

Molecular characterization of multidrug-resistant ESBL-producing and carbapenem-resistant *Klebsiella* spp. clinical isolates from urine and wound infections in Togo

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Abstract

Klebsiella spp. are World Health Organization critical-priority pathogens owing to their remarkable ability to acquire and disseminate antimicrobial resistance determinants, particularly extended-spectrum β -lactamases (ESBLs) and carbapenemases. Although antimicrobial resistance is increasing across West Africa, comprehensive molecular epidemiological data describing resistance determinants in clinical *Klebsiella* isolates remain scarce in Togo. This study aimed to provide the first multi-centre molecular characterization of ESBL-producing and carbapenem-resistant *Klebsiella* spp. circulating in the country.

A multi-center cross-sectional study was conducted from October 2024 to June 2025 in 16 healthcare facilities in Togo. A total of 161 non-duplicate clinical *Klebsiella* isolates, including 153 ESBL-producing and 8 carbapenem-resistant strains, were recovered from urine and wound specimens. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to CLSI guidelines. Eighteen antimicrobial resistance genes were detected using real-time PCR.

Klebsiella pneumoniae subsp. *pneumoniae* was the predominant species (78.9%), followed by *K. oxytoca* (12.4%), *K. pneumoniae* subsp. *ozaenae* (7.5%), and *K. pneumoniae* subsp. *rhinoscleromatis* (1.2%). Multi-drug resistance was observed in 99.4% of isolates, whereas one isolate (0.6%) exhibited an extensively drug-resistant phenotype. The most prevalent β -lactamase genes were *bla*_{SHV} (62.1%), *bla*_{CTX-M} (38.5%), and *bla*_{TEM} (25.5%). Carbapenemase genes included *bla*_{NDM} (14.3%), *bla*_{KPC} (8.1%), *bla*_{OXA-48} (6.2%), and *bla*_{VIM} (0.6%). The quinolone resistance genes *qnrA* and *qnrB* were detected in 49.1% and 32.9% of isolates, respectively. The colistin resistance gene *mcr-1* was identified in 11.2% of isolates and was correlated with *bla*_{KPC} ($p = 0.75$). Resistance was highest to trimethoprim-sulfamethoxazole (85.1%) and ceftriaxone (81.4%), whereas amikacin, carbapenems, and nitrofurantoin remained largely effective.

Clinical *Klebsiella* spp. isolates in Togo exhibit an alarming burden of multi-drug resistance and harbor diverse resistance determinants, including carbapenemase and colistin resistance genes. These findings highlight the need for routine molecular surveillance and strengthened antimicrobial stewardship programs in Togo and across West Africa.

Introduction

Antimicrobial resistance (AMR) constitutes one of the most pressing global health challenges of the twenty-first century, with an estimated 4.95 million deaths associated with bacterial AMR in 2019 alone, a burden disproportionately concentrated in low- and middle-income countries [1, 2]. Among the Gram-negative pathogens prioritized by the World Health Organization (WHO), Extended-Spectrum β -Lactamase (ESBL)-producing and carbapenem-resistant *Enterobacterales* particularly *Klebsiella pneumoniae* occupy a critical position owing to their high attributable mortality, propensity for nosocomial transmission, and progressively narrowing therapeutic options [3, 4].

Klebsiella spp. are opportunistic pathogens responsible for a broad spectrum of healthcare- and community-associated infections, including urinary tract infections, wound infections, pneumonia and bloodstream infections. Their clinical importance is largely driven by an exceptional capacity to acquire mobile genetic elements carrying multiple antimicrobial resistance determinants, thereby facilitating the rapid dissemination of multidrug-resistant clones within healthcare settings [4–6]. Among ESBL enzymes, CTX-M-type β -lactamases have become the predominant resistance mechanism worldwide, progressively replacing the historically prevalent TEM- and SHV-derived ESBLs in many geographical regions [5, 7]. The widespread dissemination of these enzymes has substantially reduced the effectiveness of third-generation cephalosporins and has increased reliance on carbapenems for the treatment of severe infections caused by ESBL-producing *Enterobacterales*. The therapeutic landscape has become increasingly complex following the global dissemination of carbapenemase-producing *Enterobacterales*, mainly driven by *bla*_{NDM}, *bla*_{KPC} and *bla*_{OXA-48}-like genes [8, 9]. Their dissemination is mediated by both successful clonal expansion and horizontal transfer of mobile genetic elements, facilitating their rapid spread across healthcare settings [9, 10]. In addition to β -lactam resistance, *Klebsiella* isolates frequently accumulate resistance determinants targeting other clinically important antimicrobial classes, including fluoroquinolones, aminoglycosides, sulfonamides, tetracyclines and polymyxins [11–13]. The coexistence of these determinants may considerably restrict therapeutic options and complicate empirical antimicrobial treatment, particularly in resource-limited settings. Although resistance determinants have also been

identified in animal and environmental reservoirs, the epidemiology of clinical multidrug-resistant *Klebsiella* remains insufficiently characterised in several West African countries, including Togo [11, 12, 14].

Despite the growing body of evidence describing antimicrobial resistance among Enterobacterales in sub-Saharan Africa, molecular epidemiological data specifically addressing clinical *Klebsiella* isolates in Togo remain extremely limited. Most available studies have relied primarily on phenotypic antimicrobial susceptibility testing, with limited characterization of the underlying resistance genes, thereby restricting the understanding of local resistance mechanisms and limiting evidence available to support surveillance and antimicrobial stewardship programmes [11, 15]. Furthermore, little is known about the coexistence of clinically relevant resistance determinants within individual isolates or about the concordance between molecular findings and phenotypic antimicrobial susceptibility profiles in this setting. Addressing these knowledge gaps is important for improving local surveillance strategies and guiding empirical antimicrobial therapy. To address these knowledge gaps, we conducted a multicentre cross-sectional study to perform the molecular and phenotypic characterization of clinical *Klebsiella* spp. isolates recovered from urine and wound infections across 16 healthcare facilities in Togo. Specifically, we aimed to (i) determine the distribution of *Klebsiella* species and their multi-drug resistance profiles according to internationally recognized MDR/XDR/PDR criteria; (ii) characterize the prevalence of eighteen clinically relevant antimicrobial resistance genes using real-time PCR; (iii) evaluate patterns of resistance gene co-occurrence and their relationship with phenotypic antimicrobial susceptibility; and (iv) identify antimicrobial agents that retain activity against these isolates. By integrating molecular and phenotypic data within a multicentre collection, this study provides baseline evidence to support antimicrobial resistance surveillance and stewardship initiatives in Togo.

Methods

Study design, setting, and ethical considerations

A multi-center cross-sectional study was conducted between October 2024 and June 2025 across sixteen healthcare facilities, including tertiary, regional and private hospitals distributed throughout Togo. The study aimed to characterize the molecular and phenotypic antimicrobial resistance profiles of ESBL-producing and carbapenem-resistant *Klebsiella* spp. isolated from urine and wound specimens. Ethical approval was obtained from the National Committee for Health Research Ethics of Togo (reference no. 036/2024/CRBS, dated 4 July 2024). To ensure data consistency and reliability, all isolates underwent centralized quality control. Confirmation of bacterial identification and verification of antimicrobial susceptibility testing were conducted at reference laboratories in Togo, including the National Hygiene Institute. All molecular analyses including genomic DNA extraction, Nanopore-based quantification, and real-time PCR for resistance gene detection were performed at the Centro de Investigación Biomédica de Granada (CIBM), University of Granada, Spain (Department of Pharmacology; ibs. GRANADA). Centralized molecular processing ensured standardized analytical conditions and enhanced the reproducibility and scientific robustness of genotypic findings [16].

Sample collection, bacterial isolation, and species identification

Clinical specimens were collected following standardized protocols aligned with international clinical microbiology guidelines [16, 17]. Midstream urine samples were obtained from adult patients following hygiene counselling, while sterile, age-appropriate collection devices were used for pediatric patients. Wound specimens were obtained by deep swabbing or aspiration of purulent material following cleansing of the lesion with sterile saline. Specimens were processed within 24 hours of collection, with interim storage at 4–8°C when required.

Specimens were inoculated onto MacConkey agar, eosin methylene blue agar, and blood agar, and incubated aerobically at 35–37°C for 18–24 hours. Presumptive identification of *Klebsiella* spp. was based on colony morphology (large, mucoid, lactose-fermenting colonies) and Gram staining. Definitive species- and subspecies-level identification was achieved through a panel of conventional biochemical tests (oxidase, catalase, motility, indole production, urease activity, citrate utilization), with confirmation using the API 20E system or the VITEK 2 automated platform (bioMérieux, France) [18, 19].

Inclusion and exclusion criteria

Isolates were eligible for inclusion in the present molecular and phenotypic analysis if they (i) were identified as *K. pneumoniae* (subsp. *pneumoniae*, *ozaenae*, or *rhinoscleromatis*) or *K. oxytoca*, (ii) were recovered from urine or wound/pus specimens, and (iii) displayed a confirmed ESBL-producing or carbapenem-resistant phenotype on combined phenotypic testing (see below). Isolates identified other *Klebsiella* spp as *Klebsiella aerogenes* or *Klebsiella quasipneumoniae* were excluded from the analysis owing to their distinct taxonomic position within the genus and their comparatively limited representation in the overall collection. A total of 161 non-duplicate isolates (one isolate per patient) met these criteria and constituted the final analytic dataset, comprising 153 ESBL-producing isolates (95.0%) and 8 carbapenem-resistant isolates (5.0%).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed by the Kirby Bauer disk diffusion method on Mueller–Hinton agar, in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition (2024) [20]. Bacterial suspensions were standardized to a 0.5 McFarland turbidity prior to inoculation, and plates were incubated aerobically at 35–37°C for 16–18 hours. Inhibition zone diameters were measured and categorized as susceptible (S), intermediate (I), or resistant (R) according to current CLSI breakpoints. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as quality control reference strains for every batch of testing [20, 21].

A panel of 36 antibiotics, spanning 13 antibiotic classes, was tested in all 161 isolates: penicillins (amoxicillin, ticarcillin, piperacillin); penicillin– β -lactamase-inhibitor combinations (amoxicillin–clavulanate, ticarcillin–clavulanate, piperacillin–tazobactam); cephalosporins (cephalothin, cefoxitin, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, cefixime, cefepime, ceftaroline); monobactams (aztreonam); carbapenems (imipenem, meropenem, ertapenem); aminoglycosides (tobramycin, amikacin, gentamicin); fluoroquinolones (nalidixic acid, norfloxacin, pefloxacin, ofloxacin, ciprofloxacin, levofloxacin); sulfonamides (trimethoprim-sulfamethoxazole); tetracyclines (tetracycline, doxycycline); amphenicols (chloramphenicol/thiamphenicol); polymyxins (colistin); phosphonates (fosfomycin); and nitrofurans (nitrofurantoin, nitrofurantoin).

Phenotypic definition of ESBL and carbapenem-resistant isolates

Phenotypic ESBL production was confirmed using the double-disk synergy test (DDST), in which an enhancement of the inhibition zone between an amoxicillin–clavulanate disk and adjacent third-generation cephalosporin disks (ceftriaxone, cefotaxime, ceftazidime) was interpreted as indicative of clavulanate-inhibitable ESBL activity, in accordance with CLSI guidelines [20, 22]. Carbapenem-resistant isolates were defined as those displaying resistance to at least one of the three carbapenems tested (imipenem, meropenem, or ertapenem) according to CLSI M100-2024 breakpoints [20].

Multi-drug, extensive drug, and pan-drug resistance classification

Isolates were classified according to a 13 antibiotic-class framework adapted from the international expert proposal of Magiorakos et al. [23] to match the panel tested in this study (penicillins; penicillins + inhibitors; cephalosporins; monobactams; carbapenems; aminoglycosides; fluoroquinolones; sulfonamides; tetracyclines; amphenicols; polymyxins; phosphonates; nitrofurans). An isolate was classified as multidrug-resistant (MDR) if it was non-susceptible to at least one agent in at least 3 of the 13 classes; as extensively drug-resistant (XDR) if it remained fully susceptible to all agents in no more than 2 of the 13 classes; and as pan-drug-resistant (PDR) if it was non-susceptible to at least one agent in all 13 classes.

DNA extraction

Bacterial isolates collected from Togolese healthcare facilities were transported under appropriate bio-safety conditions to the Centro de Investigación Biomédica de Granada (CIBM), University of Granada, Spain, where all subsequent molecular analyses were conducted. Genomic DNA was extracted from overnight cultures using the boiling lysis method. DNA concentration and

purity were subsequently determined using NanoDrop 2000, following the manufacturer's recommendations. DNA samples were stored at – 20°C until molecular analysis [24].

Real-time PCR for resistance gene detection

A panel of 18 resistance genes spanning six functional classes was investigated by real-time PCR (qPCR): three ESBL genes (*bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}); five carbapenemase genes (*bla*_{NDM}, *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP}); two plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*); one aminoglycoside resistance gene (*aac*(6')-*Ib-cr*); two sulfonamide resistance genes (*sul1*, *sul2*); two tetracycline resistance genes (*tetA*, *tetB*); one trimethoprim resistance gene (*dhfr1*); one colistin resistance gene (*mcr-1*); and one amphenicol resistance gene (*catI*) [25].

All real-time PCR amplifications were performed at the CIBM, University of Granada (Granada, Spain), using the Eco™ Real-Time PCR System (Illumina, San Diego, CA, USA) and KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems, Wilmington, MA, USA). Each reaction was carried out in a final volume of 20–25 µL containing 20 ng of genomic DNA (or 2 µL of template DNA, depending on the assay), a 2× SYBR Green master mix, optimised concentrations of forward and reverse primers, with amplification performed at the primer-specific annealing temperature. Thermal cycling consisted of an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C and combined annealing/extension at 60°C, with fluorescence acquisition at each cycle [26, 27]. Primer sequences targeting the 18 investigated resistance genes were selected from previously validated assays reported in the literature [28–35], with amplicon sizes ranging from 210 to 747 base pairs (Table 1). All primers were custom-synthesized as Invitrogen™ Custom DNA Oligos (Thermo Fisher Scientific, Carlsbad, CA, USA; order reference no. 80411402).

The bacterial 16S rRNA gene was used as an endogenous internal control to confirm DNA integrity, the presence of bacterial DNA, and the absence of PCR inhibition in each reaction. An isolate was considered positive for a resistance gene when three criteria were simultaneously fulfilled: (i) a specific amplification curve, (ii) a single melting peak corresponding to the expected melting temperature (*T*_m) for the targeted amplicon, and (iii) a reproducible amplification signal within the established cycle threshold (*C*_t) range. The ΔC_t value, calculated as the absolute difference between the *C*_t value of the target resistance gene and the *C*_t value of the 16S rRNA gene, was used as an additional quality control parameter; a $\Delta C_t \leq 5$ was considered indicative of consistent target amplification relative to bacterial DNA content. This multi-criteria interpretation approach is consistent with current MIQE 2.0 recommendations for qualitative real-time PCR-based detection of antimicrobial resistance genes [26, 27].

Escherichia coli ATCC 25922 (negative control), *K. pneumoniae* ATCC 700603 (positive ESBL control), and a no-template control were included in every run, with amplification specificity confirmed by melting curve analysis [25, 26].

Table 1

Primer sequences (forward and reverse), amplicon sizes, and original source references for the 18 resistance genes investigated by real-time PCR.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon size (bp)	Tm	Source
<i>bla</i> _{SHV}	GGGTTATTCTTATTTGTCGC	TTAGCGTTGCCAGTGCTC	747	56	[28]
<i>bla</i> _{TEM}	ATGAGTATTCAACATTTCCG	CCAATGCTTAATCAGTGAGG	445	58	[28]
<i>bla</i> _{CTX-M}	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG	593	88	[28]
<i>bla</i> _{OXA-48}	GCGTGGTTAAGGATGAACAC	CATCAAGTTCAACCCAACCG	438	60	[29]
<i>bla</i> _{VIM}	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG	390	56	[30]
<i>bla</i> _{IMP}	GGAATAGAGTGGCTTAAYTCTC	GGTTTAAYAAAAACAACCACC	232	55	[30]
<i>bla</i> _{NDM}	GGTTTGGCGATCTGGTTTTTC	CGGAATGGCTCATCACGATC	621	62	[30]
<i>bla</i> _{KPC}	CATTCAAGGGCTTTCTTGCTGC	ACGACGGTGTGTTCATTTC	538	66	[30]
<i>qnrA</i>	GATAAAGTTCTCACGCCAGG	TTCAGCAAGAGGATTTCTCAC	580	60	[31]
<i>qnrB</i>	GATCGTGAAAGCCAGAAAGG	ACGATGCCTGGTAGTTGTCC	264	62	[31]
<i>mcr-1</i>	GCTGCGGTGGTATGATTATG	CATATCGGCAAGCAGCACT	309	58	[32]
<i>aac(6')-lb-cr</i>	TTGCGATGCTCTATGAGTGGCTA	CTCGAATGCCTGGCGTGTTT	482	62	[33]
<i>catI</i>	ATGAAAAAAATTATTGAAAAAGG	TTACGCAAATAAATCTGGAAACG	650	62	[34]
<i>dfA1</i>	GTTTGATGCTGCCGCTGAG	ATGCGGCCGTTGAGGTTG	500	58	[34]
<i>tetA</i>	GGTTCACCTCGAACGACGTCA	CTGTCCGACAAGTTGCATGA	210	60	[34]
<i>tetB</i>	CATTAATAGCACCAGCAGC	GTAATGGGCCAATAACACCG	659	60	[34]
<i>sul1</i>	CGGCGTGGGCTACCTGAACG	GCCGATCGCGTGAAGTTCCG	433	66	[35]
<i>sul2</i>	TCCGGTGAGGCCGGTATCTGG	CGGGAATGCCATCTGCCTTGAG	721	70	[35]

Primers were custom-synthesized as Invitrogen™ Custom DNA Oligos (Thermo Fisher Scientific, Carlsbad, CA, USA; order reference no. 80411402).

Statistical analysis

Categorical variables are presented as frequencies and percentages. Associations between categorical variables were evaluated using Pearson's χ^2 test or Fisher's exact test, as appropriate. Pairwise associations between resistance genes were assessed using Spearman's rank correlation coefficient (ρ). Statistical analyses were performed using R (version 4.5.1) and IBM SPSS Statistics version 30. All tests were two-sided and statistical significance was established at $p < 0.05$.

Results

Study population, socio-demographic characteristics, and multi-drug resistance classification.

A total of 161 non-duplicate *Klebsiella* spp. clinical isolates recovered between October 2024 and June 2025 from 16 healthcare facilities across Togo met the inclusion criteria and constituted the final analytic dataset (Table 2). Of these, 153 (95.0%) were phenotypically confirmed as ESBL-producing and 8 (5.0%) as carbapenem-resistant. The cohort included 90 male (55.9%) and 71 female (44.1%) patients, yielding a sex ratio of 1.27. The median patient age was 48.5 years (IQR: 17.5–65.0). The age

distribution was bimodal: patients older than 60 years constituted the largest group ($n = 60$; 37.3%), followed by children aged 0–15 years ($n = 37$; 23.0%), patients aged 46–60 years ($n = 25$; 15.5%), patients aged 31–45 years ($n = 23$; 14.3%), and young adults aged 16–30 years ($n = 15$; 9.3%). Urine specimens predominated ($n = 126$; 78.3%), with wound and pus samples accounting for the remainder ($n = 35$; 21.7%). Applying the 13-antibiotic-class framework, 160 of 161 isolates (99.4%) met the criteria for multidrug resistance (MDR), one isolate (0.6%) was classified as extensively drug-resistant (XDR), and no isolate met the criteria for pan-drug resistance (PDR) (Table 2).

Species distribution by specimen type

Klebsiella pneumoniae subsp. *pneumoniae* was the predominant species ($n = 127$; 78.9%), with 98 isolates (77.2%) recovered from urine and 29 (22.8%) from wound specimens (Table 2). *Klebsiella oxytoca* ranked second ($n = 20$; 12.4%; urine: 80.0%; wound: 20.0%). *Klebsiella pneumoniae* subsp. *ozaenae* contributed 12 isolates (7.5%), with a marked urinary tropism ($n = 11$; 91.7%; wound: $n = 1$; 8.3%). *Klebsiella pneumoniae* subsp. *rhinoscleromatis* was the rarest taxon ($n = 2$; 1.2%), equally distributed between urine and wound specimens (Table 3).

Prevalence and distribution of antimicrobial resistance genes

Real-time PCR, conducted at the Centro de Investigación Biomédica de Granada (CIBM), University of Granada, Spain, using a multi-criteria positivity assessment (specific amplification curve, single melting peak at the expected T_m , reproducible C_t signal, and $\Delta C_t \leq 5$ relative to the 16S rRNA endogenous control), detected resistance genes from six functional classes across the 161 isolates (Fig. 1A).

ESBL genes. *bla*_{SHV} was the most prevalent gene overall ($n = 100$; 62.1%), followed by *bla*_{CTX-M} ($n = 62$; 38.5%) and *bla*_{TEM} ($n = 41$; 25.5%).

Carbapenemase genes. *bla*_{NDM} was the most frequently detected carbapenemase gene ($n = 23$; 14.3%), followed by *bla*_{KPC} ($n = 13$; 8.1%) and *bla*_{OXA-48} ($n = 10$; 6.2%). *bla*_{VIM} was identified in a single isolate ($n = 1$; 0.6%). *bla*_{IMP} was not detected in any isolate (0/161; 0.0%).

Co-resistance genes. *qnrA* was the most prevalent non- β -lactam resistance determinant ($n = 79$; 49.1%), followed by *qnrB* ($n = 53$; 32.9%), *sul1* ($n = 52$; 32.3%), *aac(6')-Ib-cr* ($n = 48$; 29.8%), *dfA1* ($n = 40$; 24.8%), *tetA* ($n = 38$; 23.6%), *tetB* ($n = 34$; 21.1%), and *mcr-1* ($n = 18$; 11.2%). The mean number of resistance genes per isolate was $3.8 (\pm 3.4)$; range: 0–12. *sul2* and *catI* were not detected in any isolate (0/161; 0.0%); ΔC_t values for these two genes were consistently above the positivity threshold of 5 in all 161 isolates, confirming complete absence of target amplification.

ESBL gene combination profiles

Among the 153 ESBL-producing isolates, analysis of *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} combination profiles revealed eight distinct patterns (Table 4). It was revealed the absence of all three targeted genes in 44 isolates (28.8%) despite a confirmed ESBL phenotype. Among isolates carrying at least one gene, *bla*_{SHV} carried alone was the most common single-gene profile ($n = 41$; 26.8%), followed by the triple-gene combination *bla*_{CTX-M}+*bla*_{TEM}+*bla*_{SHV} ($n = 34$; 22.2%), *bla*_{CTX-M}+*bla*_{SHV} ($n = 18$; 11.8%), *bla*_{CTX-M} alone ($n = 9$; 5.9%), *bla*_{TEM}+*bla*_{SHV} ($n = 5$; 3.3%), and two single-isolate profiles (*bla*_{CTX-M}+*bla*_{TEM} and *bla*_{TEM} alone; $n = 1$ each; 0.7% each).

Resistance gene co-occurrence patterns

Spearman correlation analysis of the 16 genes with detectable prevalence revealed the strongest positive correlations between *bla*_{KPC} and *mcr-1* ($\rho = 0.75$) and between *bla*_{CTX-M} and *aac(6')-Ib-cr* ($\rho = 0.75$) (Fig. 1B). Hierarchical clustering identified a coherent multi-resistance cluster comprising *bla*_{TEM}, *qnrB*, *bla*_{CTX-M}, and *aac(6')-Ib-cr* (pairwise ρ range: 0.43–0.59), and a *sul1*–*dfA1* association ($\rho = 0.41$). Conversely, *bla*_{SHV} showed strong negative correlations with *mcr-1* ($\rho = -0.58$) and *bla*_{KPC} ($\rho = -0.33$).

Resistance gene prevalence by species

Klebsiella pneumoniae subsp. *pneumoniae* harboured the broadest resistance gene repertoire, with elevated prevalence of *bla*_{SHV} (64%), *qnrA* (51%), *bla*_{CTX-M} (41%), and *sul1* (34%) (Fig. 1C). *K. oxytoca* displayed comparatively lower gene carriage across most classes, with the exception of *bla*_{SHV}, consistent with its chromosomal background. *K. pneumoniae* subsp. *ozaenae* showed intermediate gene prevalence, including *bla*_{CTX-M} (50%) and *qnrA* (58%). The two *K. pneumoniae* subsp. *rhinoscleromatis* isolates were insufficient in number for species-level gene analysis. *bla*_{IMP}, *sul2*, and *catI* were absent across all species (0/161; 0.0%).

Phenotypic antimicrobial susceptibility profiles

All 36 antibiotics were tested across all 161 isolates; results were interpreted according to CLSI M100-2024 breakpoints (Fig. 2A).

Highest resistance rates: Resistance was most frequent for trimethoprim-sulfamethoxazole (TMP-SXT; 137/161; 85.1%), ceftriaxone (131/161; 81.4%), gentamicin (117/161; 72.7%), ceftazidime (117/161; 72.7%), ciprofloxacin (111/161; 68.9%; intermediate: 2/161; 1.2%), amoxicillin-clavulanate (99/161; 61.5%), aztreonam (98/161; 60.9%; intermediate: 1/161; 0.6%), amoxicillin (96/161; 59.6%), cefotaxime (88/161; 54.7%), and tobramycin (86/161; 53.4%).

Intermediate resistance rates

Resistance rates between 15% and 50% were recorded for cefepime (76/161; 47.2%), levofloxacin (71/161; 44.1%), cephalothin (42/161; 26.1%), nalidixic acid (41/161; 25.5%), ceftazidime (40/161; 24.8%), cefuroxime (35/161; 21.7%), colistin (32/161; 19.9%), ticarcillin-clavulanate (27/161; 16.8%), and chloramphenicol (26/161; 16.1%).

Lowest resistance rates: The lowest resistance rates were observed for ceftaroline (1/161; 0.6%), nitrofurantoin (4/161; 2.5%), imipenem (6/161; 3.7%), nitrofurantoin (6/161; 3.7%), cefixime (6/161; 3.7%), meropenem (7/161; 4.3%), amikacin (10/161; 6.2%), ertapenem (16/161; 9.9%), pefloxacin (16/161; 9.9%), piperacillin-tazobactam (17/161; 10.6%; intermediate: 1/161; 0.6%), and fosfomycin (19/161; 11.8%).

Genotype–phenotype correlation

Among the 15 genes–antibiotics pairs evaluated across all 161 isolates (Fig. 2B), only the *bla*_{OXA-48}–ertapenem pair reached statistical significance: 30.0% of *bla*_{OXA-48}-positive isolates were ertapenem-resistant compared with 4.7% of non-carriers ($p < 0.05$, Fisher's exact test). All remaining 14 pairs were non-significant, including *bla*_{NDM}–imipenem (0.0% vs 2.2%), *bla*_{KPC}–meropenem (0.0% vs 4.9%), *aac(6')-Ib-cr*–gentamicin (77.1% vs 70.6%), *aac(6')-Ib-cr*–tobramycin (43.8% vs 54.4%), *mcr-1*–colistin (5.6% vs 22.4%), *qnrA*–ciprofloxacin (73.4% vs 59.5%), *qnrB*–ciprofloxacin (77.4% vs 61.9%), *sul1*–TMP-SXT (80.8% vs 85.9%), *tetA*–tetracycline (13.2% vs 16.7%), *tetB*–tetracycline (20.6% vs 13.4%), *bla*_{CTX-M}–ceftazidime (67.7% vs 75.9%), *bla*_{CTX-M}–ceftriaxone (80.6% vs 81.5%), *bla*_{SHV}–ceftriaxone (84.0% vs 62.5%), and *bla*_{TEM}–ceftriaxone (87.8% vs 77.3%).

Table 2
Socio-demographic, clinical, and multi-drug resistance characteristics of patients with ESBL-producing and carbapenem-resistant *Klebsiella* spp. infections ($n = 161$)

Characteristic	Category	<i>n</i> (%)
Resistance phenotype	ESBL-producing	153 (95.0%)
	Carbapenem-resistant	8 (5.0%)
Sex	Male	90 (55.9%)
	Female	71 (44.1%)
Specimen type	Urine	126 (78.3%)
	Wound/Pus	35 (21.7%)
Age group (years)	0–15	37 (23.0%)
	16–30	15 (9.3%)
	31–45	23 (14.3%)
	46–60	25 (15.5%)
	> 60	60 (37.3%)
Median age, years (IQR)		48.5 (17.5–65.0)
MDR/XDR/PDR	MDR	160 (99.4%)
	XDR	1 (0.6%)
	PDR	0 (0.0%)
<i>n</i> : number of strains; (%): percentage of <i>n</i>		

Table 3
Distribution of ESBL-producing and carbapenem-resistant *Klebsiella* spp. by species and specimen type ($n = 161$)

Species	Urine, <i>n</i> (%)	Wound/Pus, <i>n</i> (%)	Total
<i>K. pneumoniae</i> subsp. <i>pneumoniae</i>	98 (77.2%)	29 (22.8%)	127
<i>K. oxytoca</i>	16 (80.0%)	4 (20.0%)	20
<i>K. pneumoniae</i> subsp. <i>ozaenae</i>	11 (91.7%)	1 (8.3%)	12
<i>K. pneumoniae</i> subsp. <i>rhinoscleromatis</i>	1 (50.0%)	1 (50.0%)	2
Total	126 (78.3%)	35 (21.7%)	161
<i>n</i> : number of strains; (%): percentage of <i>n</i>			

Table 4
ESBL gene combination profiles (ESBL-producing isolates, $n = 153$)

Gene combination profile	n (%)
None of the three genes detected	44 (28.8%)
<i>bla_{SHV}</i> alone	41 (26.8%)
<i>bla_{CTX-M}</i> + <i>bla_{TEM}</i> + <i>bla_{SHV}</i>	34 (22.2%)
<i>bla_{CTX-M}</i> + <i>bla_{SHV}</i>	18 (11.8%)
<i>bla_{CTX-M}</i> alone	9 (5.9%)
<i>bla_{TEM}</i> + <i>bla_{SHV}</i>	5 (3.3%)
<i>bla_{CTX-M}</i> + <i>bla_{TEM}</i>	1 (0.7%)
<i>bla_{TEM}</i> alone	1 (0.7%)
n : number of strains; (%): percentage of n	

Discussion

A near-universal MDR burden, precisely quantified

The formal application of the 13-antibiotic-class MDR/XDR/PDR framework to this collection revealed that 99.4% of isolates (160/161) met the criteria for multi-drug resistance (Table 2). While this figure is consistent with the inclusion of ESBL-producing and carbapenem-resistant phenotype, its quantification within a standardized 13-class framework enables direct comparison with published cohorts and avoids the heterogeneous MDR designations prevalent in the regional literature [23, 36–38]. Only a single isolate (0.6%) reached XDR status, and none reached PDR, indicating that a combination regimen typically a carbapenem with amikacin, with or without fosfomycin remains theoretically active against the majority of isolates in this collection [23, 39].

The bimodal age distribution, dominated by patients older than 60 years (37.3%) and children aged 0–15 years (23.0%), mirrors the recognized vulnerability spectrum for invasive *Klebsiella* infections. Elderly patients face disproportionate exposure through indwelling urinary catheters, recurrent hospitalization, and cumulative antibiotic pressure, while pediatric patients in resource-limited settings are at risk through neonatal and pediatric ward environments with constrained infection control capacity [6, 40, 41]. The predominance of urinary specimens (78.3%) is consistent with the established role of ESBL-producing *Klebsiella* spp. as a leading cause of complicated urinary tract infections in Africa [5, 42].

Species epidemiology: known dominance, unexpected niches

The dominance of *K. pneumoniae* subsp. *pneumoniae* (78.9%) reproduces the global pattern in which this subspecies represents the principal vehicle for ESBL and carbapenemase gene dissemination among *Enterobacterales*, owing to its capacity for acquiring and stably maintaining large conjugative plasmids [6, 38]. The substantial representation of *K. pneumoniae* subsp. *ozaenae* (7.5%) and its striking urinary predominance (91.7%) is, however, unexpected. Historically associated with chronic rhinological infection rather than urinary pathology, the predominantly urinary recovery of this subspecies in a multicenter Togolese collection raises the possibility of an under-recognized urinary niche for *K. pneumoniae* subsp. *ozaenae* in West African healthcare settings a finding that, to our knowledge, has not been reported at this scale and merits dedicated prospective investigation [17, 43]. The detection of two *K. pneumoniae* subsp. *rhinoscleromatis* isolates further illustrates the diagnostic depth achievable through a multi-center network with centralized confirmatory identification [17, 18].

The ESBL gene landscape: chromosomal ambiguity and plasmid convergence

The 62.1% prevalence of *bla*_{SHV} the most frequently detected gene in this study must be interpreted in the context of *Klebsiella* genomic biology. *bla*_{SHV} exists as an intrinsic chromosomal gene in virtually all *K. pneumoniae* strains, and primers targeting conserved gene regions cannot discriminate the wild-type *bla*_{SHV-1} allele from ESBL-conferring point-mutant variants (*bla*_{SHV-5}, *bla*_{SHV-12}) [7, 37, 44]. The 62.1% figure therefore likely overestimates the true ESBL-type *bla*_{SHV} prevalence and should be treated as an upper bound pending allele-level sequencing. In contrast, *bla*_{CTX-M} (38.5%) and *bla*_{TEM} (25.5%) are unambiguously acquired determinants.

Gene combination analysis (Table 3) revealed that the triple-gene combination *bla*_{CTX-M}+*bla*_{TEM}+*bla*_{SHV}, identified in 34 isolates (22.2%), was the most frequent genuinely acquired multi-gene profile. This finding is consistent with the co-mobilisation of multiple β -lactamase genes on IncF-type conjugative plasmids that dominate the *K. pneumoniae* ESBL plasmidome across Africa [4, 11, 38]. Notably, 44 isolates (28.8%) were phenotypically confirmed as ESBL-producing but harboured none of the three targeted genes. This gap most plausibly reflects divergent *bla*_{CTX-M} allele groups (groups 2, 9, or 25) not captured by the primers used, alternative plasmid-mediated β -lactamases (*bla*_{CMY}, *bla*_{DHA}, *bla*_{PER}), or unconfirmed *bla*_{SHV} ESBL-variant alleles [5, 45, 46]. This subgroup constitutes the primary target for future whole-genome sequencing in this collection.

A silent carbapenemase reservoir within the ESBL population

The detection of *bla*_{NDM} (14.3%), *bla*_{KPC} (8.1%), *bla*_{OXA-48} (6.2%), and *bla*_{VIM} (0.6%) almost exclusively among phenotypically ESBL-classified isolates strains that routine laboratories would report as carbapenem-susceptible is among the most clinically relevant findings of this study. The *bla*_{NDM} prevalence of 14.3% exceeds the pooled sub-Saharan African estimate of 12.4% from a recent systematic review [8], indicating that *bla*_{NDM} dissemination in Togo is neither rare nor isolated. The detection of *bla*_{KPC} (8.1%) is equally noteworthy, as KPC-type carbapenemases are associated with globally disseminated high-risk *K. pneumoniae* clones particularly ST258, ST307, and ST11 that have driven carbapenem-resistant outbreaks across multiple continents [6, 10, 23]. Their presence within phenotypically ESBL-classified isolates suggests that genetic prerequisites for transition toward XDR *K. pneumoniae* are already established in the Togolese hospital environment, even though full phenotypic expression has not yet occurred in the majority of carriers.

Spearman correlation analysis (Fig. 1B) further sharpened this concern. The strong positive correlation between *bla*_{KPC} and *mcr-1* ($\rho = 0.75$) indicates that isolates carrying carbapenemase genes in this collection are substantially more likely to simultaneously carry the plasmid-mediated colistin resistance gene *mcr-1* that is, resistance to both last-resort antibiotic classes tends to co-occur in the same strains. While this association has been reported from clinical collections in Asia, Europe, and South America, it has been rarely described in West African clinical settings [13, 42]. Its detection here, in a multi-center Togolese clinical series, suggests that some of the structural conditions favouring the emergence of pan-drug-resistant *K. pneumoniae* emergence co-localization of carbapenemase and *mcr* genes on shared mobile elements under intense antibiotic selection pressure are already in place, even in the absence of observed PDR phenotype (Table 2).

The strong correlation between *bla*_{CTX-M} and *aac*(6')-Ib-cr ($\rho = 0.75$), together with the broader cluster comprising *bla*_{TEM}, *qnrB*, *bla*_{CTX-M}, and *aac*(6')-Ib-cr ($\rho = 0.43-0.59$), is consistent with the co-localization of β -lactam, fluoroquinolone-protective, and aminoglycoside-modifying genes within the same resistance regions on IncF-type plasmids of *K. pneumoniae* high-risk clones [4, 11, 43]. The *sul1-dfA1* correlation ($\rho = 0.41$) reflects their canonical co-localization in the 3' conserved segment of class 1 integrons, jointly explaining the 85.1% phenotypic TMP-SXT resistance documented in Fig. 2A [47, 48]. The absence of *sul2* (0/161) confirms that sulfonamide resistance in this collection is exclusively *sul1*-mediated, in contrast to the *sul2*-dominant patterns frequently reported in veterinary and environmental *Enterobacterales* from the region [14]. Finally, the negative correlations between *bla*_{SHV} and both *mcr-1* ($\rho = -0.58$) and *bla*_{KPC} ($\rho = -0.33$) suggest the co-existence of two epidemiologically distinct sub-populations within this collection: one characterized by chromosomal *bla*_{SHV} dominance with a comparatively limited acquired-resistance burden, and a second harbouring extensive plasmid-mediated multidrug resistance including *bla*_{KPC}, *mcr-1*, *bla*_{CTX-M}, and *aac*(6')-Ib-cr. Resolving this bipolarisation through multi-locus sequence typing or whole-genome sequencing represents a priority for future work [6, 49].

Species-level heterogeneity in resistance gene carriage

Klebsiella pneumoniae subsp. *pneumoniae* harboured the broadest resistance gene repertoire, consistent with its broader plasmid host range (Fig. 1C) [6, 38]. The elevated *bla*_{CTX-M} (50%) and *qnrA* (58%) prevalence observed in *K. pneumoniae* subsp. *ozaenae* despite representing only 7.5% of the collection is unexpected and, considered alongside its predominant urinary recovery (Table 3), raises the possibility that this subspecies is acquiring resistance plasmids through ecological niches shared with *K. pneumoniae* subsp. *pneumoniae*. Given the very limited prior molecular characterization of *K. pneumoniae* subsp. *ozaenae* resistomes in the literature, this observation adds novel exploratory data to the field, although the modest sample size (n = 12) warrants confirmation in larger cohorts [17, 43].

Phenotypic resistance profiles and preserved therapeutic options

The phenotypic susceptibility data (Fig. 2A) translate molecular findings into clinical terms. TMP-SXT resistance (85.1%) was the highest recorded across all 36 antibiotics exceeding even ceftriaxone (81.4%) and is mechanistically anchored by the *sul1-dfA1* co-carriage documented in Fig. 1A and Fig. 1B (32.3% and 24.8%, respectively). Given that TMP-SXT remains among the most prescribed and lowest-cost oral agents for urinary tract infection in Togo, this finding has direct implications for empirical prescribing guidelines [47, 50].

Within the aminoglycoside class, gentamicin (72.7%) and tobramycin (53.4%) resistance contrasted sharply with amikacin resistance (6.2%). This differential is consistent with the substrate specificity of *aac*(6')-*lb-cr*-type acetyltransferases (29.8% prevalence; Fig. 1A), which preferentially modify gentamicin and tobramycin while sparing amikacin in most allelic variants [51, 52]. With 93.8% of isolates remaining amikacin-susceptible, this agent is among the most robust preserved options for combination therapy of severe ESBL *Klebsiella* infections [51].

Carbapenem resistance rates remained low imipenem (3.7%), meropenem (4.3%), ertapenem (9.9%) but must be interpreted alongside the molecular data: 14.3%, 8.1%, and 6.2% of isolates silently carry *bla*_{NDM}, *bla*_{KPC}, and *bla*_{OXA-48}, respectively (Fig. 1A). The colistin phenotypic resistance rate (19.9%) considerably exceeds the *mcr-1* prevalence (11.2%), indicating that approximately 14 isolates harbour colistin resistance through alternative mechanisms most plausibly chromosomal modifications of the *mgrB*, *pmrAB*, or *phoPQ* regulatory systems [53, 54].

Nitrofurantoin (3.7% resistant) and nitrofuran (2.5% resistant) retained near-complete activity. Given the predominantly urinary origin of this collection (78.3%), nitrofurantoin represents a viable, low-cost, orally available option for uncomplicated ESBL *Klebsiella* urinary tract infections [46, 50]. Fosfomycin (11.8% resistance) similarly emerges as a credible alternative for lower urinary tract infections, consistent with its retained activity against ESBL *Enterobacterales* in recent series [44, 46].

Genotype–phenotype correlation: resolving apparent paradoxes

Among the 15 gene–antibiotic pairs evaluated, only the *bla*_{OXA-48}–ertapenem association reached statistical significance (30.0% vs 4.7%; *p* < 0.05, Fisher's exact test) (Fig. 2B). This confirms the preferential hydrolytic activity of OXA-48-type carbapenemases against ertapenem relative to imipenem and meropenem, a well-documented enzymatic property attributable to the structural features of ertapenem's side chain [8, 55]. The practical implication is direct: laboratories relying solely on imipenem or meropenem for carbapenem screening will systematically miss *bla*_{OXA-48}-producing isolates. Inclusion of ertapenem in routine carbapenem susceptibility panels is therefore essential [8, 55].

The absence of phenotypic carbapenem resistance in *bla*_{NDM}-positive (0.0% vs 2.2% for imipenem) and *bla*_{KPC}-positive isolates (0.0% vs 4.9% for meropenem) is consistent with the phenomenon of silent carbapenemase carriage, in which gene presence does not translate into elevated MIC under standard disk diffusion conditions [23, 32]. Such isolates are clinically invisible to phenotypic surveillance yet carry transferable resistance determinants; subsequent porin loss, efflux pump up regulation, or exposure to antibiotic selection pressure could unmask full carbapenem resistance in the same or a recipient strain [23, 32, 53].

The non-significant associations between ESBL genes and cephalosporin resistance reflect a statistical ceiling effect: with baseline ceftriaxone resistance already at 81.4% across the entire cohort (Fig. 2A), and 28.8% of gene-negative isolates already phenotypically ESBL-positive through undetected mechanisms (Table 4), discriminatory power between gene-positive and gene-negative groups is intrinsically limited. Larger sample sizes or a broader resistance gene panel would be required in future studies to overcome this constraint [46].

The inverse relationship between *mcr-1* carriage and phenotypic colistin resistance (5.6% in carriers vs 22.4% in non-carriers) reflects two recognized phenomena: the modest MIC elevation conferred by *mcr-1* alone (4- to 8-fold), which frequently falls below disk diffusion breakpoints [53, 56]; and the likely dominance of chromosomal resistance mechanisms (*mgrB*, *phoPQ*, and *pmrAB* mutations) as primary drivers of phenotypic colistin resistance in this collection [53, 54]. This dissociation reinforces the recommendation that colistin susceptibility be assessed by broth micro-dilution MIC in reference settings, irrespective of *mcr* gene status [56].

Regional and global context

The resistance gene profile documented here is broadly consistent with, yet in several respects exceeds, recent regional benchmarks. The *bla*_{CTX-M} prevalence (38.5%) falls toward the lower end of the 45–60% range reported from Ghana, Nigeria, and Cameroon [11, 15, 45]. The *qnrA* prevalence (49.1%) substantially exceeds the pooled West African PMQR estimate of 20–40% [11], suggesting intense fluoroquinolone selection pressure in the Togolese clinical environment. The *bla*_{NDM} prevalence (14.3%) places Togo at or above the most recent pooled sub-Saharan African estimate (12.4%) [8]. The *bla*_{KPC}–*mcr-1* co-occurrence ($p = 0.75$; Fig. 1B) represents one of the earliest such reports from a multicenter West African clinical collection [13, 42]. These comparisons position Togo within a regional trajectory of increasingly complex, multi-gene, multi-class resistance that is already well advanced.

Implications for clinical practice and public health policy

Three recommendations follow directly from these findings. First, ertapenem should be incorporated as a mandatory component of carbapenem susceptibility panels for *Klebsiella* spp. in Togolese laboratories, given its unique sensitivity for detecting *bla*_{OXA-48} producers (Fig. 2B) [8, 55]. Second, molecular screening for *bla*_{NDM}, *bla*_{KPC}, and *bla*_{OXA-48} should be considered for ESBL-confirmed isolates from intensive care and surgical wards, to identify the silent carbapenemase reservoir before phenotypic emergence [23, 32]. Third, empirical treatment guidelines should be revised to reflect the preserved activity of amikacin, carbapenems, fosfomycin, and nitrofurantoin for urinary indications, while de-emphasising TMP-SXT, ceftriaxone, ciprofloxacin, and gentamicin pending confirmatory susceptibility data [46, 50]. At the policy level, these findings provide a multicenter baseline to inform Togo's national AMR action plan and to contribute molecular surveillance data to the WHO GLASS platform [2, 3].

Limitations and Perspectives

This study has several methodological limitations that should be considered when interpreting the findings. First, its cross-sectional design provides only a snapshot of the resistance landscape and does not permit the evaluation of temporal changes in resistance gene prevalence or the investigation of clonal transmission dynamics among the sixteen participating healthcare facilities. Second, although the real-time PCR assay targeted eighteen clinically relevant resistance genes, it was unable to discriminate between wild-type and ESBL-producing variants of *bla*_{SHV}, since both are naturally encoded in the chromosome of *Klebsiella* spp. As a result, the observed prevalence of *bla*_{SHV} may overestimate the actual burden of ESBL-associated variants. Furthermore, the assay did not include several alternative β -lactamase families, such as *bla*_{CMY}, *bla*_{DHA}, and *bla*_{PER}, nor all divergent *bla*_{CTX-M} allele groups, which may partly explain the absence of targeted genes in a proportion of phenotypically ESBL-positive isolates. Third, the Δ Ct-based threshold used for gene detection generated qualitative results only and did not allow differentiation between plasmid-mediated and chromosomally integrated resistance determinants, nor did it provide information on gene expression levels. In addition, the limited number of carbapenem-resistant isolates and the very small representation of certain species restricted the statistical power of subgroup analyses and may have hindered the identification of significant genotype–phenotype associations. Finally, the lack of linked clinical information, including prior antibiotic exposure, duration of hospitalization, and patient outcomes, limited the clinical interpretation of the resistance profiles identified in this study.

Despite these limitations, the findings provide a strong foundation for future investigations. Whole-genome sequencing of clinically important isolates, particularly those co-harboring *bla*_{KPC} and *mcr-1*, carrying *bla*_{OXA-48}, or belonging to the genotype-negative ESBL subgroup, would help resolve several molecular uncertainties by enabling allele-level characterization of resistance genes, plasmid replicon typing, and clonal lineage determination through multilocus sequence typing. Expanding

surveillance within a One Health framework to include isolates from animals, food products, and hospital wastewater would also improve understanding of the circulation and potential transmission pathways of *mcr-1* and carbapenemase genes across human and non-human reservoirs in Togo. Furthermore, longitudinal surveillance studies conducted at regular intervals would provide valuable insight into the evolution and dissemination of resistance determinants over time, particularly the emergence of silent carbapenemase reservoirs that may not yet be phenotypically expressed. Finally, the integration of these findings into antimicrobial stewardship programs, including the development of updated empirical treatment guidelines and routine molecular screening strategies for carbapenemase-producing isolates, should be implemented and evaluated across healthcare facilities to strengthen national efforts against antimicrobial resistance.

Conclusion

This multi-center cross-sectional study provides the first integrated molecular and phenotypic characterization of ESBL-producing and carbapenem-resistant *Klebsiella* spp. clinical isolates from Togo, encompassing 161 isolates from 16 healthcare facilities, 36 antibiotics across 13 classes, and 18 resistance genes spanning six functional categories. The central findings are threefold. First, multi-drug resistance was near-universal, yet pan-drug resistance was absent, defining a precise and currently navigable but rapidly narrowing therapeutic window. Second, carbapenemase genes were detected almost exclusively within phenotypically ESBL-classified, carbapenem-susceptible isolates, constituting a clinically silent but genetically transferable reservoir, with *bla*_{KPC} showing a striking co-occurrence with the colistin resistance gene *mcr-1*. Third, the sole statistically significant genotype–phenotype correlation *bla*_{OXA-48} with ertapenem resistance carries an immediate, actionable diagnostic implication for carbapenem susceptibility panel design. Amikacin, carbapenems, fosfomycin, and nitrofurantoin emerged as the most reliably preserved therapeutic options, while TMP-SXT, ceftriaxone, ceftazidime, gentamicin, and ciprofloxacin are no longer suitable for empirical use against this pathogen population in Togo. These findings provide a robust, quantitative evidence base to inform the revision of national empirical treatment guidelines, to justify the systematic introduction of carbapenemase gene screening in reference laboratories, and to support Togo's integration into regional and global molecular AMR surveillance frameworks, including WHO GLASS. Future whole-genome sequencing of the priority subgroups identified here, combined with One Health sampling and longitudinal surveillance, will be essential to fully resolve the genomic epidemiology underlying this resistance landscape and to anticipate its further evolution.

Abbreviations

aac(6)-Ib-cr: Aminoglycoside acetyltransferase gene, type 6'-Ib

AMR: Antimicrobial resistance

API 20E: Analytical Profile Index 20 Enterobacteriaceae

AST: Antimicrobial susceptibility testing

ATBEF: Togolese Association for Family Welfare

ATCC: American Type Culture Collection

*bla*_{CTX-M} (or *bla*_{CTX-X}): Gene encoding CTX-M-type extended-spectrum β-lactamase

*bla*_{KPC}: Gene encoding *Klebsiella pneumoniae* carbapenemase

*bla*_{NDM}: Gene encoding New Delhi metallo-β-lactamase

*bla*_{OXA-48}: Gene encoding OXA-48-type carbapenemase

*bla*_{SHV}: Gene encoding sulfhydryl variable β-lactamase

*bla*_{TEM}: Gene encoding Temoniera β-lactamase

*bla*_{VIM}: Gene encoding Verona integron-encoded metallo-β-lactamase

CIBM: Centro de Investigación Biomédica de Granada

CLSI: Clinical and Laboratory Standards Institute

Ct: Cycle threshold

DDST: Double-disk synergy test

DNA: Deoxyribonucleic acid

ESBL: Extended-Spectrum β-Lactamase

GLASS: Global Antimicrobial Resistance and Use Surveillance System

IncFIIk : Incompatibility group FIIk plasmid replicon type

IQR: Interquartile range

KPC: *Klebsiella pneumoniae* carbapenemase

MDR: Multidrug-resistant

MIC: Minimum inhibitory concentration

MIQE: Minimum Information for Publication of Quantitative Real-Time PCR Experiments

MLST: Multilocus sequence typing

NDM: New Delhi metallo-β-lactamase

OXA-48: Oxacillinase-48-type carbapenemase

PCR: Polymerase chain reaction

PDR: Pan-drug-resistant

PMQR: Plasmid-mediated quinolone resistance

qnr: Plasmid-mediated quinolone resistance gene

qPCR: Quantitative real-time polymerase chain reaction

rRNA: Ribosomal ribonucleic acid

ST: Sequence type

sul: Sulfonamide resistance gene (e.g., *sul1*, *sul2*, *sul3*)

TMP-SXT: Trimethoprim-sulfamethoxazole

UTIs: Urinary Tract Infections

VIM: Verona integron-encoded metallo-β-lactamase

VITEK: Automated microbial identification and antimicrobial susceptibility testing system

WGS: Whole-genome sequencing

WHO: World Health Organization

XDR: Extensively drug-resistant

ΔCt : Delta cycle threshold ($Ct[\text{target}] - Ct[16S \text{ rRNA}]$)

Declarations

Ethical considerations

This study was approved by the National Committee for Health Research Ethics of Togo (reference no. 036/2024/CRBS, dated 4 July 2024). Written informed consent was obtained from all participants or their legal guardians prior to sample collection.

Clinical data were handled with strict confidentiality and anonymization in accordance with established ethical principles and relevant regulations. All samples were collected as part of routine clinical care, and all necessary administrative authorizations were obtained from each participating laboratory facility prior to sample collection and data utilization.

In accordance with the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization, authorization was obtained from the Ministry of Environment and Forest Resources of Togo for the international transfer of bacterial isolates for research purposes (Ministerial Order No. 014/MEFR/SG/DRF, issued on 13 March 2025).

All procedures were conducted in accordance with the principles of the Declaration of Helsinki and relevant national guidelines.

Clinical Trial

Not applicable.

Consent for publication

Not Applicable.

Availability of data and material

The raw data generated and/or analyzed in the current study are publicly available in an open-access repository at Figshare (DOI:10.6084/m9.figshare.33368206).

Competing interests

The authors declare that they have no conflict of interests.

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Author contributions

Tchilabalo Bouyo and Komi Koukoura Komi conceived and designed the study. Tchilabalo Bouyo and Luckman Gbati collected and curated the laboratory and molecular data. Tchilabalo Bouyo, Jorge García-García performed the formal statistical analysis and generated all figures and tables. Tchilabalo Bouyo and Sandrine Tene Salifou contributed to sample collection and processing. Tchilabalo Bouyo, Luckman Gbati, Jorge García-García, Alba Rodriguez Nogales and Komi Koukoura Komi wrote the original draft of the manuscript. Alba Rodriguez Nogales, Komi Koukoura Komi and Tchadjobo Tchacondo supervised the study and critically reviewed and edited the manuscript. All authors read, critically revised, and approved the final version of the manuscript.

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Figures

Figure 1. Molecular epidemiology of antimicrobial resistance genes
Klebsiella spp. ESBL and carbapenem-resistant isolates (N = 161)

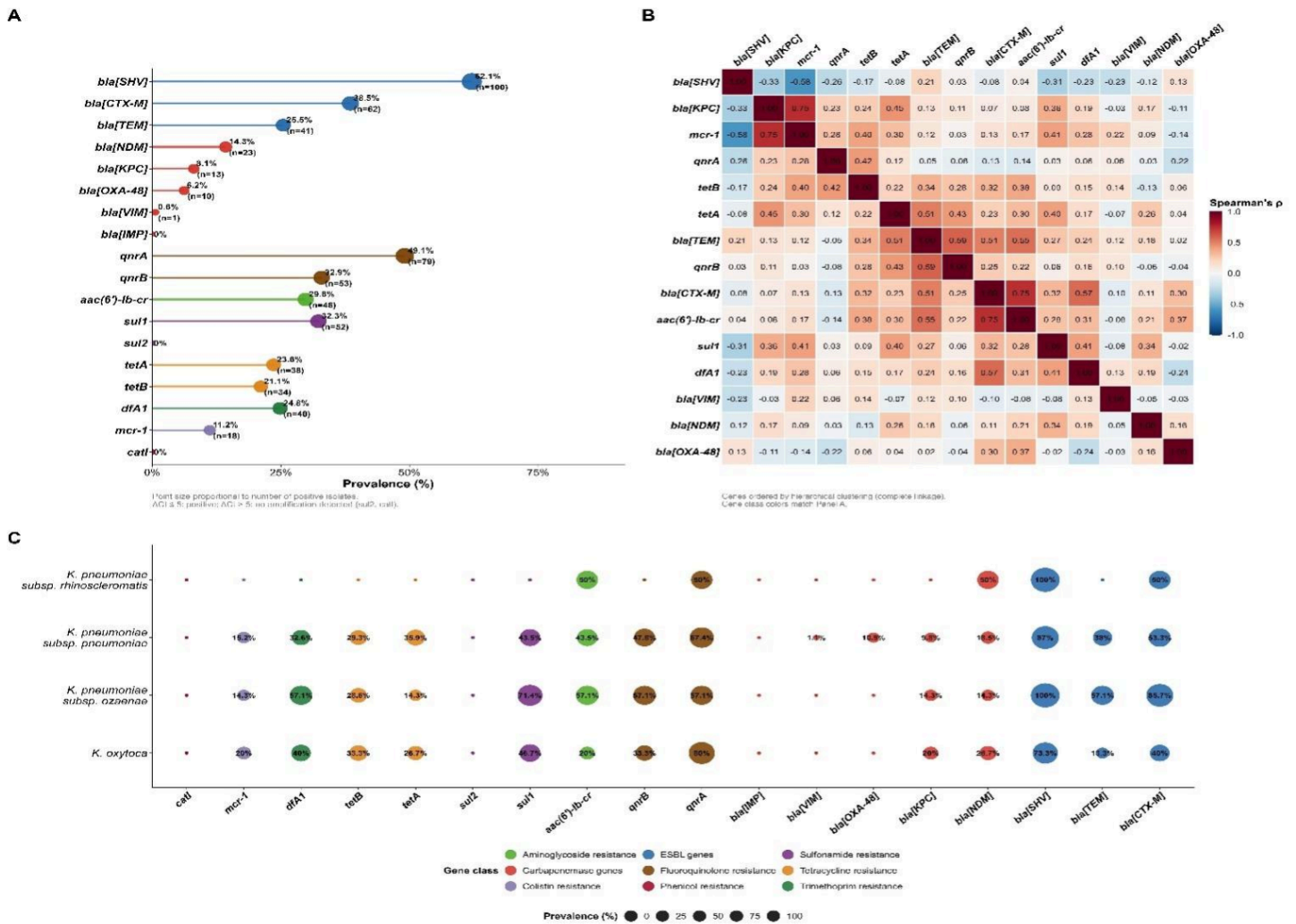


Figure 2. Phenotypic resistance and genotype–phenotype correlation
Klebsiella spp. ESBL and carbapenem-resistant isolates (N = 161)

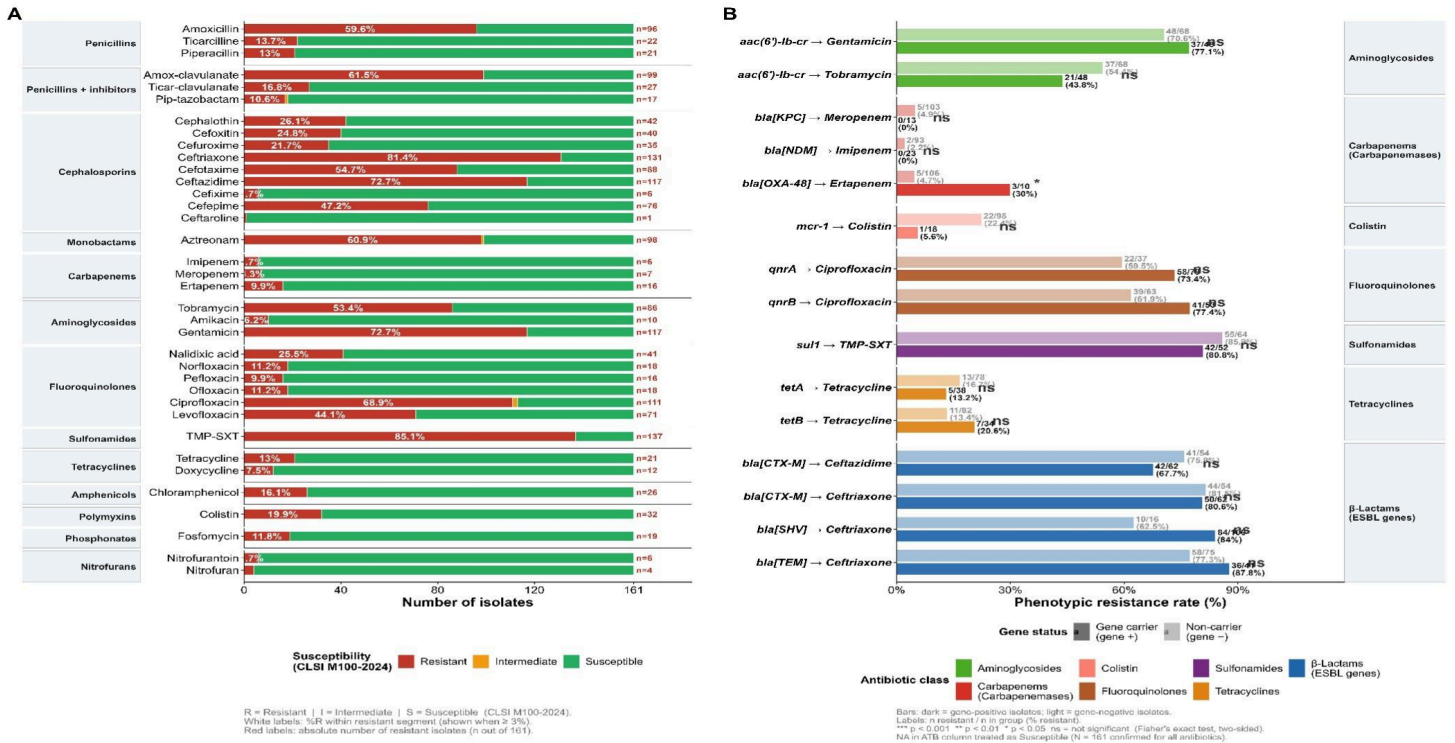


Figure 2

Phenotypic resistance (A) and genotype-phenotype correlation