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Original Research

Prevalence and Molecular Characterization of β-Lactamase and Quinolone Resistance Genes in Imipenem-Non-Susceptible Uropathogenic *Escherichia coli* (UPEC) Isolates

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Abstract

Background: *Escherichia coli* is the leading cause of urinary tract infections (UTIs), and the increasing prevalence of antimicrobial resistance represents a major public health concern. The dissemination of multidrug-resistant uropathogenic *E. coli* (UPEC), frequently harboring transferable resistance determinants, poses an urgent clinical challenge. **Methods**: This study investigated the prevalence of β-lactamase genes (*blaTEM*, *blaSHV*, *blaCTX-M*, *blaCMY*, and *blaDHA*) and plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) in 86 imipenem-non-susceptible UPEC isolates using multiplex and single PCR assays. Gene distribution and co-occurrence were examined across *E. coli* phylogenetic groups, and pairwise associations were evaluated using correlation analysis. Principal component analysis (PCA) was applied to explore global relationships between antibiotic susceptibility profiles, extended-spectrum β-lactamase (ESBL) phenotype, and resistance determinants. **Results**: Overall, 74.4% of imipenem-non-susceptible isolates carried at least one β-lactamase gene. *blaTEM* was the most prevalent (62.8%), followed by *blaCMY II* (12.8%). *blaSHV*, *blaCTX-M* group I, and *blaCTX-M* group II showed comparable prevalence (10.5% each). The B2 phylogroup showed the greatest diversity of β-lactamase profiles, with phylogroup E representing the second most frequent reservoir. Among quinolone resistance genes, *qnrB* was the most prevalent (20.9%), followed by *qnrD* (5.8%), *qnrS* (4.7%), *qnrA* (3.5%), and *qnrC* (1.2%). All *qnrC*-positive isolates were resistant to all tested quinolones. No statistically significant associations were observed between β-lactamase genes and *qnr* genes. Significant within-class correlations were detected for *blaCTX-M* group II–*blaCMY II* ($\varphi = 0.893$, $q = 9.33 \times 10^{-9}$) and *qnrC*–*qnrA* ($\varphi = 0.57$, $q = 0.374$). **Conclusions**: A high prevalence of β-lactamase and *qnr* determinants was observed among imipenem-non-susceptible UPEC, primarily driven by *blaTEM* and *qnrB*, frequently detected in B2 isolates. The co-occurrence and correlation of multiple resistance genes highlight the complexity of resistance architectures and underscore the need for ongoing molecular surveillance and strengthened antimicrobial stewardship to limit the dissemination of resistant UPEC.

Keywords: uropathogenic *Escherichia coli* (UPEC); urinary tract infection (UTI); antimicrobial resistance; β-lactamase genes; extended-spectrum β-lactamases (ESBL); plasmid-mediated quinolone resistance (PMQR); phylogroups; public health

1. Introduction

Escherichia coli, a member of the *Enterobacteriaceae* family, is one of the most prevalent gastrointestinal commensals in humans and a major cause of both community- and hospital-acquired infections, particularly urinary tract infections (UTIs) [1,2]. Uropathogenic *E. coli* (UPEC), within the broader extraintestinal pathogenic *E. coli* (ExPEC) pathotype, is the leading etiological agent of UTIs and can also contribute to severe invasive infections, such as bloodstream infections and, less frequently, meningitis. Its pathogenicity is driven by a wide repertoire of virulence determinants, such as adhesins, toxins, siderophores, capsules, and biofilm-associated traits, often occurring alongside multidrug resistance, which enhances persistence under antimicrobial pressure [3].

Several antimicrobial agents are routinely used for UTI treatment, including β-lactams (penicillins, β-lactam/β-lactamase inhibitor combinations, and cephalosporins), fosfomycin, trimethoprim/sulfamethoxazole, nitrofurantoin, quinolones, and fluoroquinolones [4,5]. However, extensive and frequently inappropriate antimicrobial use has accelerated the emergence and spread of resistant UPEC, complicating management, limiting therapeutic options, and representing a major public health concern.

Antimicrobial resistance (AMR) is increasingly recognized as a major global health emergency, with recent global syntheses highlighting a substantial and rising burden across multiple infection syndromes. In particular, recent systematic analyses have quantified the worldwide

burden of bacterial AMR and provided forecasts for future trends, emphasizing the urgent need for strengthened surveillance and stewardship interventions [4]. In parallel, the WHO GLASS reports provide updated global surveillance data on resistance prevalence and trends, including urinary tract infections, supporting the relevance of locally generated datasets for guiding empiric therapy and containment strategies [5].

Resistance to β-lactams is commonly mediated by the production of β-lactamases, including those encoded by the *blaTEM*, *blaSHV*, and *blaCTX-M* gene families, as well as plasmid-borne AmpC β-lactamase genes, such as *blaCMY* and *blaDHA* [4,6]. Quinolones and fluoroquinolones are often used as alternatives when β-lactam resistance is present, yet increasing resistance to these agents has been reported worldwide [7]. Quinolone resistance may arise through chromosomal mutations in target genes, efflux pump overexpression, and plasmid-mediated quinolone resistance (PMQR) mechanisms, including *qnr* genes [8]. The dissemination of *E. coli* strains co-harboring β-lactamase and PMQR determinants is associated with treatment failures, increased healthcare burden, and poorer clinical outcomes [9]. In Algeria, resistant UPEC strains are increasingly reported in both community and hospital settings, further complicating empirical therapy for UTIs [10–14].

Beyond resistance profiling, investigating the distribution of resistance determinants across *E. coli* phylogenetic groups can provide insights into the epidemiology and dissemination of resistant lineages. In parallel, phenotypic identification of extended-spectrum β-lactamase (ESBL)- and AmpC-producing isolates remains essential to confirm functional resistance and inform antibiotic selection. Because resistance traits frequently cluster and co-vary, this study aimed to characterize antimicrobial resistance patterns and to investigate the molecular basis of resistance by screening for major β-lactamase genes (*blaTEM*, *blaSHV*, *blaCTX-M*, *blaCMY*, and *blaDHA*) and PMQR determinants (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) in UPEC isolates recovered from urine samples of UTI patients, categorized as imipenem non-susceptible by disk diffusion according to CLSI criteria, in the Setif region of northeastern Algeria. In addition, this report evaluated gene co-occurrence and distribution across *E. coli* phylogenetic groups and applied multivariate analysis to explore overall associations between antibiotic susceptibility patterns and resistance determinants, including ESBL production.

2. Materials and Methods

2.1 Study Overview

This study forms part of a broader investigation into the prevalence of clinically relevant antibiotic-resistant bacteria in the Setif region, with a focus on elucidating molecular resistance mechanisms. The study was approved by the Ethics and Deontology Committee at University Ferhat Abbas of Setif 1 under the number UFAS1/09/03/2023/ETH-Deon-A-301, and an informed written consent was taken from each participant.

Between January 2021 and December 2023, a total of 402 bacterial isolates were recovered from urine samples collected across three healthcare areas of the Setif province (east, west, and north). The entire collection was analyzed for imipenem non-susceptibility using the Modified Hodge Test protocol reported by Aminul *et al.* [15] and the imipenem-EDTA combined test protocol reported by Radhika *et al.* [16]. From this analysis, 86 uropathogenic *Escherichia coli* (UPEC) isolates, categorized as imipenem non-susceptible, were selected for detailed phenotypic and molecular characterization. Samples were obtained from patients of both sexes and all age groups, and relevant demographic/clinical data (age, sex, prior antibiotic exposure, and sampling date) were recorded when available.

Urine cultures were processed under aseptic conditions. Only pure cultures yielding $\geq 10^5$ CFU/mL of *E. coli* were included. Isolate purity was verified by subculturing and routine laboratory quality checks.

Isolates were cultured on nutrient agar and MacConkey agar and incubated at 37 °C for 18–24 h. Presumptive identification was based on colony morphology and Gram staining, and *E. coli* identity was confirmed by conventional biochemical testing, including the IMViC panel (indole, methyl red, Voges–Proskauer, citrate utilization) as well as catalase and urease tests, nitrate reduction, motility, triple sugar iron (TSI) reactions, and gas production. Confirmed isolates were stored in nutrient broth supplemented with sterile glycerol at –20 °C until further analyses.

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar, following the guidelines of the European Committee on Antimicrobial Susceptibility Testing [17] as previously described [2].

2.3 Phenotypic Detection of ESBL and AmpC-Producing Strains

Phenotypic ESBL production was evaluated using a standard confirmatory approach as previously described [2], based on the synergy between third-generation cephalosporins and clavulanic acid. Presumptive AmpC β-lactamase production was screened phenotypically among isolates showing reduced susceptibility to cefoxitin (30 µg) in routine disk diffusion testing. In accordance with EUCAST interpretive criteria, a cefoxitin inhibition zone diameter >18 mm is considered susceptible, whereas ≤ 18 mm is interpreted as resistant. Isolates meeting the cefoxitin resistance criterion were classified as cefoxitin-resistant/presumptive AmpC-producing for downstream analyses.

Table 1. Target genes, primer sequences, amplicon sizes, annealing temperatures and reference used for *E. coli* phylogenetic grouping.

Reaction	Target genes	Primer sequences (5′–3′)	Amplicon size (bp)	Tm (°C)	Reference
Quadruplex	*chuA*	F: ATGGTACCGGACGAACCAAC R: GCCGCCAGTACCAAAGACA	288	59	[13]
	yjaA	F: AAACGTGAAGTGTCAGGAG R: AATGCGTTCCTCAACCTGTG	211	59	[14]
	tspE4.C2	F: CACTATTCGTAAGGTCATCC R: AGTTTATCGCTGCGGGTCGC	152	59	[14]
	arpA	F: AACGCTATTCGCCAGCTTGC R: TCTCCCCATACCGTACGCTA	400	59	[14,15]
Group E	*arpA*	F: AACGCTATTCGCCAGCTTGC R: TCTCCCCATACCGTACGCTA	301	57	[16]
Group C	*trpA*	F: AGTTTTATGCCCAGTGCGAG R: TCTGCGCCGGTCACGCCC	219	57	[16]
Internal control	*trpA*	F: GCGATAAAGACATCTTCAC R: GCAACGCGGCCTGGCGGAAG	489	57	[17]

2.4 Phylogenetic Grouping

Phylogenetic grouping of *E. coli* isolates was performed using the Clermont *et al.* [18] multiplex PCR method, as previously described [2]. Primer sequences and PCR conditions, including annealing temperatures, are summarized in Table 1 (Ref. [13–17]), and phylogroups were assigned according to the Clermont classification algorithm. Primers were synthesized by BMR Genomics (Padova, Italy).

2.5 Molecular Assays for Resistance Gene Screening by Multiplex PCR

Total DNA was obtained from pure colonies using the heat-lysis method adapted from Woodman *et al.* (2016) [19]. Briefly, 1–2 colonies from overnight cultures grown on nutrient agar (Oxoid, Milan, Italy) were suspended in 100 µL of sterile water, heated at 99 °C for 15 min, and centrifuged at 10,000 ×g for 10 min. The resulting supernatant was used as PCR template. DNA integrity was assessed by agarose (Sigma-Aldrich, St. Louis, MO, USA) gel electrophoresis, and DNA concentration and purity were measured using a NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). DNA suitability for amplification was verified by PCR targeting the 16S rRNA gene (primers F1/R12) [20].

β-lactamase genes were investigated using two multiplex PCR assays as described by Kim *et al.* (2009) [21]. Multiplex Set 1 targeted *blaCTX-M* group IV, *blaTEM*, *blaOXA*, and *blaSHV*, whereas Multiplex Set 2 targeted *blaCMY II*, *blaCTX-M* group I, *blaCTX-M* group II, and *blaDHA*. Plasmid-mediated quinolone resistance determinants (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) were detected by singleplex PCR. Each PCR reaction was performed in a final volume of 25 µL containing DreamTaq buffer, dNTPs, primers (according to multiplex or singleplex format), DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), and 2 µL of DNA template. Primers were synthesized by BMR Genomics (Padova, Italy).

Cycling conditions consisted of an initial denaturation step followed by 35 cycles of denaturation, primer-specific annealing, and extension, with a final elongation step. Primer sequences, expected amplicon sizes, annealing temperatures, and reference are reported in Table 2 (Ref. [20–22]).

2.6 Biofilm Production

Biofilm formation was quantified using a microtiter-plate crystal violet assay, measuring OD570 after 24 h incubation to classify strains as non-, weak, moderate, or strong biofilm producers based on an OD cut-off [2]. *E. coli* ATCC 25922 served as the control, and all experiments were performed in triplicate.

2.7 Statistical Analysis

Statistical analyses were performed using SPSS Statistics (Armonk, New York, USA (Headquarters) and R (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria; R Project website). Associations between categorical variables were assessed using Fisher's exact test (two-tailed). For gene–phenotype analyses, antimicrobial susceptibility results were dichotomized as non-susceptible (I+R) versus susceptible (S); phenotypic screening variables (ESBL production and cefoxitin-based presumptive AmpC screening) were also treated as binary outcomes. Effect sizes were reported as odds ratios (OR) with 95% confidence intervals. Pairwise associations between resistance genes (presence/absence) were quantified using the phi coefficient (φ), and significance for each gene–gene pair was evaluated using Fisher's exact test. To account for multiple comparisons across pairwise tests, p-values were adjusted using the Benjamini–Hochberg false discov-

Table 2. Target genes, primer sequences, amplicon sizes, annealing temperatures and reference used for screening of the resistance gene in UPEC isolates.

Target gene	Primer Sequence (5′–3′)	Amplicon Size (bp)	Tm (°C)	Reference
16S rDNA	F: GAGTTTGATCCTGGCTCAG R: ACGGCTACCTTGTTACGACT	1402	56	[20]
CTX-M group IV	F: GACAAAGAGAGTGCAACGGATG R: TCAGTGCGATCCAGACGAAA	501	61	[21]
TEM	F: AGTGCTGCCATAACCATGAGTG R: CTGACTCCCCGTCGTGTAGATA	431	61	[21]
OXA	F: ATTATCTACAGCAGCGCCAGTG R: TGCATCCACGTCTTTGGTG	296	61	[21]
SHV	F: GATGAACGCTTTCCCATGATG R: CGCTGTTATCGCTCATGGTAA	214	61	[21]
CMY II	F: AGCGATCCGGTTCACGAAATA R: CCCGTTTTATGCACCCATGA	695	61	[21]
CTX M group I	F: TCCAGAATAAGGAATCCCATGG R: TGCTTTACCCAGCGTCAGAT	621	61	[21]
CTX M group II	F: ACCGCCGATAATTCGCAGAT R: GATATCGTTGGTGGTGCCATAA	588	61	[21]
DHA	F: GTGGTGGACAGCACCATTAAA R: CCTGCGGTATAGGTAGCCAGAT	314	61	[21]
qnrA	F: ATTTCTCACGCCAGGATTTG R: TGCCAGGCACAGATCTTGAC	516	60	[22]
qnrB	F: CGACCTKAGCGGCACTGAAT R: GAGCAACGAYGCCTGGTAGYTG	515	50	[22]
qnrC	F: GGGTTGTACATTTATTGAATC R: TCCACTTTACGAGGTTCT	446	50	[22]
qnrD	F: CGAGATCAATTTACGGGGAATA R: AACAAGCTGAAGCGCCTG	581	50	[22]
qnrS	F: GACGTGCTAACTTGCGTGAT R: TGGCATTGTTGGAAACTTG	118	62	[22]

ery rate (FDR) method and FDR-adjusted q values were reported. For visualization, gene–gene associations were summarized by plotting the top 10 pairs ranked by $|\varphi|$, with corresponding FDR-adjusted q values and significant pairs ($q < 0.05$) highlighted. Gene–phenotype (gene–antibiotic) associations were summarized using a forest plot reporting ORs with 95% confidence intervals on a logarithmic scale, with FDR-adjusted q values annotated; only associations meeting the selected FDR threshold ($q < 0.05$) were displayed. All figures were generated in R using the tidyverse package (version 2.0; CRAN) and ggplot2 (version 4.0.1; CRAN).

3. Results

3.1 Distribution of β-Lactamase Genes

Among the 86 imipenem non-susceptible UPEC isolates, *blaTEM* was the most prevalent β-lactamase gene (54/86; 62.8%), followed by the plasmid-mediated AmpC determinant *blaCMY II* (11/86; 12.8%). *blaSHV* (9/86; 10.5%) and ESBL-associated *blaCTX-M* group I and *blaCTX-M* group II were detected at comparable and lower frequencies, while *blaDHA* (7/86; 8.1%), *blaOXA* (6/86; 7.0%) and *blaCTX-M* group IV (3/86; 3.5%) were less frequent. A consolidated overview of the prevalence of all resistance genes screened (β-lactamases and PMQR determinants) is provided in Table 3 (Ref. [23–26]).

Across phylogenetic groups, phylogroup B2 showed the broadest repertoire of β-lactamase determinants and represented the main reservoir for most detected *bla* genes (Table 4).

When gene-positive isolates were stratified by phylogroup, *blaTEM* was mainly detected within B2 (27/54; 50.0%) and E (15/54; 27.8%), and it was the only β-lactamase gene observed in phylogroup D (1/54; 1.9%). Likewise, *blaOXA* was predominantly found in B2 (5/6; 83.3%). For *blaSHV*, *blaCTX-M* group I, and *blaCTX-M* group II, most positive isolates also belonged to B2 (5/9; 55.6% each), while phylogroup E represented the second most frequent reservoir for *blaCTX-M* group I (4/9; 44.4%). In contrast, phylogroups A, B1, and D showed limited *bla* gene diversity, and no *bla* genes were detected among Clade I and Clade I/II isolates (Table 4).

As shown in **Supplementary Table 1**, the most frequent pattern was *blaTEM* + *blaCTX-M* group I (6/86,

Table 3. Prevalence of screened β-lactamase and PMQR genes among imipenem non-susceptible UPEC isolates (n = 86).

Resistance gene	n	N	n/N (%)	Expected phenotype (typical)
blaTEM	54	86	62.8%	Penicillinas e (AMP/AMX) ± ESBL variants (PCR does not resolve variant) [23,24]
blaSHV	9	86	10.5%	Penicillinas e ± ESBL variants (PCR does not resolve variant) [23,24]
blaOXA	6	86	7.0%	OXA-type β-lactamase (often narrow) → AMP/AMX; may affect inhibitor combos [23,24]
blaCTX-M group I	9	86	10.5%	ESBL → 3GC + aztreonam; usually clavulanate-inhibitable [23]
blaCTX-M group II	9	86	10.5%	
blaCTX-M group IV	3	86	3.5%	ESBL → 3GC + aztreonam; usually clavulanate-inhibitable [23,24]
blaCMY II	11	86	12.8%	pAmpC → cefoxitin + many cephalosporins; poor clavulanate inhibition [25]
blaDHA	7	86	8.1%	
qnrA	3	86	3.5%	
qnrB	18	86	20.9%	
qnrC	1	86	1.2%	PMQR (Qnr) → low-level quinolone/FQ; facilitates QRDR selection [26]
qnrD	5	86	5.8%	
qnrS	4	86	4.7%	

Note: For each determinant, the table reports the absolute number of gene-positive isolates (n), the total number tested (N), and the corresponding prevalence [n/N (%)]. The "Expected phenotype" column summarizes the typical resistance pattern associated with each gene family (β-lactamases: *blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M* groups I/II/IV, *blaCMY II*, *blaDHA*; PMQR: *qnrA/B/C/D/S*).

Table 4. Distribution of β-lactamase genes among imipenem-resistant UPEC phylogenetic groups.

Variables (Single gene)	A	B1	B2	Clade I	Clade I or II	D	E	Unknown	Total (%)
blaCTX-M group IV (n = 3)	0	0	1 (33.3)	0	0	0	1 (33.3)	1 (33.3)	100
blaTEM (n = 54)	3 (5.5)	1 (1.9)	27 (50)	0	0	1 (1.9)	15 (27.8)	7 (13)	100
blaOXA (n = 6)	1 (16.7)	0	5 (83.3)	0	0	0	0	0	100
blaSHV (n = 9)	1 (11.1)	1 (11.1)	5 (55.6)	0	0	0	1 (11.1)	1 (11.1)	100
blaCTX-M group I (n = 9)	0	0	5 (55.6)	0	0	0	4 (44.4)	0	100
blaCTX-M group II (n = 9)	1 (11.1)	1 (11.1)	5 (55.6)	0	0	0	0	2 (22.2)	100
blaCMY II (n = 11)	1 (9.1)	1 (9.1)	5 (45.5)	0	0	0	1 (9.1)	3 (27.3)	100
blaDHA (n = 7)	1 (14.3)	1 (14.3)	3 (42.8)	0	0	0	1 (14.3)	1 (14.3)	100

7.0%), followed by *blaTEM* + *qnrB* (5/86, 5.8%), whereas all remaining profiles occurred in only one or two isolates, indicating substantial heterogeneity.

When stratified by phylogroup (**Supplementary Table 2**), β-lactamase determinants frequently co-occurred within the same isolates. The most common co-carriage pattern was *blaTEM* with *blaCTX-M* group I, detected in B2 (25%) and E (50%) isolates. Additional combinations included *blaTEM/blaDHA*, *blaTEM/blaSHV*, and *blaOXA/blaCMY II/blaCTX-M* group II (each 12.5%). Within phylogroup E, combinations such as *blaTEM/blaSHV/blaCMY II/blaCTX-M* group II, *blaTEM/blaDHA*, and *blaCTX-M* group IV/*blaTEM* occurred at comparable frequencies (16.7% each), whereas B1 displayed a single combination (*blaSHV/blaCMY II/blaCTX-M* group II). In phylogroup A, two patterns were observed (*blaTEM/blaSHV* and *blaTEM/blaOXA/blaCMY II/blaCTX-M* group II; 50% each).

3.2 Association Between bla Gene Profiles and Resistance to β-Lactam Antibiotic

Phenotypic testing identified ESBL production in 42/86 (48.8%) imipenem non-susceptible UPEC isolates and a presumptive AmpC screening phenotype, based on cefoxitin (CX 30) non-susceptibility in 27/86 (31.4%) isolates. Plasmid-mediated AmpC genes (*blaCMY II* and/or *blaDHA*) were detected in 18/86 (20.9%) isolates. The cefoxitin-based presumptive AmpC phenotype showed a non-significant trend toward association with *blaCMY II/blaDHA* carriage, suggesting an enrichment of pAmpC genes among cefoxitin-non-susceptible isolates, while also indicating that reduced cefoxitin susceptibility may reflect additional mechanisms not captured by the targeted pAmpC gene panel. the corresponding 2 × 2 concordance counts are reported in Table 5.

Overall, 68.6% of isolates carried at least one *bla* gene; 40.7% harbored a single gene, 22.1% carried two genes, and 5.8% carried three or four genes (Table 4).

To assess genotype–phenotype associations within the β-lactam class, six representative agents were analyzed: amoxicillin (AMX), amoxicillin/clavulanate (AMC), ticarcillin (TC), ticarcillin/clavulanate (TCC), piperacillin (PRL), and piperacillin/tazobactam (TPZ). These compounds were selected as biologically relevant substrates for the β-lactamase families detected in this cohort. Other β-lactams were not included because their susceptibility

Table 5. Concordance between phenotypic ESBL/AmpC screening and genotypic determinants.

Analysis	No. of isolates				Statistical analysis	
	+/+	+/–	–/+	–/–	OR (95% CI)	Fisher's
ESBL production/ESBL genes (strict)	21	21	3	41	13.23 (3.40–77.12)	0.00077
AmpC screen (CX30 NS)/pAmpC genes (*blaCMY II* and/or *blaDHA*)	9	18	9	50	2.74 (0.82–9.23)	0.085

Note: The ESBL phenotype was compared with ESBL gene carriage (strict panel). The presumptive AmpC phenotype was defined as non-susceptibility to cefoxitin (CX30) and compared with the presence of plasmid-mediated AmpC genes (*blaCMY II* and/or *blaDHA*). Odds ratios (OR) with 95% confidence intervals (CI) and Fisher's exact test *p*-values are reported. + and – indicate isolates positive or negative for the phenotype and/or gene presence, respectively.

profiles can be strongly influenced by additional mechanisms (e.g., permeability changes, efflux, and co-existing enzymes), which may mask gene-specific effects.

As shown in Fig. 1 (and detailed in **Supplementary Table 2**), β-lactamase gene carriage had a gene-dependent impact on non-susceptibility (I+R) to the selected β-lactams. *blaTEM* showed the most consistent association: *TEM*-positive isolates displayed higher non-susceptibility to AMX (94.4% vs. 71.9%), AMC (63.0% vs. 50.0%), TC (94.4% vs. 68.8%), TCC (96.3% vs. 65.6%), and PRL (83.3% vs. 56.3%), with significant differences for the comparisons reaching $p < 0.05$ (Fisher's exact test). *blaSHV* was also associated with elevated non-susceptibility to AMX, TC, TCC, and PRL (all $\geq$88%), although contrasts versus *SHV*-negative isolates were attenuated by the high baseline resistance in this population.

In contrast, *blaCTX-M* groups (I, II, IV) did not clearly discriminate non-susceptibility levels for these β-lactams, as rates remained uniformly high in both *blaCTX-M*-positive and -negative isolates (often >80–90%), limiting statistical separation. Among AmpC determinants, *blaCMY II* was associated with a strong phenotype: *CMY II*-positive isolates showed 100% non-susceptibility to AMX, TC, TCC, and PRL, and higher non-susceptibility to AMC compared with *CMY II*-negative isolates (90.9% vs. 53.3%; Fisher's exact test, $p < 0.05$). Although *blaDHA* carriage was associated with increased non-susceptibility to some agents (e.g., AMC 71.4%), its low prevalence (n = 7) limited statistical power.

Overall, measurable genotype-associated phenotypic effects were mainly observed for *blaTEM* and *blaCMY II* (and to a lesser extent *blaSHV*), whereas *blaCTX-M* groups and *blaDHA* contributed less to between-group discrimination in this highly resistant cohort.

3.3 Distribution of qnr Genes Among Imipenem-Resistant UPEC Phylogenetic Groups

A total of 36.05% (31/86) of imipenem non-susceptible *E. coli* isolates carried at least one plasmid-mediated quinolone resistance (*qnr*) determinant. Within *qnr*-positive isolates, *qnrB* (18/86, 20.9%) was the most frequently detected variant, followed by *qnrD* (5/86, 5.8%), while *qnrS* (4/86, 4.7%), *qnrA* (3/86, 3.5%), and *qnrC* (1/86, 1.2%) occurred at lower frequencies (Table 3). Phylogroup B2 represented the main reservoir of *qnr* genes, particularly *qnrB* and *qnrD*, while the remaining phylogroups showed sporadic carriage. Co-occurrence of *qnrB* and *qnrD* was observed exclusively in B2 isolates.

As shown in Fig. 2, *qnr* gene carriage displayed a determinant-specific association with quinolone non-susceptibility (I+R) to ciprofloxacin (CIP), nalidixic acid (NA), and ofloxacin (OF). *qnrB* was significantly associated with non-susceptibility to all three quinolones (Fisher's exact test): CIP (83.3% vs. 29.4%, $p = 6.6 \times 10^{-5}$), NA (77.8% vs. 26.5%, $p = 1.7 \times 10^{-4}$), and OF (100% vs. 35.3%, $p = 2.3 \times 10^{-7}$). A more restricted, drug-specific association was observed for *qnrD*, which correlated with OF non-susceptibility (100% vs. 45.7%, $p = 0.024$) but not with CIP or NA.

No statistically significant differences were detected for *qnrA*, *qnrC*, or *qnrS* (all $p > 0.05$, Fisher's exact test). Overall, these findings indicate that *qnrB* is the *qnr* determinant most consistently associated with quinolone non-susceptibility in this collection, whereas *qnrD* shows a narrower, drug-dependent effect; contributions of other *qnr* variants may be limited or masked by additional quinolone resistance mechanisms.

3.4 Associations Between qnr Determinants, β-Lactamase Genes, and Resistance Phenotypes

Pairwise gene–gene associations (presence/absence) between PMQR determinants and β-lactamase genes were quantified using the phi coefficient (φ), with Fisher's exact test and Benjamini–Hochberg FDR correction. As shown in Fig. 3A (top 10 pairs ranked by $|\varphi|$; **Supplementary Table 3**), only a small subset of co-carriage signals remained statistically supported after multiple-testing correction. The strongest association involved *blaCMY II* and *blaCTX-M* group II ($\varphi = 0.893$, $q = 9.33 \times 10^{-9}$), driven by 9/86 co-carriers and a nested distribution in which *blaCTX-M* group II was never observed alone (0/86). A second supported association was observed between *blaOXA* and *blaCTX-M* group II ($\varphi = 0.503$, $q = 0.0314$; 4/86 co-carriers). Other top-ranked pairs showed moderate φ values but did not retain statistical support after FDR correction, consistent with heterogeneous co-carriage patterns and limited power for rare determinants (**Supplementary Table 3**).

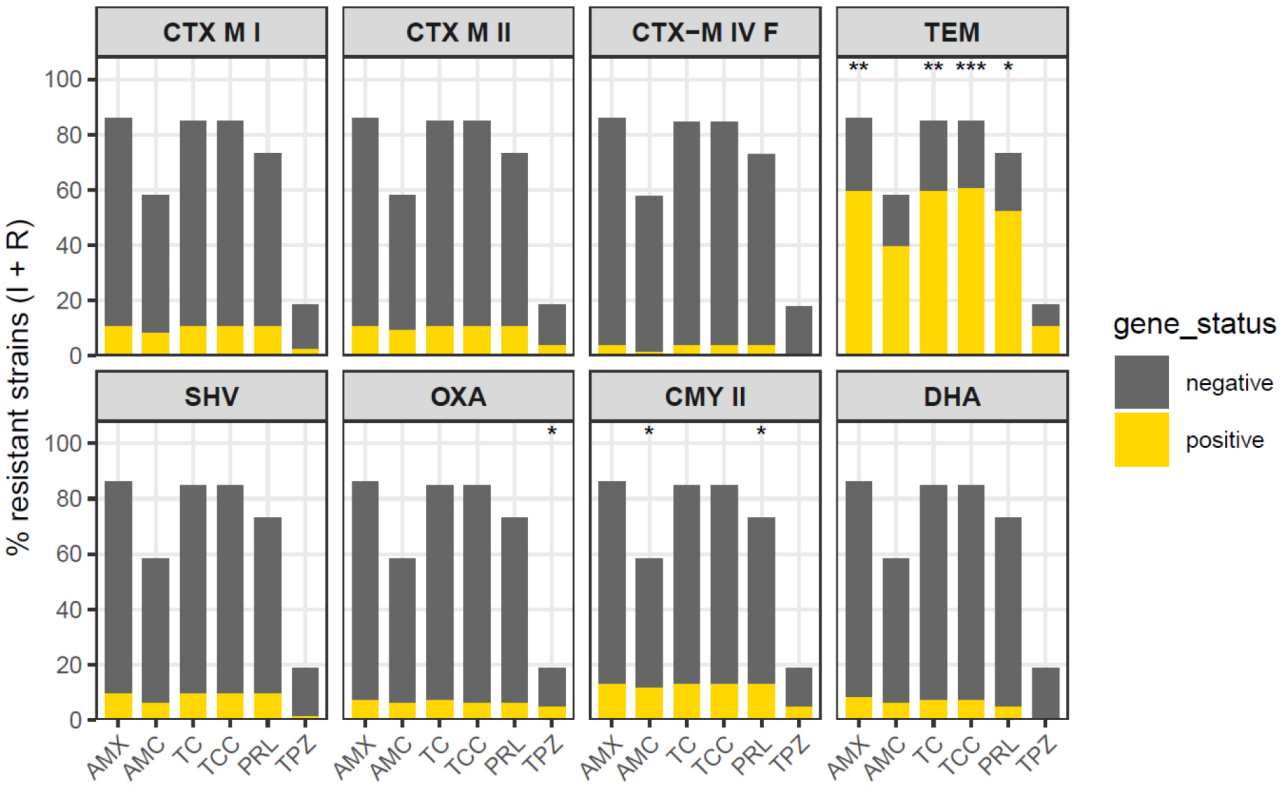

Fig. 1. β-lactam non-susceptibility (I+R) in β-lactamase gene–positive versus gene–negative UPEC isolates. For each β-lactamase gene (*blaCTX-M* group I, *blaCTX-M* group II, *blaCTX-M* group IV, *blaTEM*, *blaSHV*, *blaOXA*, *blaCMY II*, and *blaDHA*), bars show the proportion of isolates classified as non-susceptible (intermediate + resistant) to six β-lactam agents (AMX, AMC, TC, TCC, PRL, TPZ) among gene-negative (grey) and gene-positive (yellow) groups. Asterisks indicate statistically significant differences between groups (Fisher's exact test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$); only significant comparisons are annotated. This analysis highlights the differential contribution of individual β-lactamase determinants to resistance against penicillins and β-lactam/β-lactamase inhibitor combinations. AMX, amoxicillin; AMC, amoxicillin/clavulanate; TC, ticarcillin; TCC, ticarcillin/clavulanate; PRL, piperacillin; TPZ, piperacillin/tazobactam; UPEC, Uropathogenic *Escherichia coli*.

Gene–phenotype associations were assessed by testing gene carriage (0/1) versus antimicrobial non-susceptibility (I+R vs S) using Fisher's exact test, reporting odds ratios (OR) with 95% confidence intervals and applying FDR correction (Fig. 3 and **Supplementary Table 4**). Among PMQR determinants, *qnrB* was the only variant significantly associated with quinolone/fluoroquinolone non-susceptibility, including ofloxacin (OR = ∞, 95% CI 7.06–∞; $q = 6.4 \times 10^{-5}$), ciprofloxacin (OR = 11.62, 95% CI 2.86–69.36; $q = 0.0091$), and nalidixic acid (OR = 9.42, 95% CI 2.54–44.52; $q = 0.0156$). Among β-lactamase determinants, *blaTEM* was significantly associated with ticarcillin/clavulanate non-susceptibility (OR = 13.17, 95% CI 2.56–132.48; $q = 0.0156$). Complete results for all comparisons are provided in **Supplementary Tables 3,4**.

3.5 Multivariate Analysis of Antibiotic Resistance, ESBL Status, and Biofilm Formation

To investigate the global structure of antimicrobial resistance and its relationship with ESBL production and biofilm formation, a principal component analysis (PCA) was performed using antibiotic susceptibility variables, resistance determinants, and the biofilm phenotype (Fig. 4). The first two principal components explained 21.0% (Dim1) and 10.5% (Dim2) of the total variance. Given the limited variance captured by the Dim1–Dim2 plane (31.5%), the PCA was interpreted as an exploratory low-dimensional summary of multivariate patterns. A trend toward separation between ESBL-negative and ESBL-positive isolates was observed primarily along Dim1, suggesting that ESBL status and closely related β-lactam resistance markers contribute to phenotypic–genotypic variability in this cohort. ESBL-positive isolates tended to cluster on the positive side of Dim1, whereas ESBL-negative isolates were positioned toward the negative side, consistent with comparatively more susceptible profiles, although partial overlap was observed.

The correlation circle (Fig. 4B) showed that β-lactam-related variables were the main contributors to the multivariate structure. Ticarcillin/clavulanic acid (TCC 85), cefotaxime (CTX 30), cefepime (FEP 30), and aztreonam

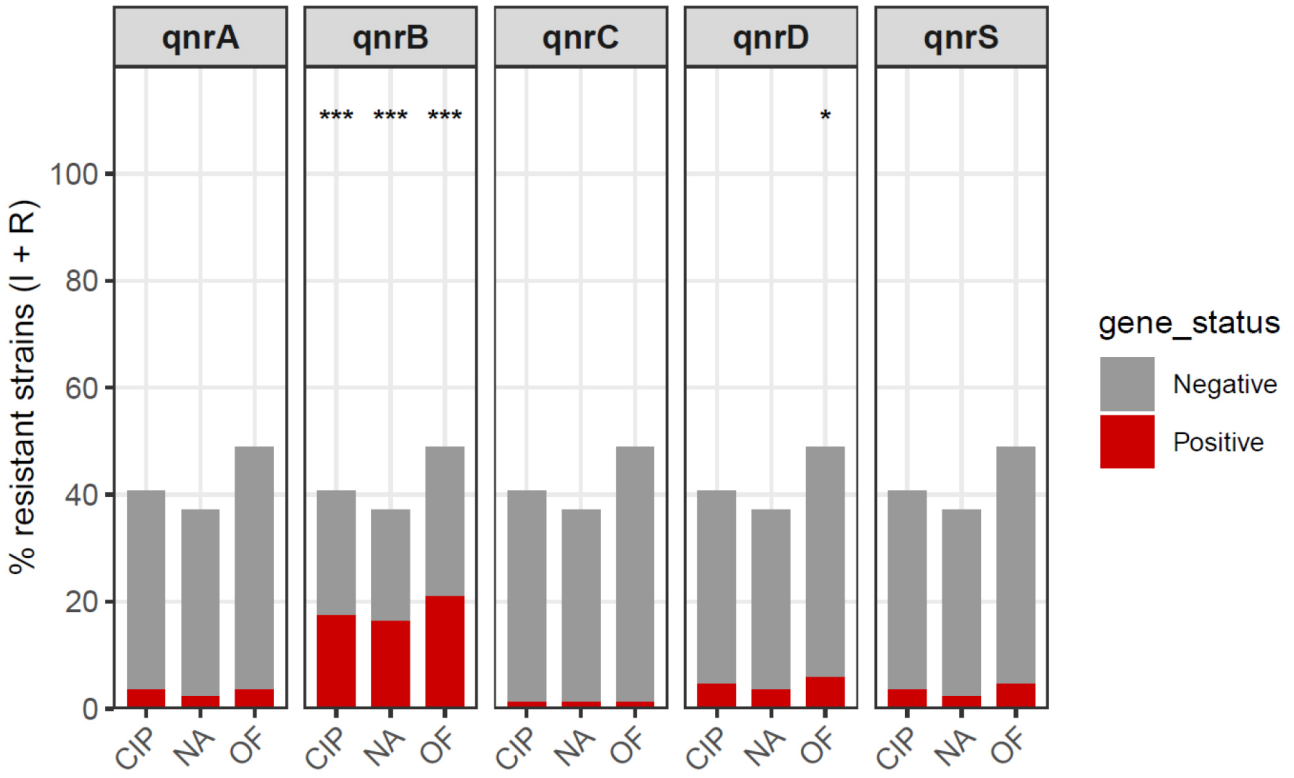

Fig. 2. Quinolone non-susceptibility (I+R) according to *qnr* gene carriage in imipenem non-susceptible *E. coli*. Bar plots show the proportion of isolates classified as non-susceptible (intermediate + resistant) to ciprofloxacin (CIP), nalidixic acid (NA), and ofloxacin (OF) among *qnr*-negative (grey) and *qnr*-positive (red) groups, stratified by each determinant (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*). Differences between gene-positive and gene-negative groups were assessed using Fisher's exact test; asterisks denote statistically significant comparisons ($^{*}p < 0.05$, $^{***}p < 0.001$), and only significant results are annotated.

(ATM 30) displayed the highest $\cos^2$ values and aligned predominantly with Dim1, confirming their strong representation on the Dim1–Dim2 plane and their major contribution to the observed variance and group separation. In contrast, variables such as biofilm formation and nitrofurantoin (NIT 300) projected toward the negative side of Dim1 and exhibited lower $\cos^2$ values, indicating weaker representation in the two-dimensional space. Dim2 captured additional, secondary variability associated with other determinants and phenotypes, including *blaTEM*, aminoglycoside response variables, and *qnrD*. Overall, the PCA supported a robust multivariate distinction between ESBL-producing and non-ESBL-producing UPEC isolates and identified β-lactam susceptibility markers as the dominant drivers of variability within this Algerian collection.

4. Discussion

β-lactamase production remains one of the most important mechanisms driving resistance to β-lactam antibiotics in *Escherichia coli*. In particular, ESBLs and plasmid-mediated AmpC enzymes compromise the activity of penicillins and extended-spectrum cephalosporins and are frequently carried on mobile genetic elements that also harbor determinants of resistance to other antimicrobial classes, thereby promoting multidrug-resistant (MDR) phenotypes [27]. In the present study, at least one β-lactamase gene was detected in 74.4% of imipenem non-susceptible UPEC isolates, a prevalence higher than previously reported in Algeria (32.5%) [11] and Burkina Faso (44.8%) [28]. Comparable frequencies have been documented in Morocco (≈80%) [29] and Egypt (≈67–74%) [30], supporting substantial regional variability likely influenced by antibiotic exposure, local stewardship policies, and circulation of successful resistant lineages.

Direct comparisons across studies should be interpreted cautiously because prevalence estimates vary by setting and inclusion criteria (e.g., community vs. hospital cohorts and antimicrobial selection). Importantly, our cohort represents a high-risk subset of UPEC (imipenem non-susceptible isolates) and was not intended to estimate population-level gene prevalence in unselected UPEC. Therefore, prevalence comparisons should be interpreted as descriptive within this clinically relevant subset rather than as representative of overall UPEC epidemiology in Algeria. In high-income settings, ESBL/β-lactamase-mediated resistance in UTI-causing Enterobacterales is generally lower than in many African and Asian reports, but it is increasing. In the United States, a multicenter prospective study

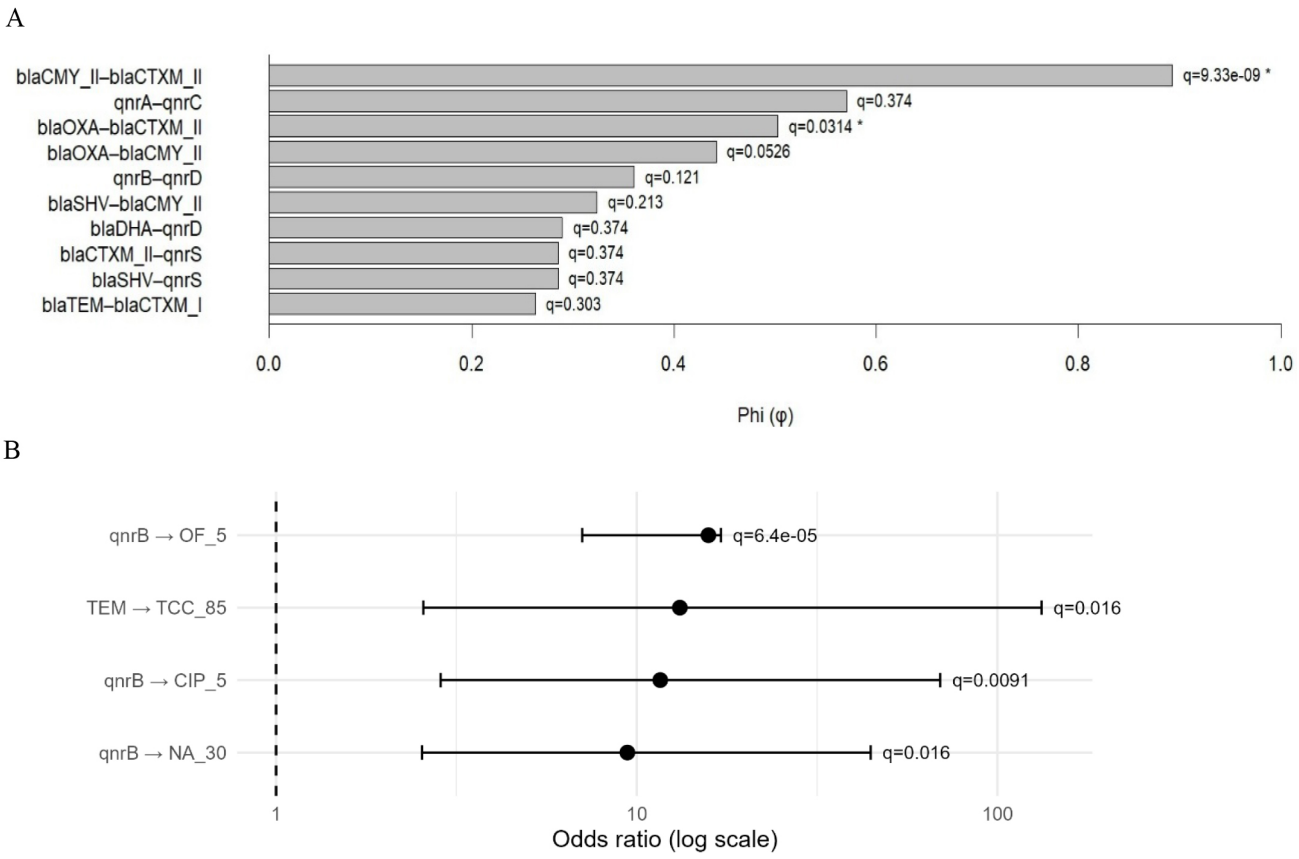

Fig. 3. Associations between *qnr* determinants, β-lactamase genes, and resistance phenotypes. (A) Top gene–gene associations among resistance determinants in imipenem-non-susceptible UPEC isolates. The bar plot reports the 10 strongest pairwise associations between resistance genes, ranked by the absolute phi coefficient (φ), a measure of association for binary variables (presence/absence). Statistical significance was evaluated using Fisher's exact test, and *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR); corresponding FDR-adjusted *q* values are displayed beside each bar. Asterisks indicate associations remaining significant after FDR correction ($q < 0.05$). Positive φ values indicate co-carriage (tendency of two genes to occur together). (B) Significant gene–antibiotic associations after FDR correction. Forest plot showing odds ratios (OR) with 95% confidence intervals for associations between resistance gene carriage (presence/absence) and antimicrobial non-susceptibility (I+R vs S) assessed by Fisher's exact test. The vertical dashed line indicates OR = 1 (no association). Labels report Benjamini–Hochberg FDR-adjusted *q* values; only associations meeting the predefined FDR threshold ($q < 0.05$) are displayed. Antibiotics are reported with disk content (µg).

of adults hospitalized for UTI (2018–2019) reported ESBL-producing Enterobacteriaceae in 17.2% of cases [31]. In Europe, multicountry collections of urinary isolates show substantial heterogeneity; for example, a large surveillance-based dataset reported an ESBL phenotype in 17.9% of *E. coli* UTI isolates from Europe [32], while European surveillance summaries highlight marked geographic gradients and temporal trends in resistance relevant to ESBL epidemiology [33]. Conversely, Asian settings frequently show higher burdens; for example, a multicenter Chinese study on strictly defined community-acquired UTIs reported ESBL-positive *E. coli* in ~38% of isolates [34].

Among β-lactamase determinants, *blaTEM* was the most prevalent gene, whereas *blaSHV* and *blaCTX-M* groups were detected at lower but comparable frequencies. *blaOXA* was detected in 7.0% of isolates, consistent with its sporadic but clinically relevant contribution within MDR UPEC backgrounds. Similar distributions have been reported across multiple geographic settings, including Pakistan [35], Iraq [36], Nigeria [37], as well as in Sudan [38,39], and Egypt [40], although prevalence values vary markedly between studies [39,40].

Phylogenetic analysis further indicated that phylogroup B2 harbored the broadest repertoire of β-lactamase genes and accounted for the largest share of *bla*-positive isolates in this collection, consistent with previous reports describing B2 ExPEC lineages as highly adapted to persist in the urinary tract and to acquire resistance under antimicrobial pressure [5,38,41].

The heterogeneity observed between phylogroups B2 and E may also reflect differences in the underlying population structure of UPEC and in dissemination dynamics.

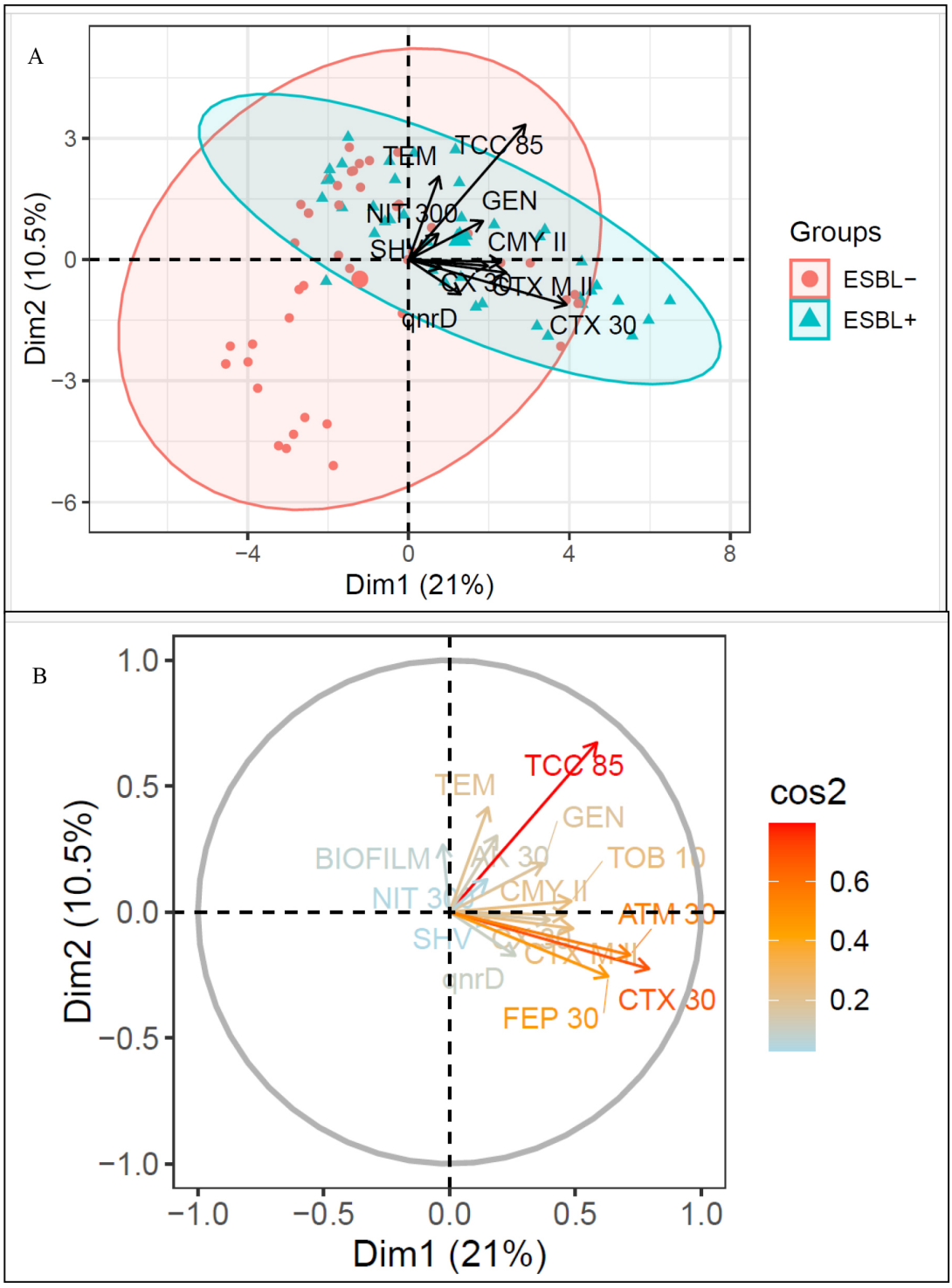

Fig. 4. Multivariate structure of antibiotic resistance, ESBL status, and biofilm formation in imipenem non-susceptible UPEC. (A) PCA biplot showing the distribution of isolates by ESBL status, with separation occurring mainly along Dim1 (21.0% variance explained). (B) PCA correlation circle showing projection of resistance determinants, antibiotic non-susceptibility variables, and biofilm formation on the Dim1–Dim2 plane (Dim1 = 21.0%, Dim2 = 10.5%). The color gradient indicates the quality of representation (cos^2), with higher values corresponding to better representation in the two-dimensional space. Variables with the highest cos^2 values (including TCC 85, CTX 30, FEP 30, and ATM 30) align predominantly with Dim1 and contribute most to the multivariate structure, whereas biofilm formation and NIT 300 show lower cos^2 and are less well represented on the Dim1–Dim2 plane.

Phylogroup B2 is a hallmark ExPEC background and is often enriched in virulence-associated traits that support urinary tract colonization and persistence, which may increase opportunities for acquisition and long-term maintenance of resistance plasmids under antimicrobial pressure [42,43]. In addition, successful clonal lineages frequently belonging to phylogroup B2 (e.g., globally disseminated MDR UPEC lineages) may contribute to the overrepresentation of resistance determinants through clonal expansion and repeated antibiotic exposure [44]. In contrast, phylogroup E is less consistently linked to classical ExPEC virulence profiles, and its contribution to resistance in our collection may be compatible with the expansion of specific resistant lineages and/or the local circulation of particular mobile genetic elements within healthcare or community networks [18,45]. Therefore, the distribution of β-lactamase and PMQR determinants across B2 and E likely reflects a combination of lineage success, selective pressure, and plasmid-mediated dissemination rather than phylogroup membership alone [46,47]. ESBL/PMQR co-carriage in the North Africa region was highlighted also in previous studies [12,13]. Prevalence of plasmid-mediated quinolone resistance (PMQR) determinants among extended spectrum beta-lactamase (ESBL)-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Aleppo, Syria [14].

The frequent co-occurrence of *blaTEM* with *blaCTX-M* group I within B2 isolates is compatible with co-carriage on mobile genetic elements and/or co-selection under antimicrobial pressure, which may facilitate the accumulation of multiple resistance determinants in successful UPEC lineages [12,48,49].

Regarding AmpC, cefoxitin non-susceptibility (presumptive AmpC screening) showed only a non-significant enrichment of plasmid-mediated AmpC genes (*blaCMY II* and/or *blaDHA*), consistent with the plasmid-borne nature and variable distribution of *blaCMY*-type determinants [50]. Reduced cefoxitin susceptibility may also involve additional mechanisms (e.g., permeability changes and/or co-existing β-lactamases) not captured by the targeted pAmpC panel [51].

With respect to quinolone resistance, *qnrB* emerged as the most frequent determinant among *qnr*-positive isolates, followed by *qnrD*, whereas *qnrA*, *qnrS*, and especially *qnrC* were rare. This pattern is consistent with reports from Algeria and other countries, including Iraq, Togo, Nigeria, Iran and Tunisia [11,52–55]. In our collection, *qnr* determinants were mainly detected in B2 isolates, in line with the frequent linkage between ExPEC-associated backgrounds, virulence traits, and multidrug resistance [42,43,47]. Importantly, after multiple-testing correction *qnrB* was the only PMQR determinant retaining statistically supported associations with quinolone/fluoroquinolone non-susceptibility, whereas *blaTEM* showed the strongest β-lactam association (ticarcillin/clavulanate), consistent with their expected functional roles.

The persistence of quinolone resistance among *qnr*-negative isolates indicates that additional mechanisms contribute to the overall phenotype, most notably chromosomal alterations in the quinolone resistance–determining regions (QRDR) of *gyrA* and *parC*, potentially combined with efflux/porin changes and/or other plasmid-mediated determinants (e.g., *aac(6′)-Ib-cr*, *qepA*, *oqxAB*) [26,53]. Although *qnrB* was associated with quinolone/fluoroquinolone non-susceptibility, substantial resistance was also observed among *qnr*-negative isolates; however, QRDR mutations were not investigated in this study, representing an important limitation [26,56]. Therefore, in our collection, *qnr* genes should be interpreted as mobile resistance determinants that may modulate MICs and facilitate selection of chromosomal mutations rather than fully explaining the quinolone-resistant phenotype.

Several studies have described co-carriage of *qnr* genes with β-lactamase determinants, including combinations such as *qnrB* with *blaTEM/blaCTX-M/blaOXA* and *qnrS* with *blaCTX-M*, supporting the role of plasmids in coselection and horizontal dissemination [6,46,57–59]. In our dataset, formal gene–gene association testing indicated that only a restricted subset of co-carriage signals remained robust after FDR correction. The strongest association involved *blaCMY II* and *blaCTX-M* group II (consistent with a "nested" distribution compatible with co-localization and/or clonal enrichment), and *blaOXA* also showed a significant association with *blaCTX-M* group II; in contrast, several additional pairs displayed moderate φ values but did not retain statistical support after correction, likely reflecting low event counts and heterogeneous genetic backgrounds. Nevertheless, recurrent co-existence remains epidemiologically relevant, as plasmid-mediated co-mobilization can facilitate the spread of MDR profiles even when individual determinants do not show tight statistical coupling. Beyond univariate associations, the multivariate PCA analysis provided an exploratory integrated view of resistance structure. Because the first two dimensions explained a limited proportion of total variance (31.5% for Dim1+Dim2), the observed ESBL+ vs ESBL– separation should be interpreted as a dominant trend on the 2D projection rather than a complete representation of the dataset structure; nevertheless, β-lactam-related traits remained the main contributors to Dim1, consistent with ESBL status being a major driver of variation in this high-risk cohort. Variables contributing most strongly to the multivariate space were dominated by β-lactamrelated traits (including non-susceptibility to key β-lactams), indicating that ESBL status and associated β-lactam resistance markers represent major drivers of dataset organization. Biofilm formation and selected non-β-lactam traits contributed more modestly, suggesting additional layers of phenotypic divergence superimposed on the dominant ESBL-related axis. Overall, this multivariate pattern is consistent with the global literature describing ESBL-producing UPEC as frequently

embedded within broader MDR profiles [60,61]. Beyond clinical settings, ESBL and PMQR determinants have also been documented in animal and environmental reservoirs, supporting a One Health framework for dissemination and co-selection [62–64]. In particular, *qnrB* and ESBL/PMQR co-carriage have been reported in food-producing animal contexts, underscoring the potential for cross-sector circulation of mobile resistance elements [63–65]. From a clinical perspective, the increasing burden of quinolone resistance is particularly concerning because the use of fluoroquinolones is already constrained by safety warnings and recommendations to reserve these agents for situations where safer alternatives are unsuitable [66]. Therefore, rising resistance further narrows an already restricted therapeutic option set and reinforces the need for local surveillance to guide empiric therapy and preserve remaining effective agents. Collectively, our findings highlight the prominence of β-lactamase and PMQR determinants, especially within B2 lineages, and support the value of continued molecular monitoring combined with stewardship strategies and, where feasible, plasmid-focused tracking approaches to better understand dissemination routes and inform targeted containment.

Finally, although the present study is based on targeted molecular screening rather than whole-genome sequencing, our findings align with the broader evidence from genomic surveillance indicating that successful ExPEC/UPEC lineages can accumulate ESBL determinants—often dominated by *blaCTX-M* enzymes (including widely disseminated variants such as *blaCTX-M-15*)—together with plasmid-mediated quinolone resistance factors (e.g., *qnr* variants and *aac(6′)-Ib-cr*) on mobile genetic elements. This co-localization can promote co-selection under antibiotic pressure and accelerate the spread of multidrug-resistant UPEC, further limiting oral treatment options for UTIs. In addition, recent reports describing carbapenemase-producing UPEC strains carrying additional resistance determinants underscore the risk of increasingly difficult-to-treat lineages and reinforce the need for continued molecular surveillance and stewardship [67–69].

5. Conclusions

This study identified *blaTEM* as the predominant β-lactamase determinant among imipenem non-susceptible UPEC isolates, with other *bla* genes detected at lower frequencies. The collection exhibited extensive resistance to both β-lactams and quinolones, with plasmid-mediated quinolone resistance determinants detected among a subset of isolates, most notably *qnrB* (and less frequently *qnrD*, *qnrA*, *qnrS*, and *qnrC*). The frequent co-carriage of multiple resistance determinants within single strains highlights the growing clinical threat posed by multidrug-resistant UPEC, which reduces effective treatment options and complicates infection management. These findings support the need for continuous molecular surveillance and strengthened antimicrobial stewardship to limit dissemination of resistance determinants and to inform local empiric therapy.

These results should be interpreted in light of several limitations. The study was based on isolates collected from a single region and on a subset selected for imipenem non-susceptibility, which may limit generalizability and inflate resistance estimates compared with unselected UPEC populations. However, this targeted approach enabled us to focus on clinically relevant high-risk isolates that pose the greatest therapeutic challenge. In addition, information on prior antibiotic exposure was not systematically available, and carbapenemase production was not investigated, preventing a detailed characterization of carbapenem resistance mechanisms. Finally, PCR-based detection does not capture genetic context (e.g., plasmid vs. chromosomal location) or expression levels, and chromosomal mechanisms underlying quinolone resistance (e.g., QRDR mutations in *gyrA/parC*) were not assessed.Future studies including larger, geographically diverse cohorts, clinical metadata (including antibiotic histories), and expanded molecular characterization (e.g., plasmid profiling and/or sequencing) are warranted to better define dissemination routes and clinical impact of multidrug-resistant UPEC.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

AK, CM and ME performed the research, analyzed and interpreted the data. AK and CM wrote the draft of the manuscript. NB and RA designed the research study and supervised the study. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was reviewed and approved by the Ethics and Deontology Committee of the University Ferhat Abbas Setif 1, under approval number UFAS1/09/03/2023/ETH-Deon-A-301 (title: Ethical Approval). A total of 86 urine samples were collected from participants enrolled in the study. Written informed consent was obtained from all subjects or their legal guardians prior to sample collection and for the publication of this work. The study was carried out in accordance with the guidelines of the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest. Given her role as the Editorial Board member, Rosa Alduina had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Sung-Kun Kim.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL48045.

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