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Phenotypic and Genotypic Characterization of ESBL-, AmpC-, and Carbapenemase-Producing Klebsiella pneumoniae and High-Risk Escherichia coli CC131, with the First Report of ST1193 as a Causative Agent of Urinary Tract Infections in Algeria

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Phenotypic and Genotypic Characterization of ESBL-, AmpC-, and Carbapenemase-Producing *Klebsiella pneumoniae* and High-Risk *Escherichia coli* CC131, with the First Report of ST1193 as a Causative Agent of Urinary Tract Infections in Algeria

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Abstract: Background: High-risk *Escherichia coli* clones, such as Sequence Type (ST)131 and ST1193, along with multidrug-resistant (MDR) *Klebsiella pneumoniae*, are globally recognized for their significant role in urinary tract infections (UTIs). This study investigates the characteristics of these pathogens in the Tebessa region of Algeria. **Methods**: Forty *E. coli* and 17 *K. pneumoniae* isolates exhibiting extended-spectrum cephalosporin (ESC)-resistance were phenotypically and genotypically characterized. Whole genome sequencing (WGS) was performed on the ST1193 clone. **Results:** Among *K. pneumoniae* isolates, all except one harbored CTX-M-15, with a single isolate carrying *bla*$_{\text{CTX-M-194}}$. Additionally, two *K. pneumoniae* isolates co-harboring *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{NDM}}$ exhibited both phenotypic and genotypic hypervirulence traits. Fluoroquinolone resistance (FQR) was detected in 94.1% of *K. pneumoniae* isolates. The *E. coli* isolates carried diverse ESC-resistance genes, including CTX-M-15 (87.5%), CTX-M-27 (5%), CTX-M-1, CMY-59, and CMY-166 (2.5% each). Co-carriage of *bla*$_{\text{ESC}}$ and *bla*$_{\text{OXA-48}}$ was identified in three *E. coli* isolates, while 62.5% exhibited FQR. Phylogenetic analysis revealed that 52.5% of *E. coli* belonged to phylogroup B2, including the high-risk clonal complex (CC)131 CH40-30 (17 isolates) and ST1193 (one isolate). The virulence profile indicated that 72.5% of isolates met the criteria for extraintestinal pathogenic *E. coli* (ExPEC), while 47.5% were classified as uropathogenic *E. coli* (UPEC). Characterization of CC131 clone showed that virotypes F and E accounted for 59% of isolates. *In silico* analysis of the ST1193 genome determined O75:H5-B2 (CH14-64), and the carriage of IncI1-I(Alpha) and IncF [F-:A1:B10] plasmids. Notably, core genome single-nucleotide polymorphism (SNP) analysis demonstrated high similarity between the Algerian ST1193 isolate and a previously annotated genome from a hospital in Northwest Spain. **Conclusions:** This study highlights the spread and genetic diversity of *E. coli* CC131 CH40-30 and hypervirulent *K. pneumoniae* clones in Algeria. Additionally, it represents the first report of a CTX-M-15-carrying *E. coli* ST1193 in the country. These findings emphasize the urgent need for surveillance programs and optimized antibiotic stewardship to curb the dissemination of high-risk clones that pose an increasing public health threat in Algeria.

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Keywords: urinary tract infection (UTI); *Escherichia coli*; *Klebsiella pneumoniae*; ST131; ST1193; ESBL; carbapenemases; Algeria

1. Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide. UTIs affect millions of individuals annually and impact both men and women, however, women are particularly susceptible, with approximately 50% to 60% of women experiencing at least one UTI during their lifetime [1,2]. In 2019, the global burden of UTIs was estimated to be 405 million cases and 237,000 deaths [3]. Furthermore, UTIs are the fourth leading cause of death associated with antimicrobial resistance worldwide [4]. This poses a considerable burden on public health and healthcare systems while substantially impairing the quality of life for those affected. *Escherichia coli* is the predominant causative agent of UTIs, accounting for most cases globally, and *Klebsiella pneumoniae* is recognized as the second contributor to UTIs. Both *E. coli* and *K. pneumoniae* exhibit a variety of virulent factors that enable them to adhere to, invade, and persist in the urinary tract, often leading to acute or chronic infections [2]. These pathogens also exhibit increasing resistance, especially to cephalosporins, fluoroquinolones, and carbapenems complicating the clinical management of UTIs, leading to longer hospital stays, increased healthcare costs, and treatment failures [4].

Among *E. coli*, multidrug-resistant (MDR) high-risk clones such as ST38, ST131, ST167, ST405, ST410, ST648, and ST1193, are commonly reported in these infections [5–7]. Among them, ST131 is considered the most prominent, while ST1193 appears to be emerging as a significant clone [8]. MDR clones are often linked to resistance to fluoroquinolones (FQR), with many also producing CTX-M enzymes, which severely limit treatment options [6–9]. On the other hand, extended-spectrum beta-lactamase (ESBL) and carbapenemase-encoding *K. pneumoniae* (both together referred to as MDR-Kp) are globally disseminated and cause infections that are often difficult to treat, placing MDR-Kp and MDR-*E. coli* high on current lists of significant threats to public health by the WHO [10].

In Algeria and other low- and middle-income countries (LMICs), the prevalence of MDR uropathogens is notably high, exacerbated by the lack of robust antibiotic stewardship programs. A study conducted in the Tizi-Ouzou region of Algeria revealed that *E. coli* isolates from UTIs exhibited significant resistance to commonly prescribed antibiotics, highlighting inappropriate antibiotic utilization and underscoring the urgent need to improve prescription practices in the country [11]. Furthermore, different reports highlight the presence and spread of antibiotic-resistant MDR-Kp strains in various regions of Algeria [12–14]

This paper aims to provide an overview of the virulence, and antibiotic resistance profiles of *E. coli* and *K. pneumoniae* strains causing UTIs in the Tebessa region of Algeria. Understanding the local epidemiology is crucial to mitigate the spread of MDR pathogens and to enhance surveillance systems.

2. Results

The present study comprised 57 non-duplicate isolates, 40 *E. coli* and 17 *K. pneumoniae*, recovered from urine samples at three healthcare facilities of northeast Algeria (Table S1 and Table S2).

2.1. Escherichia coli Collection

A total of 40 non-duplicate *E. coli* isolates were recovered from patients in three healthcare facilities in the Tebessa region, Algeria, including nine isolates from male and 31 from female patients. All isolates tested positive for the *uidA* target-specific species gene [15]. Phenotypic characterization on MacConkey Lactose agar revealed that four isolates (10%) were non-lactose fermenters (NLF) (Table S1).

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2.1.1. Antimicrobial Susceptibility Testing (AST) and Genotypic Characterization of *bla* Genes

Antimicrobial susceptibility testing (AST) revealed that all *E. coli* isolates were resistant to ampicillin, cefuroxime, cefotaxime, and ceftazidime. Additionally, most of the isolates (≥ 50%) exhibited resistance to aztreonam, amoxicillin-clavulanic acid, nalidixic acid, sulfamethoxazole, ciprofloxacin, doxycycline, and tobramycin. Noticeably, four isolates (10%) were resistant to ertapenem (Figure 1). Overall, all isolates were classified as MDR, and 62.5% demonstrated FQR.

The isolates were subsequently analyzed by PCR to detect the presence of bla_{ESBL}, bla_{AmpC}, and bla_{CARBA} genes. The collection exhibited different ESC enzymes, including CTX-M-15 (87.5%), CTX-M-27 (5%), as well as CTX-M-1, CMY-59, and CMY-166 (2.5% each). The bla_{OXA-48} gene was detected in three *E. coli* isolates resistant to ertapenem, which also harbored CTX-M-27 (two isolates) and CMY-59 (one isolate). No isolates tested positive for *mcr* (1 to 5) genes, nor did they exhibit polymyxin resistance (Table S1).

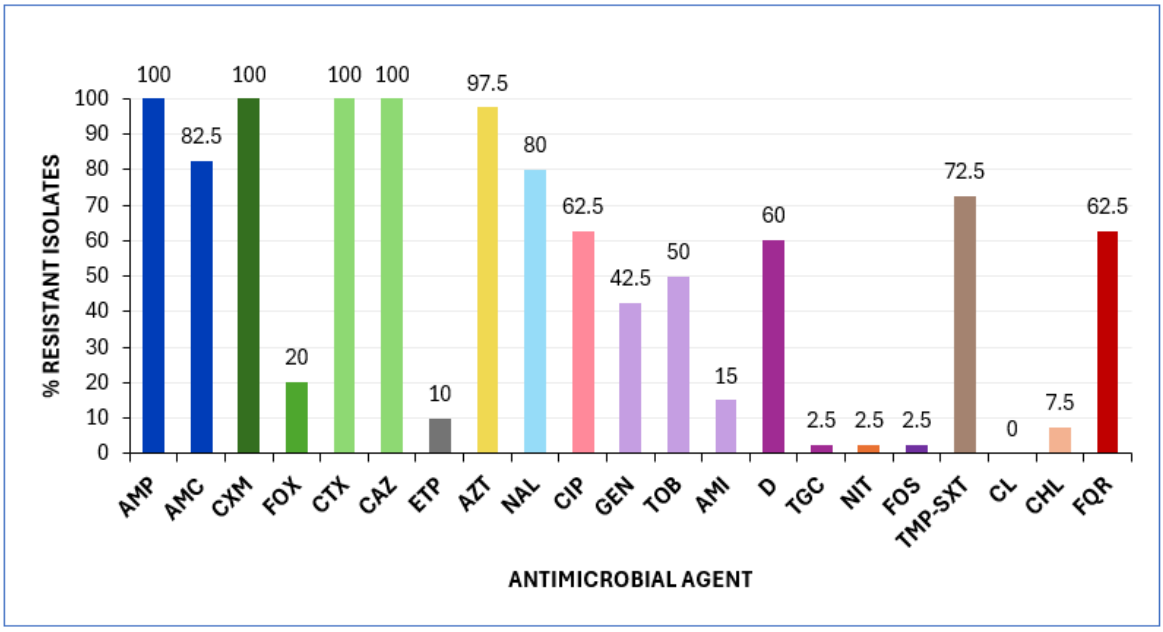

Figure 1. Prevalence of antimicrobial resistance among the 40 *E. coli* isolates analyzed in this study. Minimum inhibitory concentrations (MICs) were interpreted according to EUCAST 2025 and CLSI 2024 clinical breakpoints. Abbreviations: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ETP, ertapenem; AZT, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMI, amikacin; D, doxycycline; TGC, tigecycline; NIT, nitrofurantoin; FOS, fosfomycin; TMP-SXT, trimethoprim-sulfamethoxazole; CL, colistin; CHL, chloramphenicol; FQR, fluoroquinolone resistance.

2.1.2. Virulence Traits

The screening of virulence factors associated with enhanced urinary tract colonization indicated that 18 out of the 40 isolates (45%) met the criteria for UPEC status, defined by the presence of at least three of the specific virulence genes (*chuA*, *fyuA*, *vat*, and *yfcV*). Individually, *chuA*, *fyuA*, *vat*, and *yfcV* were detected in 77.5%, 80%, 5% and 52.5% of the isolates, respectively.

Furthermore, 72.5% of the isolates were classified as ExPEC based on the presence of at least two of the five key virulence markers: *papAH*, *sfa/focDE*, *afa/draBC*, *kpsM* II and *iutA*. The individual prevalence of these genes among the isolates was 40%, 0%, 30%, 57.5%, and 77.5%, respectively (Table S1).

2.1.3. Clonal Groups

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Using the PCR method described by Clermont et al. [16,17], five *E. coli* phylogroups were identified among the 40 isolates. Phylogroups B2 and D were the most prevalent, accounting for 52.5% and 22.5% of the isolates, respectively. The remaining isolates belonged to phylogroups B1 (12.5%), A (7.5%), and F (5%) (Table 1).

Presumptive identification of the pandemic clonal complex (CC)131 was performed via PCR screening for the *rfb*O25 and *fli*C$_{H4}$ gene markers, with 17 out of 40 isolates (42.5%) testing positive for both. Additionally, the *fli*C$_{H5}$ gene, typically associated with clones such as ST1193, was determined in one NLF isolate (2.5%) (Table S1).

Clonotyping identified 14 distinct *fumC-fimH* combinations. Notably, 42.5% of the isolates exhibited clonotype CH40-30, which is typically associated with CC131. The second most prevalent clonotype, CH26-5, was detected in 15% of the isolates. Notably, UPEC status was associated with phylogroup B2 isolates, whereas ExPEC status was linked to both B2 and D isolates (Table 1).

Table 1. Clonotypes determined among the 40 *E. coli* isolates.

Phylogroup	Clonotype (CH)[a]	No isolates	UPEC status	ExPEC status	FQR
B2	CH40-30	17	15	16	17
	CH14-64	1	1	1	1
	CH108-Neg	2	2	2	1
	CH1012-Neg	1	0	0	1
D	CH26-5	6	0	6	1
	CH26-Neg	3	0	3	0
B1	CH65-27	2	0	0	0
	CH27-54	1	0	0	0
	CH65-32	1	0	0	0
	CH7-604	1	0	0	0
A	CH11-54	1	0	1	1
	CH11-Neg	1	0	0	1
	CH7-94	1	0	0	0
F	CH88-Neg	2	0	0	2

[a]Eight distinct *fimH* alleles were determined within the 40 *E. coli*.
(Neg): Nine isolates showed no amplification of the 489-bp internal sequence [18].

2.1.3.1. Characterization of Clonal Complex (CC) 131 Isolates

The most prevalent clonal group O25b:H4-B2 (CH40-30) CC131, identified in 17 isolates, exhibited FQR (100%), carriage of *bla*$_{CTX-M-15}$ (100%), and virulence profiles accomplishing ExPEC (16 of 17, 94.1%) and UPEC (15 of 17, 88.2%) status (Table 1). Remarkably, one CC131 isolate displayed non-susceptibility to ertapenem, though the underlying genetic mechanism could not be determined. High levels of resistance were observed against tobramycin (16 isolates, 94.1%), amoxicillin/clavulanic acid (15 isolates, 88.2%), sulfamethoxazole (13 isolates, 76.5%), gentamycin (13 isolates, 76.5%), and doxycycline (10 isolates, 58.8%).

Using the virotyping scheme proposed by Dahbi et al. [19], virotype F was identified in seven isolates (41.2%), while virotype E was detected in three isolates (17.6%). Among the extraintestinal virulence markers included in this scheme, the specific pilus tip adhesin molecule type II associated with pyelonephritis (*papGII*) was present in all isolates, the secreted autotransporter toxin (*sat*) was detected in 14 isolates (82.3%), and the K5 group II capsule (*kps*M II-K5) was found in 10 isolates (58.8%). Notably, co-occurrence of *pap*GII, *cnf*1 (cytotoxic necrotizing factor 1), and *hly*A (α-hemolysin) were observed in three of virotype E isolates (17.6%) (Table 2).

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Table 2. Phenotypic resistance and virulence profile of the 17 CC131 *E. coli* isolates.

Isolate	UPEC status[a]	ExPEC status[b]	Virotype[c]	Phenotypic resistance[d]	Virulence genes profile
Ec4	+	+	NT	AMP, CXM, CTX, CAZ, AZT, NAL, CIP, TOB, AMI, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *papGII*
Ec5	+	+	NT	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, TOB, AMI, D, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *papGII*
Ec8	+	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, AMI, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*
Ec10	+	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*
Ec19 a	+	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*
Ec20 a	+	+	E	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII, cnfI, hlyA*
Ec22	+	+	F	AMP, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*
Ec23	+	-	NT	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, TOB, AMI, D, NIT, TMP-SXT	*fyuA, yfcV, chuA, papAH, papGII*
Ec28	+	+	NT	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, sat, papGII*
Ec30	+	+	NT	AMP, AMC, CXM, CTX, FOX, CAZ, ETP, AZT, NAL, CIP, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, sat, papGII*
Ec31	+	+	NT	AMP, AMC, CXM, CTX, FOX, CAZ, AZT, NAL, CIP, GEN, TOB, D, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, sat, papGII*
Ec41	-	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, D, TMP-SXT	*chuA, iutA, kpsMII*-K5, *sat, papGII*
Ec43	+	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, D TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*

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Ec45	+	+	NT	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, AMI, D, TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, sat, papGII*
Ec52	+	+	F	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB, D TMP-SXT	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII*
Ec55 a	+	+	E	AMP, AMC, CXM, CTX, CAZ, AZT, NAL, CIP, GEN, TOB	*fyuA, yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII, cnfI, hlyA*
Ec57	-	+	E	AMP, AMC, CXM, CTX, FOX, CAZ, AZT, NAL, CIP, GEN, TOB, D, TGC, FOS, TMP-SXT, CHL	*yfcV, chuA, papAH, iutA, kpsMII*-K5, *sat, papGII, cnfI, hlyA*

[a]UPEC status. + positive for ≥3 of the following genes: *chuA*, *fyuA*, *vat*, and *yfcV* [20]. [b]ExPEC status. + positive for ≥2 of of these five markers: *papAH*, *sfa/focDE*, *afa/draBC*, *kpsMII* and *iutA* [21]. [c]Virotypes following the protocol established by Dahbi et al. [19], based on the presence or absence of specific extraintestinal virulence factors (*afa/draBC*, *afa* operon FM955459, *iroN*, *sat*, *ibeA*, *papGII*, *papGIII*, *cnf1*, *hlyA*, *cdtB*, *kpsMII*-K1, -K2 and -K5). [d]Abbreviations: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ETP, ertapenem; AZT, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMI, amikacin; D, doxycycline; TGC, tigecycline; NIT, nitrofurantoin; FOS, fosfomycin; TMP-SXT, trimethoprim-sulfamethoxazole; CL, colistin; CHL, chloramphenicol.

2.1.3.2. In silico characterization of ST1193 isolate

The NLF isolate B2-CH14-64 underwent WGS and *in silico* analysis using bioinformatics tools from the Center for Genomic Epidemiology. Table 3 summarizes its genomic characteristics.

Serotyping analysis using SerotypeFinder identified the O75:H5 serotype while CHTyper confirmed its clonotype as CH14-64. Multilocus sequence typing (MLST) based on the seven-gene of the Atchman scheme assigned the sequence type (ST)1193, while the alternative MLST scheme using eight genes (*dinB*, *icdA*, *pabB*, *polB*, *putP*, *trpA*, *trpB*, and *uidA*) [22] predicted ST53. Core genome MLST (cgMLST) analysis, based on 2,513 loci, assigned the isolate to cgST140226.

Resistance gene profiling using ResFinder confirmed the presence of $bla_{\text{CTX-M-15}}$, along with chromosomal mutations associated with FQR (*gyrA* p.S83L, *gyrA* p.D87N, *parC* p.S80I, and *parE* p.L416F), consistent with the observed phenotypic resistance. Additionally, acquired resistance genes, including *aac(3)-IIa*, *aac(6')-Ib-cr*, $bla_{\text{OXA-1}}$, and *catB3* were identified.

Virulence profiling using VirulenceFinder identified a broad array of virulence factors, including *vat*, *fyuA*, *yfcV*, *chuA*, *iutA*, and *kpsM* II, confirming its UPEC and ExPEC status. Plasmid analysis revealed the presence of IncI1-I(Alpha) and IncF [F-:A1:B10] plasmids, along with small Col-like plasmids.

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Table 3. *In silico* characterization and phenotypic resistance of the ST1193 genome.

ID Code for isolate/genome[a]	Ec1a/ LREC-468
O:H antigens[b]	O75:H5
ST#1 / ST#2[c]	1193/53
cgMLST[d]	140226
Acquired resistances and point mutations (in bold)[e]	*aac(3)-IIa*, *aac(6')-Ib-cr*, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *catB3* ***gyrA* p.S83L, *gyrA* p.D87N, *parC* p.S80I, *parE* p.L416F**
Plasmid content: Inc. group [pMLST][f]	IncF [F-:A1:B10], IncI1-I [ST Unknown], ColBS512-like
Virulence genes[g]	*aslA, chuA, cia, csgA, fdeC, fimH, fyuA, gad, iha, irp2, iucC, iutA, kpsE, kpsMII_K1, neuC, nlpI, ompT, papA_F43, sat, shiA, sitA, terC, tia, usp, vat, yehA, yehB, yehC, yehD, yfcV*
Phenotypic resistance[h]	AMP, AMC, CXM, CAZ, CTX, ATM, NAL, CIP, GEN

[a]Isolate and genome (LREC) identification. [b]O and H antigen prediction with SerotypeFinder 2.0.1 [c]Sequence types (ST#1 and ST#2) based on two different MLST schemes *E. coli* #1 and *E. coli* #2, respectively, and retrieved with MLST 2.0.9. [d]Core genome ST obtained with cgMLSTFinder1.2 Software run against the Enterobase database. [e]Acquired antimicrobial resistance genes retrieved using ResFinder 4.6: Acquired resistance genes: beta-lactam: *bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$; aminoglycosides: *aac(3)-IIa*; phenicols: *catB3*; fluoroquinolones: *aac(6')-Ib-cr*. Point mutations: quinolones and fluoroquinolones: *gyrA* S83L: TCG-TTG, *gyrA* D87N: GAC-AAC, *parC* S80I: AGC-ATC, *parE* L416F: CTT-TTT. [f]Plasmid content retrieved using PlasmidFinder 2.1 [g]Virulence determinants via VirulenceFinder 2.0: *aslA*: putatitive sulfatase; *chuA*: outer membrane hemin receptor; *cia*: colicin ia; *csgA*: curlin major subunit CsgA; *fdeC:* intimin-like adhesin FdeC; *fimH:* type 1 fimbriae; *fyuA:* siderophore receptor; *gad*: glutamate decarboxylase; *iha*: adherence protein; *irp2*: high molecular weight protein 2 non-ribosomal peptide synthetase; *iucC*: aerobactin synthetase; *iutA*: ferric aerobactin receptor; *kpsE*: capsule polysaccharide export inner-membrane protein; *kpsMII_K1*: polysialic acid transport protein group 2 capsule; *neuC*: polysialic acid capsule biosynthesis protein; *nlpI*: lipoprotein NlpI precursor; *ompT*: outer membrane protease (protein protease 7); *papA_F43*: major pilin subunit F43; *sat*: secreted autotransporter toxin; *shiA*: homologs of the Shigella flexneri SHI-2 pathogenicity island gene shiA; *sitA*: iron transport protein; *terC*: tellurium ion resistance protein; *usp*: uropathogenic specific protein; *vat*: vacuolating autotransporter toxin; *yehA*: outer membrane lipoprotein, YHD fimbriael cluster; *yehB*: usher, YHD fimbriael cluster; *yehC*: chaperone, YHD fimbriael cluster; *yehD*: major pilin subunit, YHD fimbriael cluster; *yfcV*: fimbrial protein. [h]Abbreviations: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; AZT, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin.

To investigate the genomic relationship between the Algerian ST1193 isolate and five ST1193 recently recovered from a hospital in Northwest Spain, we performed a single nucleotide polymorphism (SNP) comparison of their core genomes, which represented 95.65% of the reference genome LREC-269 (5.4Mb). The analysis was conducted using CSI Phylogeny 1.4 (Figure 2A; Table S3). The Algerian ST1193 isolate (LREC-468) exhibited a minimum distance of 78 SNPs from LREC-269 and a maximum distance of 128 SNPs from LREC-270.

Additionally, on December 20, 2024, we accessed Enterobase (https://enterobase.warwick.ac.uk/) to search for ST1193 genomes assigned to cgST140226. We retrieved one genome (ESC_RA5887AA), part of BioProject PRJEB21277, registered in 2020 by the University of Oxford. A subsequent SNP comparison analysis (Figure 2B, Table S4), including the Algerian (LREC-468), five Spanish and ESC_RA5887AA ST1193 genomes, revealed that 82.6% of nucleotide positions in the reference genome (LREC-269) were present in all analyzed genomes. The Algerian LREC-468 genome exhibited 55 SNP compared to ESC_RA5887AA.

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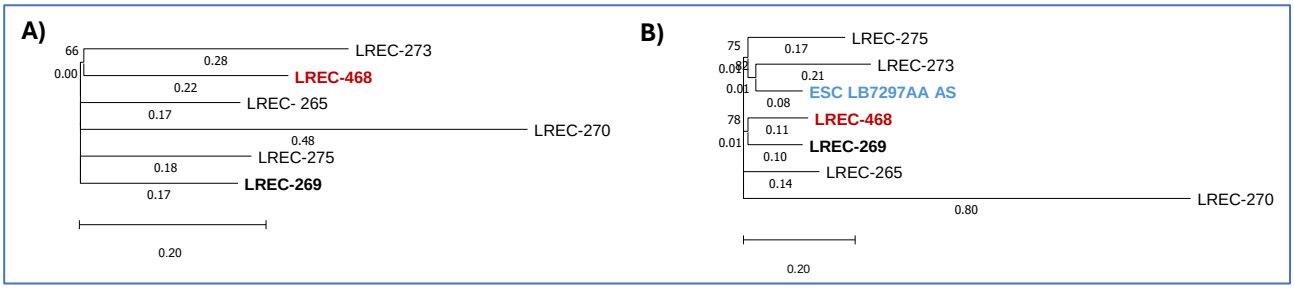

Figure 2. Phylogenetic dendrogram based on whole-genome SNP analysis.

The phylogenetic tree was constructed with the CSI Phylogeny version 1.4 (CGE, https://cge.cbs.dtu.dk/services/CSIPhylogeny/ with the following parameters: min. depth at SNP positions ×10; min. relative depth at SNP positions: ×10; min. distance between SNPs (prune): 10 bp; min. SNP quality: 30; min. read mapping quality: 25, a min. Z-score of 1.96 and by ignoring heterozygous SNPs). Branch values represent substitutions per site.

(A) Phylogenetic dendrogram based on SNP counts per substitution within the core genome of the Algerian and Spanish ST1193 isolates. The WGS comparison resulted in a core genome covering 95.65% of the reference genome LREC-269 (5.4Mb).

(B) Phylogenetic dendrogram incorporating the Algerian, Spanish and ESC_RA5887AA ST1193 isolates, showing SNP counts per substitution. The WGS comparison resulted in a core genome covering 82.6% of the reference genome LREC-269 (5.4Mb).

2.2. Klebsiella Pneumoniae Collection

Seventeen *K. pneumoniae* isolates were recovered from patients across three healthcare facilities in the Tebessa region of Algeria, comprising five isolates from male and 12 from female patients. Sixteen isolates tested positive for the *kp50233* species-specific gene. The single negative isolate was confirmed as *K. pneumoniae* using the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF).

2.2.1. AST and Genotypic Characterization of *bla* Genes

All isolates exhibited resistance to ampicillin, cefuroxime, cefotaxime, and aztreonam. Additionally, a majority (≥ 60%) were resistant to ceftazidime, ciprofloxacin, fosfomycin, sulfamethoxazole, and amoxicillin-clavulanic acid. Notably, two isolates (11.8%) demonstrated resistance to ertapenem (Figure 3). Overall, all isolates were classified as MDR, and 94.1% exhibited FQR.

PCR analysis was conducted to detect the presence of $bla_{ESBL/AmpC/CARBA}$ genes. The CTX-M-15 gene was identified in 94.1% of the isolates, with one iso-late harbouring the CTX-M-194 variant. Notably, the bla_{NDM} gene was identified in two *K. pneumoniae* isolates that exhibited ertapenem resistance, which also carried the CTX-M-15 gene. Additionally, two isolates possessed both the CTX-M-15 and SHV-148 genes. No isolates tested positive for *mcr* (1 to 5) genes, nor did they exhibit polymyxin resistance (Table S2).

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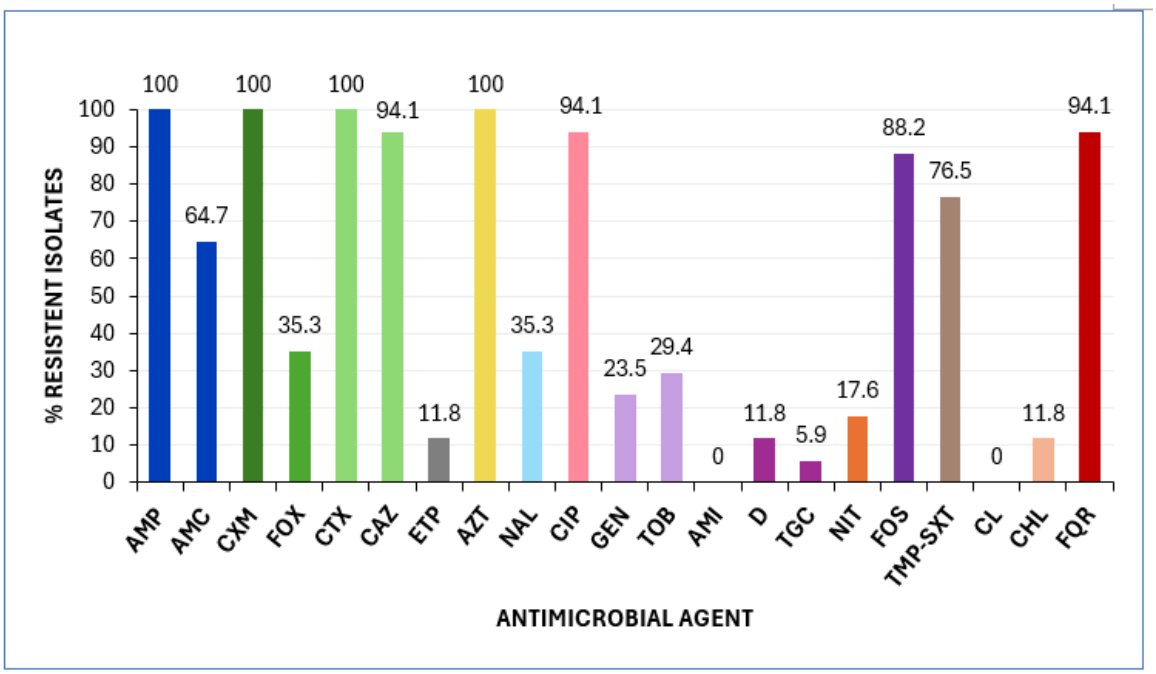

Figure 3. Prevalence of antimicrobial resistance among the 17 *K. pneumoniae* analyzed in this study.MICs were interpreted according to EUCAST 2025 and CLSI 2024 clinical breakpoints. Abbreviations: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ETP, ertapenem; AZT, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMI, amikacin; D, doxycycline; TGC, tigecycline; NIT, nitrofurantoin; FOS, fosfomycin; TMP-SXT, trimethoprim-sulfamethoxazole; CL, colistin; CHL, chloramphenicol; FQR, fluoroquinolone resistance.

2.1.2. Virulence Traits

The hypermucoviscous (HMV) phenotyping of the 17 *K. pneumoniae* isolates was assessed using the string test described by Shon et al. [23]with modifications on four different culture media: Mueller-Hinton (MH) agar, MacConkey (ML) agar, trypticase soy agar (TSA), and Columbia agar (5% sheep blood). Among the isolates, four were identified as HMV-positive in at least two of the tested culture conditions (Table 4 and S2). Notably, two isolates exhibited the HMV-positive phenotype across all culture conditions.

PCR screening was conducted to detect virulence genes commonly associated with hypervirulent *K. pneumoniae* (hvKp) [24]. All isolates tested negative for *iroB*, *peg-589*, *peg-1631*, and *rmpA2* genes. However, over 50% were positive for *terB* and *rpmA*. Notably, the two *K. pneumoniae* isolates exhibiting the HMV phenotype across all culture conditions tested positive for *iucA*, *peg-344*, and *rmpA* genes, and co-harbored both *bla*$_{NDM}$ and *bla*$_{CTX\text{-}M\text{-}15}$ (Table 4).

Table 4. Main virulence and AMR traits of the four phenotypic hypermucoviscous *K. pneumoniae*.

	Hypermucoviscous Phenotype (HMV) [a]						
Isolate	**ML**	**MH**	**TSA**	**CA**	**Phenotypic resistance[b]**	**Virulence genes[c]**	***bla* genes**
KP6b	+	-	+	-	AMP, CXM, CTX, CAZ, AZT, CIP, FOS	*terB*	CTX-M-15
KP10c	+	+	+	+	AMP, AMC, CXM, FOX, CTX, CAZ, ETP, AZT, NAL, CIP, GEN, TOB, NIT, FOS	*iucA*, *peg-344*, *rmpA*	NDM, CTX-M-15

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KP16	+	+	+	+	AMP, AMC, CXM, FOX, CTX, CAZ, ETP, AZT, NAL, CIP, GEN, TOB, TGC, NIT, FOS, TMP-SXT, CHL	*iucA, peg-344, rmpA*	NDM, CTX-M-15
KP20a	+	+	+	-	AMP, CXM, CTX, CAZ, AZT, CIP, FOS, TMP-SXT	*terB*	CTX-M-15

[a]The HMV phenotype was evaluated using Mueller-Hinton (MH) agar, MacConkey (ML) agar, trypticase soy agar (TSA), and Columbia agar (5% sheep blood) (CA) medium culture. + and – indicates positive or negative result, respectively. [b]AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; ETP, ertapenem; AZT, aztreonam; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; TGC, tigecycline; NIT, nitrofurantoin; FOS, fosfomycin; TMP-SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol. [c] *iucA*: aerobactin siderophore biosynthesis, *rmpA*: regulator of the mucoid phenotype via increased capsule production, *peg-344*: putative transporter, *terB*: tellurite resistance.

3. Discussion

Urinary tract infections (UTIs) continue to represent a significant public health concern globally, with the pathogens *E. coli* and *K. pneumoniae* being the primary causative agents [2,3]. Particularly in Algeria, UTIs and pyelonephritis were reported as the fifth leading cause of death in 2021, accounting for 1,290 fatalities. *E. coli* and *K. pneumoniae* were the primary pathogens responsible for 40.5% of the total deaths (370 and 153, respectively). Among these, 84% of deaths were attributed to resistant *E. coli* and *K. pneumoniae* (321 and 117, respectively) [25]. Given the significant role of these bacteria in the etiology of UTIs, their global contribution to the dissemination of MDR, and the limited data on their impact in Algeria, this study aimed to investigate the molecular characteristics of *E. coli* and *K. pneumoniae* exhibiting ESC resistance associated with UTIs in the province of Tebessa, an Algerian city where data on such infections remains scarce.

CTX-M-15 and OXA-48-like enzymes are the most prevalent and widely disseminated ESBLs and carbapenemases, respectively, posing a significant global public health threat [26,27]. In this study, *bla*$_{\text{CTX-M-15}}$ was detected in 87.5% of *E. coli* isolates, a prevalence similar to that reported in other regions of Algeria for ESBL-producing *E. coli* in UTIs [28,29]. The *bla*$_{\text{OXA-48}}$ carbapenemase gene, which is endemic in North Africa and highly reported in Algeria [30], was found here in three *E. coli* isolates.

Notably, we identified two *E. coli* isolates *bla*$_{\text{CTX-M-27}}$, which also harbored *bla*$_{\text{OXA-48}}$; this would represent the first report of the CTX-M-27 variant in Algeria, whose emergence and global spread have been reported in regions such as Japan and Europe [26,31]. The third OXA-48 *E. coli* analyzed here showed a co-carriage of CMY-59. Furthermore, we observed FQR in 62.5% of ESC-producing *E. coli*, which aligns with findings from Sétif [28] but is higher than the 30% resistance prevalence reported by Zenati et al [29]in Tlemcen, Algeria.

The *E. coli* population is classified into distinct phylogenetic groups, namely A, B1, B2, C, D, F, and G, along with cryptic clades [16,17]. UPEC isolates predominantly belong to phylogroups B2 and D, which are typically associated with the carriage of a higher number of virulence genes compared to other phylogroups [32,33]. Accordingly, our ESC-producing *E. coli* isolates were classified into A, B1, B2, D, and F, with B2 being the most prevalent (52.5%), notably including high-risk clones such as CC131 and ST1193. Phylogroup D was the second, comprising 22.5% of the isolates. Of the 21 B2 isolates, 19 met the criteria for UPEC status, and 20 for ExPEC status. Additionally, all nine phylogroup D isolates fulfilled the criteria for ExPEC status.

The CC131 and ST1193 are reported as the most prevalent among FQ and cephalosporin-resistant *E. coli* isolates globally, commonly linked to MDR UTIs [7,8,34,35]. The CC131 lineage of *E. coli* has diversified into three clades: A and B, which are susceptible, and C, which exhibits FQ/cephalosporin resistance. Clade C, particularly the *fimH*30 variant (CH40-30 clonotype), is the most widely distributed. Within clade C, subclade C1 (H30*R*) includes FQR non-ESBL producers,

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whereas subclade C2 (H30*Rx*) is characterized by the presence of both FQR and the *bla*$_{CTX-M-15}$ gene [36].

In this study, we used *rfb*O25b, *fliC*$_{H4}$, *fliC*$_{H5}$, and the B2 phylogroup as PCR screening markers for CC131, along with the clonotyping, as previously described [7]. This approach allowed us to identify 17 O25b:H4-B2 *E. coli* (42.5%), all assigned to CC131 and exhibiting the CH40-30 clonotype. Moreover, all isolates displayed FQR and carried the *bla*$_{CTX-M-15}$ gene, classifying them within subclade C2 [36].

CC131 has been reported in various sources in Algeria, including human clinical samples [37,38], uropathogenic *E. coli* from non-hospitalized patients [39], fish [40], food [41], and wildlife [42], indicating its widespread distribution across the country. However, detailed molecular characterization of CC131 in Algeria remains limited. Our study provides a molecular characterization of CC131, focusing on resistance and virulence gene profiles. Thus, we found that 88.2% and 94.1% of CC131 isolates exhibited UPEC and ExPEC status, respectively. Furthermore, analysis of extraintestinal virulence factors following Dahbi et al [19] scheme, identified two distinct virotypes: F (*sat, papG II, kpsM II-K5*) in 41.2% of isolates and E (*sat, papG II, cnf1, hlyA, kpsM II-K5*) in 17.6%. Notably, the remaining seven FQR, CTX-M-15 O25b:H4-B2-CC131 (CH40-30) isolates, with the carriage of only two virulence genes within the virotype scheme, could not be virotyped.

The ST1193 clone is considered an emerging pathogenic lineage of *E. coli* [43,44] typically associated with the O75 serotype, *fimH*64 type 1 pili, and either the K1 or K5 capsular types. This clone is also characterized by FQR and lactose non-fermentation [8]. Although ST1193 has been reported in Europe [6,7,45,46], Asia [47–49], and the United States [50], its detection in Africa remains limited [51–53]. Notably, in this study, we identified one ST1193 isolate, which was lactose non-fermenting, FQR, and a CTX-M-15-producer. Genomic analysis revealed key features consistent with globally disseminated ST1193 isolates [6–8]. Specifically, the Algerian isolate carried the K1 capsular type and harbored mutations in *gyrA* (D87N, S83L) and *parC* (S80I), along with *parE* (L416F). Plasmidome analysis identified the presence of IncF [F-:A1:B10], IncI1-I, and ColBS512-like plasmids. A large-scale genomic study of ST1193 [43] classified the ST1193 clone into distinct clades based on capsule type (K1 or K5) and the presence of specific IncF plamids: clade A (K5 capsule with F−:A1:B20 plasmids), subclade B1 (K1 capsule with F−:A1:B10 plasmids) and subclade B2 (K1 capsule with F−:A1:B1 plasmids) [8,43]. According to this classification, our ST1193 isolate belongs to subclade B2.

A core genome SNP comparison between our Algerian ST1193 isolate (LREC-468) and previously characterized ST1193 isolates from a Northwest Spanish hospital [6] revealed a minimun distance of 78 SNPs with LREC-269. We then queried Enterobase for genomes assigned to cgST140226 and retrieved a single genome (ESC_RA5887AA) from BioProject PRJEB21277 (University of Oxford, 2020), which differed by only 55 SNPs.

cgMLST, which analyzes 2,513 soft-core genes, offers significantly higher resolution than MLST, making it a powerful tool for tracing transmission dynamics within outbreaks and defining population structures across different levels, including the genus level [54]. Interestingly, the detection of the same cgST in a genome presumably originating from Ireland (based on the metadata provided in the BioProject of this genome) suggests a noteworthy finding, especially considering the geographical distance from Algeria.

Phylogroup D was the second most prevalent in our *E. coli* collection, comprising 22.5% of the isolates, all classified as ExPEC. These isolates exhibited either the *fum*C26 *fim*H5 or *fum*C26 *fim*H-negative clonotypes (no amplification of the 489-bp internal sequence of the clonotype scheme) [18]. According to EnteroBase, all genomes assigned to phylogroup D (Clermont scheme) with *fum*C26 *fim*H5/negative are associated with CC38, which includes ST38, a high-risk MDR clone linked to UTI and bloodstream infections, as well as the global spread of OXA-48 [27]. ST38 has also contributed to the emergence of nosocomial and community-acquired OXA-244-producing *E. coli* ST38 in Europe [55,56]. Additionally, putative inter-host and host-environment transmission events within ST38, where genomes differed by <35 SNPs, underscore its role in maintaining and disseminating AMR genes [57]. In this study, the two D-CH26-Neg isolates carried both OXA-48-like and CTX-M-27,

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while the one D-CH26-5 isolate harboured OXA-48-like and CMY-59. ST38 OXA-48-producing *E. coli* has previously been detected in Algeria in white stork (*Ciconia ciconia*) migratory birds [58], river water [59] human clinical samples [60], and broilers [61]. Our findings further highlight the role of CC38 in the spread of AMR in this region.

All *K. pneumoniae* isolates, except one, carried the *bla*$_{\text{CTX-M-15}}$ gene. In addition, two ertapenem-resistant isolates tested positive for *bla*$_{\text{NDM}}$ gene. The co-occurrence of carbapenem and ESBL resistance in the same isolate significantly complicates treatment options and raises concerns about therapeutic failures. Specifically, *K. pneumoniae* harboring both *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{NDM}}$ gene was previously detected in two urine samples from a patient hospitalized at Annaba University hospital (Algeria) in 2014 [62]. Further studies have reported the widespread presence of *bla*$_{\text{CTX-M-15}}$ in Algeria, with a high prevalence among ESBL-producing *K. pneumoniae* isolates in clinical settings, including the University Hospital Establishment of Oran, where nearly all ESBL-positive isolates carried *bla*$_{\text{CTX-M-15}}$ [63]. Additionally, *bla*$_{\text{CTX-M}}$ *K. pneumoniae* isolates were identified in the Regional Military University Hospital of Oran, where 37.5% of isolates were ESBL producers [14]. Carbapenem-resistant *K. pneumoniae* strains carrying *bla*$_{\text{CTX-M-15}}$ have also been documented in Annaba Hospital, where OXA-48 and KPC-2 carbapenemase-producing isolates were identified in urology patients [13]. These findings underscore the ongoing emergence of multidrug-resistant *K. pneumoniae* in Algerian hospitals, necessitating enhanced surveillance and infection control measures.

In terms of virulence, *K. pneumoniae* is categorized into two distinct pathotypes: classical (cKp) and hypervirulent (hvKp). cKp is a common cause of hospital-acquired infections particularly in elderly or immunocompromised people, such as pneumonia and UTI, and is known for its ability to acquire multiple AMR genes [64]. By contrast, hvKp is more virulent, and capable of causing severe infections in healthy people such as pyogenic liver abscesses, endophthalmitis, meningitis, septic arthritis, and other unusual infections, often leading to significant morbidity and mortality [65,66]. The definition of hvKp has evolved. Initially, a hypermucoviscous phenotype with a positive string test (>5 mm) was used for identification [23,67], but this method lacks accuracy. Currently, murine infection models remain the gold standard [68], however, their high cost and ethical constraints limit their practicality. Instead, recent evidence supports the presence of five virulence plasmid-associated genes (*peg-344, rmpA, rmpA2, iroB,* and *iucA*) as the most accurate molecular markers for hvKp identification, which offer a feasible diagnostic alternative for clinical and surveillance applications [69,70]. In our *K. pneumoniae* collection, the HMV phenotype was observed in four isolates, though only two were consistently positive across all test conditions. PCR screening of the virulence markers revealed that these two isolates were the only ones carrying three of the five plasmid-associated genes (*peg-344, rmpA,* and *iucA*). Notably, both also harboured the *bla*$_{\text{NDM}}$ and *bla*$_{\text{CTX-M-15}}$ genes, further highlighting their clinical relevance.

Initially, hvKp isolates were susceptible to common antibiotics, including last generation cephalosporins and carbapenems. However, in recent years, the emergence of MDR hvKp has raised significant concerns. Notably, the European Centre for Disease Prevention and Control reported the spread of hvKp ST23 strains carrying carbapenemase genes across multiple EU/EEA countries, highlighting the convergence of hypervirulence and antimicrobial resistance [71]. Although our two HMV *bla*$_{\text{NDM}}$ and *bla*$_{\text{CTX-M-15}}$-producing *K. pneumoniae* possessed only three of the five key virulence plasmid-associated genes (*peg-344, rmpA,* and *iucA*), they exemplify the concerning trend of MDR *K. pneumoniae* strains enhancing their clinical virulence potential. This convergence of resistance and virulence factors poses significant challenges for treatment and infection control strategies.

4. Materials and Methods

4.1. E. coli and K. pneumoniae Collections

This study analyzed 57 non-duplicate isolates, comprising 40 *E. coli* and 17 *K. pneumoniae*, recovered from urine samples of both male (9 *E. coli* and 5 *K. pneumoniae*) and female (31 *E. coli* and 12 *K. pneumoniae*) patients. The samples were obtained from both outpatients and inpatients at three

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healthcare facilities in the Tebessa region of northeast Algeria. These facilities included the Bouguerra Boularess-Bekaria Public Hospital Establishment (EPH) (252 beds, 8 wards), the Khaldi Abdelaziz-Tebessa Maternity Hospital (EPH) (166 beds, 4 wards), and the private laboratory, Elite. Isolates were collected between July 2022 and January 2024.

Following incubation on MacConkey (ML) agar (Oxoid) at 37 °C for 18-24 hours, bacterial identification was performed using the API 20E system (bioMérieux, Algeria) and the Vitek 2 GN system (bioMérieux Inc., Hazelwood, MO, United). Isolates were selected based on extended-spectrum cephalosporin (ESC) resistance using the Vitek 2 AST system (bioMérieux). Selected isolates were stored on slanted nutrient agar (Difco) at room temperature until further molecular analysis at the Reference Laboratory for *E. coli* (LREC, University of Santiago de Compostela, Spain) (Tables S1 and S2).

Species confirmation was conducted by PCR amplification of the β-d-glucuronidase (*uidA*) gene for *E. coli* [15] and the putative acyltransferase (*kp50233*) gene for *K. pneumoniae* [72] (Table S5). When PCR results were inconclusive, bacterial identification was performed using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltonik, Bremen, Germany). A species-level identification was considered reliable only if the obtained score exceeded 2.

Non-lactose fermenting (NLF) *E. coli* were identified phenotypically based on their inability to ferment lactose after overnight incubation on ML agar at 37 °C.

4.2. Antimicrobial Susceptibility Testing (AST)

At the LREC (University of Santiago de Compostela, Spain), antimicrobial susceptibility testing (AST) was performed using the disc diffusion method (Becton Dickinson, Sparks, MD, USA) on Mueller-Hinton (MH) agar (Oxoid, Madrid, Spain). A total of 20 antibiotics were tested: penicillin (ampicillin), penicillin + beta-lactamase inhibitors (amoxicillin-clavulanic acid), non-broad spectrum cephalosporins (cefuroxime); broad-spectrum cephalosporins (cefoxitin, cefotaxime, ceftazidime), carbapenems (ertapenem), monobactams (aztreonam), fluoroquinolones (nalidixic acid, ciprofloxacin), aminoglycosides (amikacin, gentamicin, tobramycin), tetracyclines (doxycycline), glycylcyclines (tigecycline), phosphonic acids (fosfomycin), nitrofurans (nitrofurantoin), folate pathway inhibitors (trimethoprim-sulfamethoxazole), polymyxins (colistin), and amphenicols (chloramphenicol).

AST results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing [73] clinical breakpoints when available, or Clinical & Laboratory Standards Institute [74] as an alternative. Isolates were classified as MDR if they exhibited resistance to at least one drug in three or more antimicrobial categories [75].

4.3. Detection and Typing of Antimicrobial Resistance Genes

The isolates were screened by PCR for specific *bla* genes using TEM, SHV, CMY, and CTX-M-specific primers (Table S6), followed by sequencing as described elsewhere [76]. Additionally, PCR was performed to detect carbapenemases genes (*bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA48}$, *bla*$_{KPC}$, *bla*$_{NDM}$) [77], and mobile colistin resistance genes *mcr-1* to *mcr-5* [78,79] (Tables S6 and S7).

4.4. Molecular Characterization of E. coli: Virulence Traits, Phylogroup, Clonotype and Virotype Assignment

For the molecular characterization of the *E. coli* collection, we followed the workflow scheme proposed by García-Meniño et al. [7]. Briefly, specific virulence-associated genes statistically linked to increased efficiency in urinary tract colonization were tested by PCR. Isolates were classified as UPEC if they harbored ≥3 of the following genes: *chuA*, *fyuA*, *vat*, and *yfcV* [20] (Table S8). The designation of extraintestinal pathogenic *E. coli* (ExPEC) status was attributed to isolates positive for ≥2 of of these five markers: *papAH*, *sfa*/*focDE*, *afa*/*draBC*, *kpsM II* and *iutA* [21] (Table S8).

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The clonal structure of the *E. coli* collection was investigated using the phylogroup assignment method of Clermont et al. [16,17] (Table S9), which differentiates eight *E. coli* phylogroups (A, B1, B2, C, D, E, F, and G). Clonotype (CH) determination was performed by sequencing a 469-nucleotide (nt) internal region of the *fumC* gene (allele derived from MLST) and a 489-nt internal fragment of the *fimH* gene, encoding type 1 fimbrial adhesion [18] (Table S10).

To presumptively identify the pandemic CC131 lineage, we screened for phylogroup B2 along with *rfb*O25, *fliC*$_{H4}$, and *fliC*$_{H5}$. Additionally, the *fliCH5* flagellar-encoding gene is commonly associated with NFL *E. coli* ST1193 (Table S11). Isolates confirmed as O25b:H4-B2-CH40-30 were further characterized for their virotypes according to the protocol established by Dahbi et al. [19], based on the presence or absence of specific extraintestinal virulence factors (*afa/draBC*, *afa* operon FM955459, *iroN*, *sat*, *ibeA*, *papG II*, *papG III*, *cnf1*, *hlyA*, *cdtB*, *kpsM II*-K1, -K2 and -K5) (Tables S12 and S13).

4.5. Phenotypic and Genotypic Detection of Hypervirulent K. pneumoniae

Hypervirulent *K. pneumoniae* (hvKp) were initially screened based on the hypermucoviscous (HMV) phenotype using the string test, as described by Shon et al [23]. String tests were performed on colonies grown on MH agar, ML agar, trypticase soy (TSA) agar (Oxoid, Madrid, Spain), and Columbia agar (5 % sheep blood) (Oxoid, Madrid, Spain) incubated at 37 °C/24 h. A positive string test was defined as the formation of a viscous string ≥5 mm in length when a bacteriological inoculation loop was used to stretch bacterial colonies on an agar plate [23].

Additionally, the presence of molecular markers statistically associated with hvKp was assessed by PCR. Isolates were classified as hvKp if they carried all five hvKp virulence plasmid-associated genes: *peg-344* (putative transporter), *rmpA* and *rmpA2*(regulators of the mucoid phenotype via increased capsule production), *iroB* (salmochelin siderophore biosynthesis), and *iucA* (aerobactin siderophore biosynthesis) [24,69] (Table S14).

4.6. Whole Genome Sequencing (WGS) and Bioinformatics Analysis

The *E. coli* isolate classified as *fliC*$_{H5}$-B2 and CH14-64, was further analyzed by WGS as described by García et al. [80]. Briefly, DNA was extracted with the DNeasey Blood and Tissue Kit (Qiagen, Hilen, Germany) according to the manufacturer's instructions. After extraction, the DNA was quantified by an Invitrogen Qubit fluorimeter (Thermo Fisher Scientific, Massachusetts) and evaluated for purity using a NanoDrop ND-1000 (Thermo Fisher Scientific, Massachusetts). DNA sequencing was performed using Illumina technology with a NovaSeq 6,000 S4PE150 XP system to obtain 150 bp paired-end reads at Eurofins Genomics (Eurofins Genomics GmbH, Konstanz, Germany), after a standard library preparation. The quality of the paired-end Illumina reads was evaluated using FastQC.

The reconstruction of the genome and *in silico* analysis was performed as described elsewhere [81]. Briefly, the raw reads were assembled with the VelvetOptimiser.pl. script implemented in the "on line" version of PLAsmid Constellation NETwork (PLACNETw). The assembled contigs, with genomic size 5.0 Mbp, were analyzed using the bioinformatics tools of the Center for Genomic Epidemiology (CGE) as specified, and applying the thresholds suggested by default when required (minimum identity of 95% and coverage of 60%): for the presence of acquired genes and or chromosomal mutations mediating antimicrobial resistance (ResFinder 4.6.), for identification of acquired virulence genes (VirulenceFinder 2.0), plasmid replicon types (PlasmidFinder 2.1/pMLST 2.0), identification of clonotypes (CHTyper 1.0), and serotypes (SeroTypeFinder 2.0.1). For the phylogenetic typing, two different MLST (2.0.9) schemes were applied. Additionally, cgMLSTFinder1.2 was applied for the core genome multi-locus typing (cgMLST) from the raw reads of the isolate.

To investigate the phylogenetic relationship of the ST1193 isolate sequenced in this study, a comparative analysis was performed using *CSI Phylogeny 1.4* with five previously described ST1193 isolates from a hospital in northwest Spain. The pipeline was run with default parameters, using the

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genome of LREC-269 as the reference for single nucleotide polymorphism (SNP) calling. Bootstrap support for the consensus phylogenetic tree was calculated using 1,000 replicates [82]. The resulting SNP matrix is shown in Table S3. Finally, EnteroBase (https://EnteroBase.warwick.ac.uk/) was queried for ST1193 genomes based on the Achtman 7-gene MLST scheme. Specific core genome MLST (cgMLST) sequences were retrieved, and their raw reads were used for comparative genomic analysis (Table S4).

5. Conclusions

This study provides important insights into the epidemiology of multidrug-resistant (MDR) *E. coli* and *K. pneumoniae* in Algeria, highlighting the presence and diversity of *E. coli* CC131 CH40-30 and reporting, for the first time, *E. coli* ST1193 carrying CTX-M-15 in this region. The detection of virulent *K. pneumoniae* co-harboring carbapenemase and ESBL resistance genes is particularly concerning, as it raises the potential for increased pathogenicity and treatment failure in clinical settings.

The co-occurrence of AMR and virulence factors underscores the need for enhanced surveillance and infection control measures. A simplified surveillance method based on virulence traits in *E. coli* and *K. pneumoniae* is proposed for early detection and outbreak monitoring. Additionally, monitoring high-risk clones such as CC38 and OXA-48 is crucial for preventing further public health threats. A practical lab workflow for identifying high-risk *E. coli* clones associated with UTIs is outlined, emphasizing non-lactose fermenting isolates, AST, and phylogroup/clonotyping.

Given the significant resistance profiles observed, targeted antibiotic optimization programs should focus on rational antibiotic use, improved prescription practices, and enhanced surveillance, especially in low- and middle-income countries (LMICs) where MDR infections pose a major public health burden

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1. Metadata and main traits of the 40 UTI *E. coli* isolates analyzed in this study; Table S2. Metadata and main traits of the 17 UTI *K. pneumoniae* isolates analyzed in this study; Table S3. Pairwise distance matrix calculated from SNP in the the Algerian and Spanish ST1193 genomes; Table S4. Pairwise distance matrix calculated from SNP in the Algerian, Spanish and ESC_RA5887AA ST1193 genomes; Table S5. Targets and primers used for *Escherichia coli* and *Klebsiella pneumoniae* identification; Table S6. Primers used for the detection and/or sequencing of *bla* genes; Table S7. Primers used for the detection of *mcr* genes; Table S8. Targets and primers used to determine the UPEC and ExPEC status; Table S9. Targets and primers used for the phylogroup determination in *E. coli;* Table S10. Targets and primers to determine clonotypes (CH); Table S11. Primers used for the *rbf*O25, H4 (*fliC*$_{H4}$) and H5 (*fliC*$_{H5}$) screening; Table S12. Targets and primers used in the virotype scheme of CC131 isolates; Table S13. Virotype designation scheme for CC131 *E. coli;* Table S14. Targets and primers used for virulence genes of *Klebsiella pneumoniae.*

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Data Availability Statement: The nucleotide sequence of the ST1193 *E. coli* isolate was deposited in the European Nucleotide Archive (ENA) with the following accession ERS22141751 and BioSample SAMEA117078805 codes, as part of BioProject ID PRJEB82513.

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