinterpreted using updated CLSI breakpoints to avoid retroactive misclassification bias in longitudinal trend analyses. To ensure data integrity, extracted records were screened for duplicate entries and implausible values prior to analysis. Isolate identification and antimicrobial susceptibility profiles were independently reviewed by two microbiologists through cross-checking with microbiology bench records and routine laboratory quality assurance logs.

The study was reviewed and approved by the Aseer Institutional Review Board (IRB approval number: F7-2-2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Given the retrospective nature of the study and the use of de-identified data and isolates, the requirement for informed consent was waived by the ethics committee.

Microbiological Identification and Susceptibility Testing

Bacterial identification and antimicrobial susceptibility testing (AST) were primarily conducted using the VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France), which provides minimum inhibitory concentration (MIC)–based results, with interpretations based on CLSI M100 standards.[15] Owing to the use of automated susceptibility testing over a prolonged surveillance period and variability in testing panels and MIC reporting formats, detailed MIC distribution analyses and MIC50/MIC90 calculations were not performed. Instead, resistance was analyzed using standardized CLSI categorical susceptibility interpretations to ensure methodological consistency and comparability across species and time periods. When required for confirmation or resolution of borderline results, additional disk diffusion and gradient diffusion (Etest) methods were performed on Mueller–Hinton agar in accordance with CLSI recommendations. Molecular testing for carbapenemase genes was performed only on carbapenem-resistant *Klebsiella* isolates from 2024, as data from previous years were unavailable. Molecular characterization was therefore limited to isolates collected in 2024. Polymerase chain reaction (PCR) was performed using standard laboratory protocols as previously described.[16] Isolates selected for testing were based on imipenem and meropenem non-susceptibility, specimen relevance (eg, blood, respiratory, deep tissue), resource availability, and expert microbiology input. The GeneXpert® Carba-R PCR assay (Cepheid, Sunnyvale, CA, USA) detected *bla_KPC, bla_NDM, bla_VIM, bla_IMP*, and *bla_OXA-48* genes. Phenotypic carbapenemase confirmation assays (mCIM and eCIM) were not routinely performed, this approach was intentionally adopted to ensure methodological consistency across the full 12-year surveillance period, as categorical CLSI-based susceptibility interpretations provide greater comparability than pooled MIC distributions in long-term retrospective analyses with evolving testing panels.

Phenotypic Definitions of Resistance

Two clinically relevant resistance phenotypes were assessed in this study. Carbapenem-resistant Enterobacterales (CRE) were defined according to the Clinical and Laboratory Standards Institute (CLSI) criteria as isolates demonstrating phenotypic non-susceptibility (intermediate or resistant) to at least one carbapenem based on minimum inhibitory concentration (MIC) breakpoints, including imipenem/cilastatin (MIC ≥2 μg/mL), meropenem (MIC ≥2 μg/mL), or ertapenem (MIC ≥1 μg/mL), in accordance with CLSI M100 standards (35th edition).[15] DTR was defined according to Kadri et al[11] as in vitro non-susceptibility to all β-lactam agents, including carbapenems and fluoroquinolones. Specifically, DTR encompasses non-susceptibility to imipenem, meropenem, ertapenem, cefepime, ceftazidime, piperacillin-tazobactam, ciprofloxacin, and levofloxacin. Carbapenemase-producing Enterobacterales (CPE) refers specifically to isolates with confirmed carbapenemase gene detection or enzymatic activity.

Statistical Analysis

Descriptive statistics summarized the study population and distribution of the isolates. Continuous variables were reported as means ± SD and compared using *t*-tests, while categorical variables were expressed as counts and percentages and analyzed using chi-square or Fisher's exact test. A p-value <0.05 was considered significant.

Isolates were obtained from both hospitalized and outpatient settings. Overall analyses were conducted on the combined dataset to describe species distribution and antimicrobial resistance patterns across all clinical submissions. In addition, stratified comparative analyses between inpatient and outpatient isolates were performed to assess differences in resistance prevalence across care settings.

To assess potential progression from localized infection to bloodstream infection, we retrospectively identified cases in which a blood isolate of *Klebsiella* spp. was preceded by a non-blood isolate of the same species within the prior 3 months. To ensure temporal relevance and avoid duplication, only the closest preceding non-blood isolate per patient was

included. Due to the absence of exact specimen collection dates, time intervals were estimated using calendar month differences, approximating each month as 4.3 weeks.

Temporal trends in non-susceptibility were assessed using binary logistic regression, with susceptibility coded as 0 (susceptible) or 1 (non-susceptible), and calendar year as a continuous predictor. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported. Trends for CRE and DTR phenotypes were visualized using Python 3.11 with the matplotlib (v3.7.1) package.

Results

This surveillance study analyzed 9790 non-duplicate *Klebsiella* isolates, providing a comprehensive overview of species distribution and resistance dynamics across clinical settings. Out of 10,136 total isolates collected from 2012 to 2024, *Klebsiella pneumoniae* accounted for 93.0% (n = 9106), while *non-pneumoniae* species constituted 7.0% (n = 684). The mean patient age was similar between groups (58.8 ± 22.0 vs 59.9 ± 23.5 years), with 61.9% of isolates derived from male patients. Notably, *K. pneumoniae* was slightly more prevalent in female patients (93.7%) than males (92.6%) (Table 1).

Non-pneumoniae species were disproportionately recovered from patients with prolonged hospital stays (8.0%). ICU settings exhibited the highest *K. pneumoniae* prevalence (94.5%), followed by medical (92.9%) and surgical wards (92.3%).

Table 1 Demographic and Clinical Characteristics of Patients with *Klebsiella* spp. Infections

Characteristic	Total Samples (n = 9790)	Organism	
		***Klebsiella pneumoniae* (n = 9106)**	**Klebsiella species (n = 684)**
Age (mean ± SD)	58.86 ± 22.134	58.78 ± 22.031	59.87 ± 23.450
Sex no. (%)			
Male	6062 (61.9%)	5613 (92.6%)	449 (7.4%)
Female	3728 (38.1%)	3493 (93.7%)	235 (6.3%)
Risk factors no. (%)			
Long stay	802 (8.2%)	738 (92.0%)	64 (8.0%)
Burn	140 (1.4%)	130 (92.9%)	10 (7.1%)
ESRD (dialysis)	132 (1.3%)	123 (92.2%)	9 (6.8%)
DM	116 (1.2%)	105 (90.5%)	11 (9.5%)
Other	8600 (87.8%)	7945 (92.4%)	655 (7.6%)
Setting no. (%)			
Inpatient (INP)	8494 (86.8%)	7922 (93.3%)	572 (6.7%)
Outpatient (OPD)	1296 (13.2%)	1184 (91.4%)	112 (8.6%)
Hospitalization Ward no. (%)			
Medical wards	2914 (34.3%)	2708 (92.9%)	206 (7.1%)
Surgical wards	2605 (30.7%)	2404 (92.3%)	201 (7.7%)
ICU	2975 (35.0%)	2810 (94.5%)	165 (5.5%)

Note: Long stay refers to hospitalization exceeding 14 days.
Abbreviations: ESRD, End-Stage Renal Disease; DM, Diabetes Mellitus; ICU, Intensive Care Unit.

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Across all specimen types, *K. pneumoniae* remained dominant, comprising 90.4% to 94.9% of *Klebsiella* isolates. However, *non-pneumoniae* species were relatively more frequent in abscesses (8.4%) and skin samples (7.7%). A significant annual decline in *K. pneumoniae* recovery was observed (OR = 0.957; 95% CI: 0.935–0.979; $p < 0.001$); however, although statistically significant, the yearly decline was modest, Table 2 summarizes overall species distribution trends, reflecting the predominance of *K. pneumoniae* across the study period.

Antimicrobial resistance rose significantly over time, particularly among carbapenems. Yearly odds of non-susceptibility increased for ertapenem (OR = 1.076), imipenem (OR = 1.164), and meropenem (OR = 1.168), indicating increasing odds of non-susceptibility per year (Table 3). In outpatient urinary isolates, resistance increased for trimethoprim-sulfamethoxazole (OR = 1.055), fosfomycin (OR = 1.164), and nitrofurantoin (OR = 1.118) (Table 4).

Table 2 Temporal and Specimen Source Distribution of *K. pneumoniae* and Other *Klebsiella* spp.

Characteristic	Total Samples (n = 9790)	Organism		p-value
		***Klebsiella pneumoniae* (n = 9106)**	***Klebsiella* species (n = 684)**	
Source				0.011[a]
Respiratory	2737 (28.0%)	2567 (93.8%)	170 (6.2%)	
Skin samples	1220 (12.5%)	1126 (92.3%)	94 (7.7%)	
Urine	3818 (39.0%)	3543 (92.8%)	275 (7.2%)	
Blood	980 (10.0%)	928 (94.7%)	52 (5.3%)	
Abscess	523 (5.3%)	479 (91.6%)	44 (8.4%)	
Other	512 (5.2%)	463 (90.4%)	49 (9.6%)	
Predictor	**Exp(B) (OR)**	**95% CI for Exp(B)**	**p-value**	
Year	0.957	0.935–0.979	< 0.001[b]	

Note: [a]Chi-square test; [b]Logistic regression Organism vs Year.

Table 3 Logistic Regression Trends in Non-Susceptibility to Selected Antibiotics for All *Klebsiella* species (2012–2024)

Group	Antibiotic[a]	OR (Exp(B))	95% CI for Exp(B)	p-value
β-lactams	Cefoxitin	1.090	1.072–1.109	< 0.001
	Cefuroxime	1.001	0.981–1.022	0.900
	Cefotaxime	0.963	0.943–0.984	0.001
	Ceftazidime	0.980	0.966–0.994	0.006
	Cefepime	0.981	0.967–0.995	0.008
	Aztreonam	0.942	0.923–0.962	< 0.001
	Piperacillin-tazobactam	1.059	1.045–1.073	< 0.001
	Ertapenem	1.076	1.057–1.095	< 0.001
	Imipenem	1.164	1.148–1.180	< 0.001
	Meropenem	1.168	1.151–1.186	< 0.001

(*Continued*)

Table 3 (Continued).

Group	Antibiotic[a]	OR (Exp(B))	95% CI for Exp(B)	p-value
Fluoroquinolones	Ciprofloxacin	1.040	1.027–1.053	< 0.001
	Levofloxacin	1.047	1.031–1.063	< 0.001
Aminoglycosides	Gentamicin	0.985	0.972–0.997	0.016
	Tobramycin	0.988	0.971–1.004	0.147
	Amikacin	1.006	0.994–1.019	0.334
Others	Nitrofurantoin[b]	1.054	1.022–1.088	0.001
	Tetracycline	1.060	1.017–1.104	0.006
	Trimethoprim-sulfamethoxazole	1.033	1.020–1.046	< 0.001
	Tigecycline[c]	0.967	0.948–0.986	0.001

Notes: [a]The year is entered as a predictor variable for each antibiotic; [b]For urine samples only; [c]Tigecycline data from 2015 were excluded from the model due to an anomalously low number of non-susceptible isolates. This discrepancy is likely attributable to the underreporting or misclassification of resistant results within the laboratory information system during that year, potentially reflecting inconsistent susceptibility testing or documentation practices.

Table 4 Non-Susceptibility Trends in Oral UTI Agents in Outpatients

First-Line Oral Options for UTI	Non-Susceptibility N (%)	Exp(B) (OR)	95% CI for Exp(B)	p-value
Trimethoprim-sulfamethoxazole	465 (45.1%)	1.055	1.012–1.101	0.012
Fosfomycin	23 (26.7%)	1.164	1.006–1.347	0.041
Nitrofurantoin	218 (54.5%)	1.118	1.048–1.193	0.001

Note: logistic regression, antibiotic vs Year as predictor.

K. pneumoniae demonstrated significantly higher resistance to most β-lactams, fluoroquinolones, and aminoglycosides compared with *non-pneumoniae* species, except for cefoxitin and cefuroxime, where resistance was higher among *non-pneumoniae* isolates (Table S1). Among *non-pneumoniae* isolates, *K. aerogenes* exhibited the highest resistance to aminoglycosides, β-lactams, and fluoroquinolones (Table S2).

Resistance rates were significantly higher in inpatient than outpatient isolates across nearly all antibiotic classes, with ORs < 0.60 for most agents (Table S3). From 2012 to 2021, CRE prevalence surged from 12.4% to 69.3%, declining to 49.7% by 2024. DTR rates similarly rose from 2.1% to 50.7% by 2021 (Figure 1).

Compared with non-CRE isolates, CRE isolates were significantly more likely to be recovered from hospitalized patients, particularly those in ICU settings (46.4% vs 23.7%, $p < 0.001$), and from respiratory and bloodstream specimens. No significant difference in patient age was observed between CRE and non-CRE groups. CRE isolates were less frequently recovered from outpatient settings and urinary specimens. These findings highlight clear epidemiologic and infection control–relevant differences between CRE and non-CRE *Klebsiella* isolates (Table S4).

Among CRE isolates, resistance to ciprofloxacin (92.2%), levofloxacin (90.2%), nitrofurantoin (88.5%), and tobramycin (88.3%) was most pronounced. Amikacin and TMP-SMX resistance were 75.9% and 74.0%, respectively; fosfomycin resistance was 28.3% (Figure 2).

The mean interval from initial non-blood isolate to bloodstream infection was 4.8 weeks, with respiratory (55.9%) and urinary (30.4%) tracts as common sources. Progression to BSI occurred in 1.7% of *K. pneumoniae* and 0.6% of *non-pneumoniae* cases. A detailed summary of species distribution, source of preceding non-blood isolates, and time to bloodstream infection is provided in (Table S5).

Among carbapenem-resistant *Klebsiella* isolates recovered in 2024, molecular testing using the GeneXpert® Carba-R assay was performed on 28 isolates. bla_OXA-48-like was the most frequently detected carbapenemase gene (35.7%),

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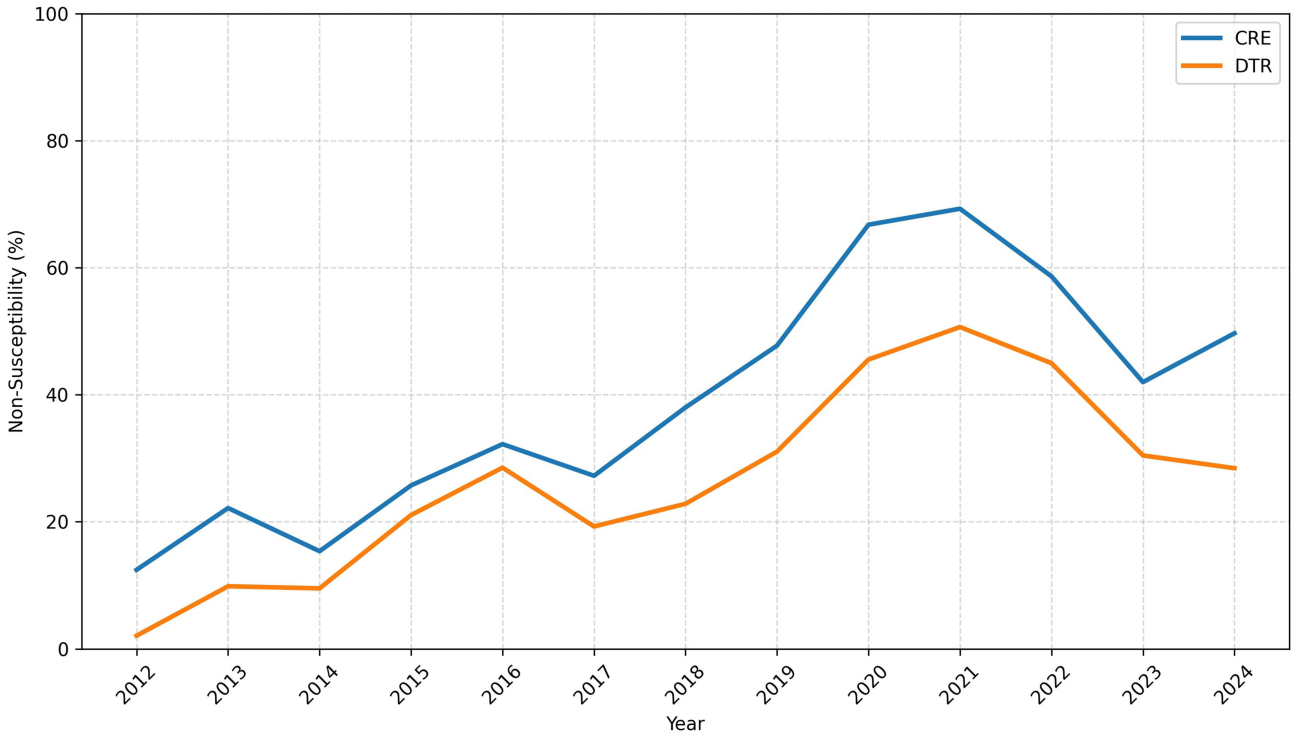

Figure 1 CRE and DTR non-susceptibility trends in all *Klebsiella* species (2012–2024).

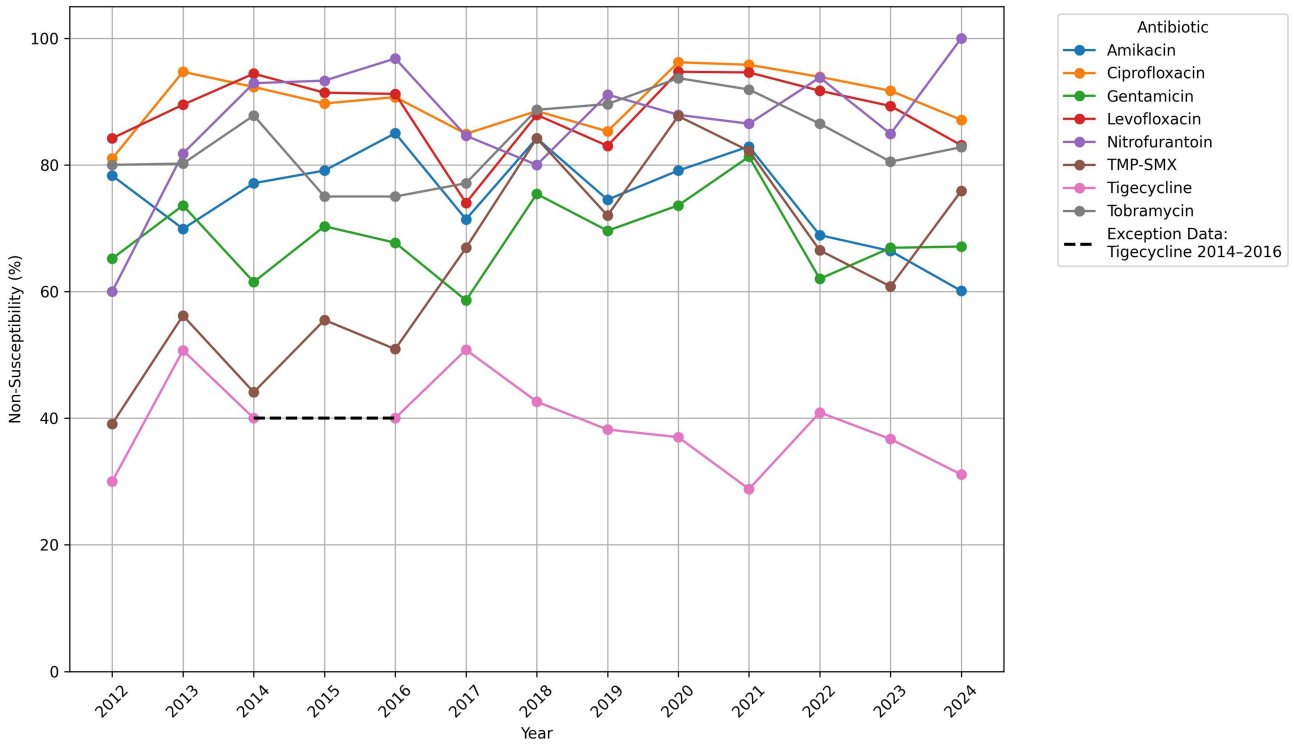

Figure 2 Non-β-lactam non-susceptibility rates in *Klebsiella* CRE isolates.

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followed by bla_NDM (17.9%) and bla_KPC (14.3%). Co-carriage of multiple carbapenemase genes was observed in 25% of tested isolates, including combinations of bla_NDM + bla_OXA-48-like and bla_NDM + bla_KPC. No bla_VIM or bla_IMP genes were detected in the tested isolates.

Discussion

This 12-year surveillance study provides a comprehensive analysis of AMR in *Klebsiella* spp. isolates from a tertiary care center in southern Saudi Arabia. The findings reveal a striking increase in carbapenem resistance and the emergence of the difficult-to-treat resistance (DTR) phenotype, reflecting complex underlying genetic mechanisms and significant implications for both empirical treatment and infection control policy. To our knowledge, this represents the first longitudinal analysis from southern Saudi Arabia demonstrating a DTR prevalence exceeding 50%, alongside species-level differentiation of antimicrobial resistance patterns across *Klebsiella* spp. over a 12-year period.

Carbapenem Resistance and DTR: A Rapidly Emerging Threat

Carbapenem resistance rose sharply from 12.4% in 2012 to 69.3% in 2021, reflecting global and regional trends in CRE. Although rates declined modestly in 2024, they remain high, especially with DTR prevalence rising from 2.1% to 50.7%, a known predictor of treatment failure and poor outcomes in BSIs.[11] These pronounced increases should be interpreted in the context of a long surveillance period, during which changes in CLSI interpretive breakpoints, antimicrobial formulary practices, and laboratory instrumentation may have contributed to apparent shifts in resistance rates.

Co-resistance in CRE isolates was also high to amikacin (75.9%), tobramycin (88.3%), and ciprofloxacin (92.2%), leaving limited options such as colistin and tigecycline.

Molecular Epidemiology: Widespread Dissemination of Carbapenemase Genes

Molecular profiling of selected CRE isolates using the GeneXpert® Carba-R assay showed predominance of *bla_OXA-48-like* (35.7%), followed by *bla_NDM* (17.9%) and *bla_KPC* (14.3%). Notably, 25% harbored multiple carbapenemase genes, including *bla_NDM* + *bla_OXA-48* and *bla_NDM* + *bla_KPC*. Co-occurrence of multiple carbapenemase genes likely reflects accumulation of mobile genetic elements (eg, IS26, ISKpn19, Tn4401) that promote inter- and intra-species gene exchange.[14] Although limited to a subset of 28 carbapenem-resistant isolates from 2024, these molecular findings suggest the predominance of plasmid-mediated carbapenemase dissemination in the study setting; however, comprehensive molecular surveillance, including whole-genome sequencing, would be required to characterize clonal relatedness, plasmid structures, and transmission dynamics over time, rather than to confirm carbapenemase gene presence alone.

These patterns align with prior reports from Saudi Arabia and the Gulf region; for example, Alshahrani et al reported that among carbapenem-resistant *K. pneumoniae* isolates from the same region, 62.5% carried bla_OXA-48-like, 25% carried bla_NDM-1, and 12.5% co-harbored both genes.[8] The presence of these enzymes, often carried on conjugative plasmids like IncL/M (*bla_OXA-48*) and IncFII/IncA/C (*bla_NDM-1*), highlights the role of horizontal gene transfer in spreading resistance. Recent regional studies in other Enterobacterales, such as extensively drug-resistant *Escherichia coli*, have highlighted the potential role of additional genetic elements, including CRISPR-encoding systems, in resistance evolution beyond carbapenemase production.[17] Similar mechanisms involving outer membrane porin loss and metallo-β-lactamase production have been reported in other clinically significant Gram-negative pathogens, including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, further emphasizing the shared molecular pathways driving carbapenem resistance across nosocomial organisms.[18]

Detection of KPC-type carbapenemases, previously rare in the region, may indicate emerging clones or plasmids introduced from high-prevalence areas (eg, the US or southern Europe). The presence of multi-carbapenemase producers raises concerns about diagnostic challenges and potential treatment failure, especially in settings without molecular testing.

Species-Level Differentiation: Clinical Relevance and Resistance Mechanisms

Although *K. pneumoniae* made up 93.0% of isolates, *non-pneumoniae* species, particularly *K. aerogenes* and *K. oxytoca*, exhibited distinct resistance profiles. *K. aerogenes* showed significantly higher resistance to third-generation

cephalosporins, aminoglycosides, and nitrofurantoin, likely due to inducible chromosomal AmpC β-lactamases, as described by Jacoby[3] and IDSA.[13] Table S1 provides detailed species-specific comparisons, highlighting statistically significant differences across multiple antibiotic classes, including the notably higher cefoxitin resistance among *non-pneumoniae* species.

Intrinsic resistance may be amplified by porin loss or efflux pump overexpression (eg, AcrAB-TolC), enhancing resistance to cephalosporins and carbapenems even without carbapenemase genes. Lack of species-level stratification in surveillance data obscures these phenotypic differences and hampers targeted empiric therapy.

Clinical Setting, Colonization Dynamics, and Community Spillover

Resistance rates were significantly higher in inpatient isolates, with the ICU showing the greatest burden, reflecting its role as a hub for MDRO transmission due to intense antibiotic use, invasive devices, and prolonged stays.[5,12]

These findings align with previous Saudi studies, including Hafiz et al, who reported significantly higher ICU admission and mortality in carbapenem-resistant *K. pneumoniae* bacteremia cases (57.9% vs 25.6%, p = 0.001).[10] More concerning is the detection of ESBL- and carbapenemase-producing *Klebsiella* in outpatient urinary and wound samples, suggesting community spread of hospital-derived clones, possibly through recently discharged patients or asymptomatic carriers. As Rodríguez-Baño et al noted, this highlights the blurring boundaries between hospital and community ecosystems.

> The presence of ESBL and carbapenemase-producing *Klebsiella* in outpatient urinary isolates or wound infections highlights the porous boundaries between hospital and community microbial ecosystems.[14]

This overlap between community and healthcare reservoirs calls for integrated surveillance and a reassessment of empirical therapy for community-acquired infections in high-AMR regions. Within the local healthcare context, empirical antibiotic prescribing is guided by institutional antimicrobial stewardship policies informed by cumulative hospital antibiogram data and national recommendations. Standard treatment pathways and infection prevention and control guidelines, including standard and transmission-based precautions, are implemented at the institutional level. However, the rapid escalation of carbapenem-resistant and difficult-to-treat *Klebsiella* phenotypes observed in this study suggests that evolving resistance patterns may outpace existing empirical therapy frameworks. These findings underscore the importance of regular review of antibiotic policies, strengthened adherence to stewardship and infection control guidelines, and closer alignment of empirical treatment algorithms with species-specific and resistance phenotype–driven surveillance data. The timing of isolation relative to hospital admission further informs resistance epidemiology. Early isolation of carbapenem-resistant or difficult-to-treat *Klebsiella* suggests community carriage or prior healthcare exposure at admission, whereas isolation after prolonged hospitalization is more consistent with healthcare-associated acquisition driven by antimicrobial exposure and in-hospital transmission. Although length of stay was not formally analyzed, this distinction highlights the combined influence of community antimicrobial use, hospital antibiotic policy, and infection prevention practices on resistance dynamics.

Progression from Colonization to Invasive Disease

A key finding was the progression from non-blood to bloodstream infections, with a mean interval of 4.8 weeks. Most preceding non-blood isolates originated from the respiratory tract (55.9%) and urinary tract (30.4%), consistent with endogenous translocation. Progression to bloodstream infection occurred in 1.7% of *Klebsiella pneumoniae* cases and 0.6% of *non-pneumoniae* cases. Overall, *K. pneumoniae* accounted for 97.5% of bloodstream infections arising from prior non-blood isolates, whereas *non-pneumoniae* species represented 2.5%, underscoring the need for active surveillance in high-risk patients to enable early detection and intervention (Table S5). These quantitative findings provide direct support for the observed progression patterns discussed and confirm that bloodstream invasion most commonly follows recent non-blood isolation from high-burden anatomical sites.

Implications for Stewardship, Diagnostics, and Policy

This study underscores the need for multifaceted strategies to contain MDR *Klebsiella*. Key priorities include: (1) integrating local resistance data into stewardship and empirical therapy, (2) mandating species-level identification and reporting of CRE/DTR phenotypes, and (3) expanding molecular diagnostics (eg, Carba-R, WGS) to support infection control and outbreak management.

National policies should address community spillover by reinforcing infection control in long-term care, regulating outpatient antibiotic use, and promoting data sharing across institutions. Linking AMR data to platforms like WHO GLASS will support early detection of emerging clones and resistance mechanisms.

Strengths and Limitations

Strengths of this study include its long duration, large sample size, inclusion of multiple *Klebsiella* species, and use of CLSI-based methods for susceptibility testing and trend analysis. Limitations involve its single-center scope, limited molecular testing, and absence of genotyping to confirm clonal links between non-blood and BSI isolates. In addition, susceptibility testing for recently approved anti-carbapenemase agents (eg, ceftazidime–avibactam, imipenem–cilastatin–relebactam, cefiderocol) and agents active against MDR *Pseudomonas aeruginosa* (ceftolozane–tazobactam) was unavailable during much of the study period due to prolonged market supply interruptions and the absence of corresponding AST cards in the hospital laboratory. Furthermore, molecular characterization was limited to isolates collected in 2024, which restricts direct genotypic–phenotypic comparisons across the full 12-year study period. This constraint was driven by resource availability and archival limitations. Nevertheless, the 2024 subset included isolates from multiple clinical sources and resistance profiles, providing a representative snapshot of contemporary carbapenem-resistant *Klebsiella* circulating in the study setting. Accordingly, the molecular findings should be interpreted as exploratory, as the limited number of tested isolates and the absence of routine phenotypic carbapenemase confirmation assays (eg, mCIM/eCIM) preclude definitive conclusions regarding the overall prevalence of CPE. Still, the findings are highly relevant and generalizable to similar tertiary care settings in the region.

Conclusion

This study addresses major gaps in *Klebsiella* epidemiology in Saudi Arabia by achieving four key objectives. First, species-level analysis showed that *K. pneumoniae* had significantly higher resistance than *non-pneumoniae* species, while *K. aerogenes* exhibited distinct AmpC-associated patterns. Second, time-series regression revealed rising resistance trends, with carbapenem and DTR rates surpassing 50% by 2024. Third, resistance varied by setting, with ICU and inpatient isolates showing significantly higher rates than outpatient ones. Fourth, molecular testing identified widespread *bla_OXA-48, bla_NDM*, and emerging *bla_KPC*, with many isolates co-harbouring multiple carbapenemase genes.

These findings highlight a worsening resistance landscape characterized by species-specific resistance, rising carbapenem and DTR rates, and community spillover of hospital-derived MDR strains. Urgent action is needed, including species-specific diagnostics, expanded molecular surveillance, and stewardship-guided empirical therapy across care settings. These data further support the need for regular updating of local and national antibiotic guidelines to reflect the high burden of carbapenem-resistant and DTR *Klebsiella* infections, particularly in inpatient and ICU populations. Future research should prioritize multicenter surveillance and genomic approaches, including whole-genome sequencing, to better define transmission dynamics and resistance evolution at the national level. The modest decline in carbapenem resistance observed after 2021 may reflect the combined impact of enhanced antimicrobial stewardship initiatives, strengthened infection prevention measures, and increased clinical awareness, although further evaluation is warranted.

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Supplementary Material

Additional data supporting the findings of this study, including detailed antimicrobial resistance comparisons, species-specific analyses, clinical stratifications, and progression from non-blood to bloodstream infection, are provided in the supplementary material. The supplementary document includes Supplementary Tables S1–S5, all of which are cited in numerical order within the main manuscript text.

Disclosure

The author(s) report no conflicts of interest in this work.

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