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Antimicrobial resistance and virulence profiling of *Klebsiella pneumoniae* isolated from urinary tract infections in community and hospital settings in Pakistan

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Abstract: Urinary tract infections (UTIs) caused by multidrug-resistant (MDR) bacteria pose escalating challenges in resource-limited settings. This cross-sectional study addresses critical gaps in molecular surveillance by directly comparing antimicrobial resistance (AMR) genotypes, virulence factors, and resistance phenotypes of *K. pneumoniae* isolates from community (CA-UTI) and hospital-acquired (HA) UTI. in Pakistan. Bacterial identification was performed using standard microbiological techniques and MALDI-TOF MS. Antimicrobial susceptibility was assessed via the Kirby-Bauer disk diffusion and broth microdilution methods, following CLSI guidelines. PCR was employed to detect AMR genes and virulence factors. Of 2,700 urine samples analyzed, 721 (26.7%) tested positive for uropathogens, with *K. pneumoniae* accounting for 128 isolates (17.8%). HA-UTI isolates exhibited significantly higher resistance to amoxicillin-clavulanic acid ($p = 0.0117$) as well as cefotaxime and ceftriaxone ($p = 0.0002$). All isolates remained fully susceptible to colistin and tigecycline. Genotypic analysis revealed HA-UTI isolates carried higher frequencies of ESBL ($bla_{CTXM-15}$: 23.1% vs. 13.5%) and carbapenemase genes (bla_{NDM}: 9.9% vs. 2.7%), alongside tetracycline (*tetB*: 64.8% vs. 54.1%) and fluoroquinolone resistance determinants (*qnrA*: 37.4% vs. 29.7%). Virulence factors *fimH* (32.0%) and *mrkD* (26.6%) were common overall with no statistical difference between CA and HA UTI cases. The burden of AMR particularly in HA-UTI isolates coupled with carbapenemase production underscore the urgent need for hospital-specific infection control and stewardship programs prioritizing carbapenem-sparing regimens in Pakistan.

Keywords: *Klebsiella pneumoniae*, Urinary tract infections (UTIs), Antimicrobial resistance (AMR), Multidrug resistance (MDR), Virulence factors

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INTRODUCTION

The global rise of multidrug-resistant bacteria that is mainly driven by widespread antibiotic misuse represents one of the most pressing public health challenges of the 21st century (Aslam *et al.*, 2020; Issa, 2024; Rasheed *et al.*, 2020). This trend is particularly concerning for managing common infections such as urinary tract infections (UTIs), which are among the most prevalent bacterial diseases worldwide. Recent estimates indicate that UTIs affect approximately 150 million individuals annually, imposing a staggering economic burden of up to $6 billion in direct healthcare costs (Liu *et al.*, 2024). With reference to AMR, the *Klebsiella pneumoniae* has emerged as a particularly concerning pathogen in UTIs. While *Escherichia coli* remains the most frequently isolated uropathogen, *K. pneumoniae* now ranks as the second-leading cause of community- and hospital-acquired UTIs globally (Herrera-Espejo *et al.*, 2024). The virulence mechanisms including biofilm formation, capsular polysaccharides, and fimbriae enable *K. pneumoniae* to persistently colonize the urinary tract (Arafa & Kandil, 2023; Mancuso *et al.*, 2024; Shehata *et al.*, 2024). The threat even becoming more worse due to the rise of multidrug-resistant (MDR) *K. pneumoniae* especially due to the rise of extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant strains which limit treatment options and are often linked with prolonged hospital stays and higher mortality (Arafa & Kandil, 2023; Car *et al.*, 2024).

The clinical burden of UTIs varies significantly between CA-UTI and HA-UTI. CA-UTIs, predominantly caused by *E. coli* (75–95% of cases), show *K. pneumoniae* prevalence rates of 15–20%, often leading to recurrent infections and substantial morbidity (Taha, 2024). In contrast, HA-UTIs are associated with invasive healthcare interventions (e.g., catheterization) and exhibit greater microbial diversity, including MDR pathogens like *Pseudomonas aeruginosa* and *K. pneumoniae* (Al-Sayaghi *et al.*, 2023). HA-UTIs are further complicated by higher resistance rates, with carbapenem resistance exceeding 50% in some regions, largely due to carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{OXA-48}) transmitted via horizontal gene transfer (Car *et al.*, 2024; Kumar *et al.*, 2023). These strains undermine last-line therapies such as cephalosporins, fluoroquinolones, and carbapenems, escalating treatment failures and mortality (Manikandan *et al.*, 2024; Naghavi *et al.*, 2024).

In Pakistan, UTIs caused by MDR *K. pneumoniae* are a growing concern, yet comprehensive data on resistance gene profiles and virulence determinants remain scarce

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(Khatoon *et al.*, 2023). Regional studies, such as a 2021 analysis in Quetta, identified *tetB* (100%), *sul1* (66.7%), and bla_{SHV} (33.3%) in *K. pneumoniae* isolates but lacked integration of AMR and virulence profiling (Fatima *et al.*, 2021). This study addresses these gaps by providing the comparative molecular characterization of *K. pneumoniae* in Pakistan, combining resistance gene profiling with virulence factor analysis in both community and hospital acquired UTI cases. This study will aid in targeted surveillance, infection control measures, and regionally tailored antibiotic guidelines to mitigate the dual threat of resistance and virulence.

MATERIALS AND METHODS

Ethical approval

The study was conducted in tertiary care private and public sector hospitals in Faisalabad and Lahore, Pakistan, between January 2023 and January 2024. Ethical approval for the study was obtained from the Ethics Review Committee (Letter No. GCUF/ERC/2022/5).

Inclusion and exclusion criteria

CA-UTI was defined according to the Infectious Diseases Society of America (IDSA) and European Society for Clinical Microbiology and Infectious Diseases (ESCMID) criteria for uncomplicated UTIs (Bonkat *et al.*, 2018; Hooton *et al.*, 2010). Eligible cases included symptomatic individuals (e.g., dysuria, urgency, fever ≥38°C) with laboratory-confirmed infection, defined as $\geq 10^3$ colony-forming units per milliliter (CFU/mL) of a single uropathogen in midstream urine, accompanied by pyuria (≥10 leukocytes/mm³). To align with World Health Organization (WHO) and CDC surveillance standards for healthcare-associated infections, CA-UTI cases excluded individuals with hospitalization, long-term care residency, dialysis, or invasive urinary procedures (e.g., catheterization) within 30 days prior to symptom onset, ensuring no healthcare exposure could confound classification (WHO, 2022). Additional exclusions for CA-UTI (e.g., recent antibiotic use within 72 hours, urinary instrumentation within 14 days) were applied per IDSA guidelines to avoid misclassification of healthcare-associated or partially treated infections.

HA-UTI was defined using the CDC/National Healthcare Safety Network (NHSN) criteria for catheter-associated and non-catheter-associated UTIs. Cases were restricted to inpatients with microbiologically confirmed infection ($\geq 10^5$ CFU/mL of a uropathogen in catheterized or midstream urine) diagnosed >48 hours after admission, consistent with the NHSN's time-based threshold to distinguish nosocomial from community-onset infections. The HA-UTI cases excluded patients with urinary tract surgery within 30 days, systemic antimicrobial therapy initiated >24 hours pre-culture, or critical illness (e.g., septic shock, ICU admission), as these factors may reflect non-nosocomial or non-infectious etiologies.

Exclusion criteria for both cohorts (pregnancy, urinary tract abnormalities, repeat samples, inability to consent) were applied per international ethical and clinical guidelines to ensure homogeneity and reduce confounding.

Sample collection, isolation and identification

Urine specimens were collected using standardized protocols to minimize contamination and ensure diagnostic accuracy. For CA-UTI cases, midstream clean-catch urine was obtained after instructing patients to cleanse the urethral meatus with a sterile chlorhexidine wipe (females) or saline solution (males), discarding the initial stream before collecting the midstream portion into a pre-labeled and sterile container. The HA-UTI specimens were aseptically collected from indwelling catheters using a needleless syringe after disinfecting the catheter port with 70% ethanol, adhering to CDC aseptic guidelines. All samples were immediately labeled with patient identifiers, collection time/date, and source (midstream/catheter) to ensure traceability.

To preserve microbial viability, specimens were transported to the laboratory within 15 minutes using insulated coolers maintained at 4°C. For unavoidable delays exceeding two hours, samples were stored in urine preservative tubes at 4°C until processing.

The specimens were inoculated onto blood agar (5% sheep blood), cystine-lactose-electrolyte-deficient (CLED) agar, and Sabouraud-dextrose agar (SDA) (Oxoid, UK) using a calibrated 1 µL disposable loop (bioMérieux, France). Plates were incubated aerobically at 37°C ± 1°C for 24-48 hours and inspected daily for colony morphology.

Bacterial identification was performed using biochemical reactions on the VITEK® 2 Compact system (bioMérieux, Marcy-l'Étoile, France) with Gram-negative identification cards, followed by confirmation via MALDI-TOF MS using the VITEK® MS platform (bioMérieux, Marcy-l'Étoile, France).

Molecular confirmation of K. pneumoniae isolates

The bacterial DNA was extracted using the FavorPrep™ Genomic DNA Extraction Kit (Favorgen Biotech Corporation, Taiwan) and stored at −20 °C for further analysis. PCR amplification of the *gyrA* gene was performed using specific primers: forward (CGCGTACTATACGCCATGAACGTA) and reverse (ACCGTTGATCACTTCGGTCAGG). The reaction targeted a 441 bp fragment with an annealing temperature of 55°C. The amplicons were separated by electrophoresis on 1.2% (w/v) agarose gels stained with ethidium bromide and visualized under ultraviolet (UV) light using a UV transilluminator. Product sizes were determined using the GeneRuler 100-bp Plus DNA ladder (Thermo Fisher Scientific, Massachusetts, USA) as a size marker. Selected PCR products were purified using the PCR Product Purification Kit (Favorgen Biotech Corp., Pingtung

County, Taiwan) and sequenced for further confirmation. The obtained sequences were compared against the GenBank database using the BLAST tool.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method with commercially sourced disks (Oxoid, UK). Tested antimicrobial agents included amikacin (AK, 30µg), gentamicin (CN, 10µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), piperacillin-tazobactam (TZP, 100/10µg), cefotaxime (CTX, 30µg), ceftriaxone (CRO, 30µg), imipenem (IPM, 10µg), meropenem (MEM, 10µg), ciprofloxacin (CIP, 5µg), doxycycline (DO, 30µg), nalidixic acid (NA, 30µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75µg), fosfomycin (FOS, 200µg) and nitrofurantoin (F, 300µg).

Minimum inhibitory concentrations (MICs) for amikacin (AK), gentamicin (CN), cefotaxime (CTX), ceftriaxone (CRO), imipenem (IPM), meropenem (MEM), ciprofloxacin (CIP), doxycycline (DO), colistin (CT), and tigecycline (TGC) were determined using broth microdilution. Susceptibility results for both methods were interpreted according to CLSI (2023) guidelines, except for tigecycline, for which FDA breakpoints (susceptible ≤2 µg/mL, intermediate 4 µg/mL, resistant ≥8 µg/mL) were applied (Khurshid *et al.*, 2020). The quality control strains used in this study were *Escherichia coli* ATCC® 25922, *Escherichia coli* ATCC® 35218 and *Pseudomonas aeruginosa* ATCC® 27853.

Screening of antimicrobial resistance and virulence genes

All *K. pneumoniae* isolates underwent screening for ESBL-encoding genes, specifically $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes. Isolates that tested positive for $bla_{\text{CTX-M}}$ were further screened for subtypes, including $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-10}}$, $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$. Additionally, class B beta-lactamases (bla_{NDM}, bla_{VIM}), and class D beta-lactamases ($bla_{\text{OXA-48}}$) were screened. Tetracycline resistance genes (*tet*A, *tet*B), quinolone resistance genes (*qnr*A, *qep*A, *qnr*B and *qnr*S,), and sulphonamide resistance genes (*sul*1, *sul*2) were detected using PCR. Additionally, screening for 16S methylases (*arm*A, *rmt*B-F) and aminoglycoside modifying enzymes (AMEs) such as *aac(6')-Ib*, *ant(2")-Ia*, and *aph(3")-Ib* was conducted. The virulence factor genes (*fim*H, *rmp*A, *mrk*D, *ycf*M) were screened using primers synthesized by Macrogen™ (South Korea). The PCR was performed using primers sequence listed in table S1 in T100 Thermal Cycler.

(Bio-Rad, USA) under the following conditions: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30s at variable temperature (table S1) for 30-45 s and 72°C for 1 min; final extension at 72°C for 107 min. Amplicons were

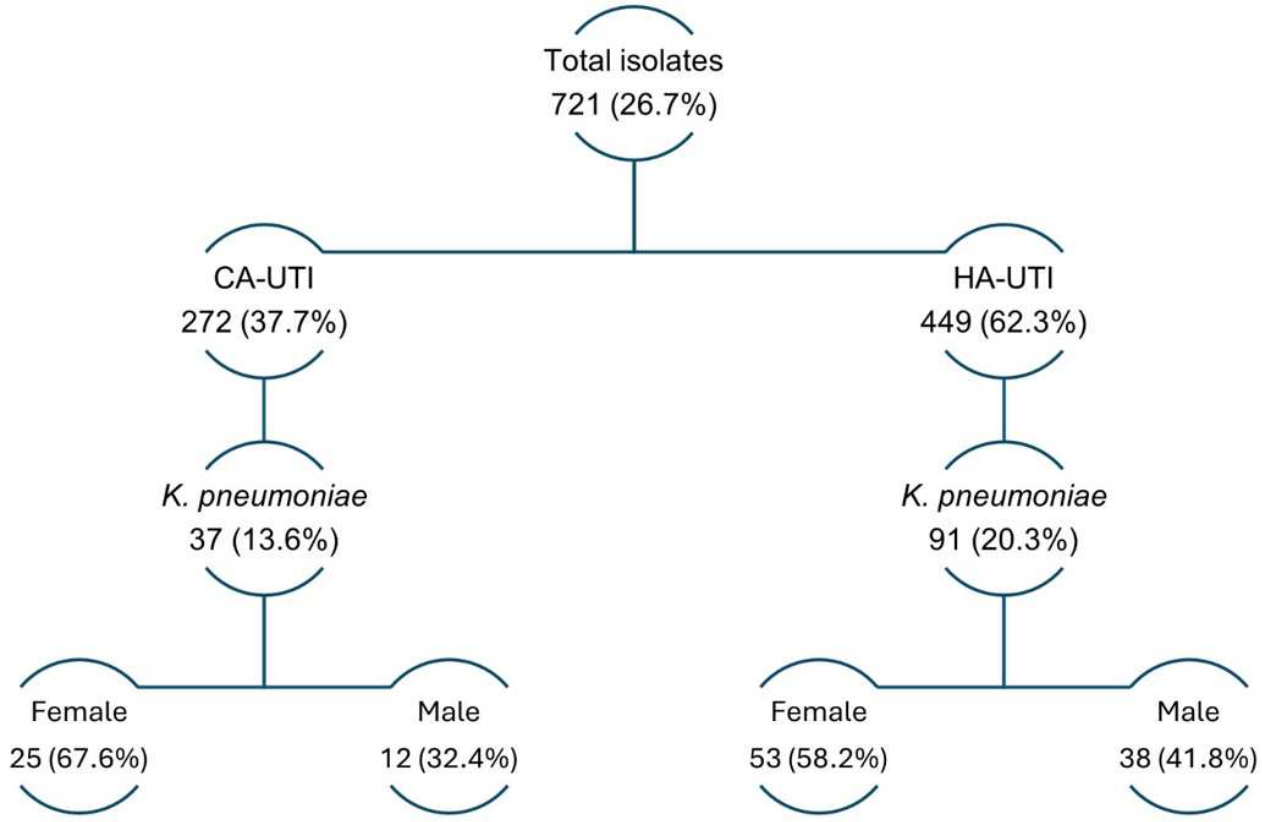

Fig. 1: Prevalence of *K. pneumoniae* among positive HA and CA samples

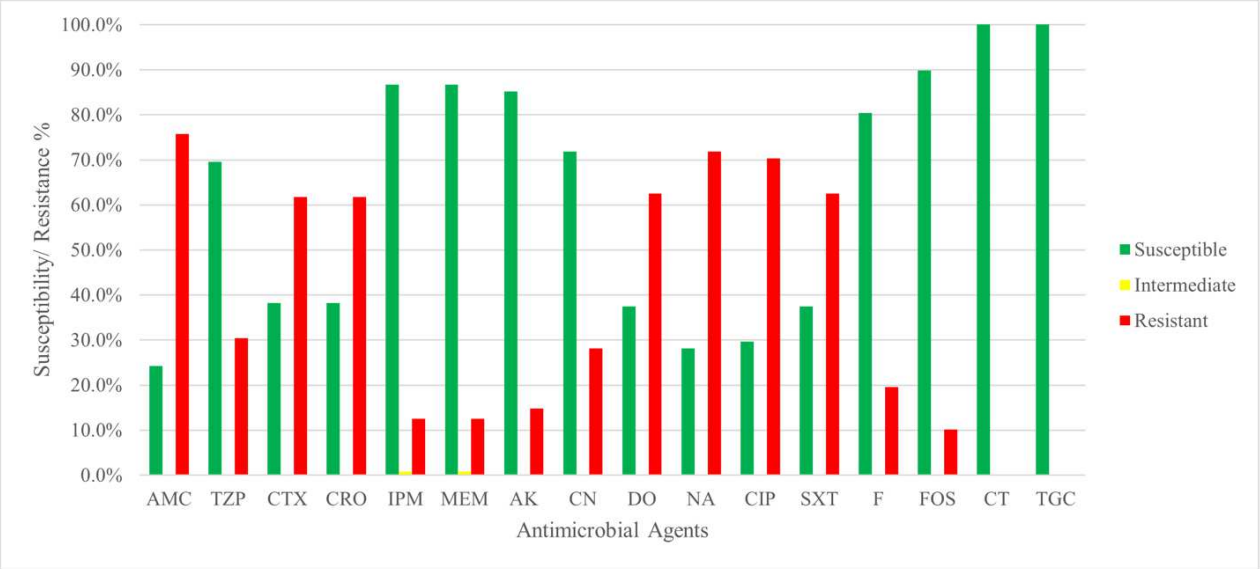

Fig. 2: Overall antimicrobial resistance trends in *Klebsiella pneumoniae* isolates

resolved on 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

RESULTS

A total of 2,700 urine samples (1,350 community-acquired, 1,350 hospital-acquired) were analyzed, with 721 (26.7%) testing positive for bacterial growth. *Klebsiella pneumoniae* was the second most prevalent uropathogen (17.8%, 128/721), following *Escherichia coli* (54.1%, 390/721). *K. pneumoniae* isolates were disproportionately identified in hospital settings, constituting 20.3% (91/449) of hospital-acquired UTIs versus 13.6% (37/272) of community-acquired cases. Female patients predominated overall (60.9%, 78/128), with a pronounced disparity in community isolates (67.6% female vs. 32.4% male) compared to hospital-acquired infections (58.2% vs. 41.8%).

Antimicrobial susceptibility patterns

The antimicrobial resistance profile of *K. pneumoniae* isolates revealed high resistance to amoxicillin-clavulanic acid (75.8%), fluoroquinolones (ciprofloxacin: 71.9%; nalidixic acid: 68.8%), trimethoprim-sulfamethoxazole (65.6%), and doxycycline (62.5%) All isolates remained fully susceptible to colistin and tigecycline (fig. 2). The antimicrobial susceptibility patterns of CA-UTI and HA-UTI isolates showed statistically significant differences in resistance using the Chi-Square Test (χ^2 Test). The results indicate significantly higher resistance in HA-UTI isolates for Amoxycillin-Clavulanic Acid (p=0.0117), Cefotaxime (p =0.0002) and Ceftriaxone (p=0.0002). While carbapenems (Imipenem and Meropenem) showed high susceptibility, no statistically significant difference was observed between CA-UTI and HA-UTI isolates (table 1). In contrast, resistance patterns for aminoglycosides (Amikacin and Gentamicin) and fluoroquinolones (Ciprofloxacin) were comparable between both groups, with p-values greater than 0.05, indicating no significant difference. Aminoglycosides (amikacin: 82.0% susceptible; gentamicin: 71.9%) and older agents like nitrofurantoin (81.3%) and fosfomycin (85.9%) demonstrated notable efficacy. Elevated MIC values for cephalosporins and carbapenems in HA-UTI isolates suggested emerging resistance trends in hospital settings (table 2).

AMR and virulence genes

The distribution of antimicrobial resistance (AMR) determinants and virulence factor genes among *Klebsiella pneumoniae* isolates from community-acquired urinary tract infections (CA-UTI) and hospital-acquired urinary tract infections (HA-UTI) was analyzed. Among the 128 isolates, 37 (28.9%) were CA-UTI, while 91 (71.1%) were HA-UTI (table 3). The prevalence of β-lactamase genes varied between the two groups, with *blaSHV* being significantly more common in HA-UTI isolates (39.6%) compared to CA-UTI isolates (18.9%) (p=0.024998). Other β-lactamase genes, including bla_{TEM} (18.9% vs. 33.0%, p=0.111979), bla_{CTXM} (13.5% vs. 26.4%, p=0.115111), and $bla_{CTXM-15}$ (13.5% vs. 23.1%, p=0.222792), showed higher prevalence in HA-UTI isolates but did not reach statistical significance. No isolates harboring bla_{CTXM-2}, bla_{CTXM-8}, bla_{CTXM-9}, $bla_{CTXM-10}$ and $bla_{CTXM-14}$ were detected in either CA-UTI or HA-UTI samples.

Carbapenemase genes, such as bla_{NDM} (2.7% vs. 9.9%, p=0.169568) and bla_{OXA-48} (5.4% vs. 5.5%, p=0.983965), were present at low frequencies in both groups. Aminoglycoside resistance determinants, including *aac(6')-Ib* (10.8% vs. 9.9%, p=0.875778) and *ant(2")-Ia* (16.2% vs. 16.5%, p=0.970470), were similarly distributed among CA-UTI and HA-UTI isolates.

Table 1: Antimicrobial Susceptibility Patterns in *K. pneumoniae* from Community-Acquired and Hospital-Acquired Urinary Tract Infections

Antimicrobial Agents	CA-UTI (37)			HA-UTI (91)		Total (128)			P-value
	Susceptible n(%)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)	Resistant n(%)	Susceptible n(%)	Resistant n(%)	Intermediate	
Amoxycillin-Clavulanic Acid	15(40.5)	22(59.5)	-	16(17.6)	75(82.4)	31(24.2)	97(75.8)	-	0.0117
Piperacillin-Tazobactam	25(67.6)	12(32.4)	-	64(70.3)	27(29.7)	89(69.5)	39(30.5)	-	0.9235
Cefotaxime	24(64.9)	13(35.1)	-	25(27.5)	66(72.5)	49(38.3)	79(61.7)	-	0.0002
Ceftriaxone	24(64.9)	13(35.1)	-	25(27.5)	66(72.5)	49(38.3)	79(61.7)	-	0.0002
Imipenem	34(91.9)	2(5.4)	1(2.7)	77(84.6)	14(15.4)	111(86.7)	16(12.5)	1(0.8	0.2272
Meropenem	34(91.9)	2(5.4)	1(2.7	77(84.6)	14(15.4)	111(86.7)	16(12.5)	1(0.8)	0.2272
Amikacin	32(86.5)	5(13.5)		77(84.6)	14(15.4)	109(85.2)	19(14.8)		1.0000
Gentamicin	27(73.0)	10(27.0)		65(71.4)	26(28.6)	92(71.9)	36(28.1)		1.0000
Doxycycline	16(43.2)	21(56.8)		32(35.2)	59(64.8)	48(37.5)	80(62.5)		0.5128
Nalidixic Acid	12(32.4)	25(67.6)		24(26.4)	67(73.6)	36(28.1)	92(71.9)		0.6353
Ciprofloxacin	12(32.4)	25(67.6)		26(28.6)	65(71.4)	38(29.7)	90(70.3)		0.8258
Trimethoprim-sulfamethoxazole	18(48.6)	19(51.4)		30(33.0)	61(67.0)	48(37.5)	80(62.5)		0.1443
Nitrofurantoin	31(83.8)	6(16.2)		72(79.1)	19(20.9)	103(80.5)	25(19.5)		0.7208
Fosfomycin	37(100)	0(00)		78(85.7)	13(14.3)	115(89.8)	13(10.2)		0.0355
Colistin	37(100)	0(00)		91(100)	0(00)	128(100)	0(00)		-
Tigecycline	37(100)	0(00)		91(100)	0(00)	128(100)	0(00)		-

Table 2: MIC Distribution of Various Antimicrobial Agents for *Klebsiella pneumoniae* in CA and HA UTIs

Antimicrobials	Isolate source	No. of isolates with MIC of (µg/mL)												MIC_{50}	MIC_{90}
		≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256		
Cefotaxime	CA	NT	NT	15	9	0	7	0	1	1	4	0	0	1	64
	HA	NT	NT	14	11	0	25	19	1	10	11	0	0	4	64
	Total	NT	NT	29	20	-	32	19	2	11	15	-	-	4	64
Ceftriaxone	CA	NT	NT	9	15	0	6	2	0	3	2	0	0	1	32
	HA	NT	NT	11	14	0	22	20	3	5	16	0	0	4	64
	Total	NT	NT	20	29	-	28	22	3	8	18	-	-	4	64
Imipenem	CA	NT	NT	25	9	1	2	0	0	0	0	0	0	0.5	1
	HA	NT	NT	67	10	0	3	3	6	2	0	0	0	0.5	8
	Total	NT	NT	92	19	1	5	3	6	2	-	-	-	0.5	4
Meropenem	CA	NT	NT	28	6	1	1	1	0	0	0	0	0	0.5	1
	HA	NT	NT	68	9	0	3	3	5	3	0	0	0	0.5	8
	Total	NT	NT	96	15	1	4	4	5	3	-	-	-	0.5	8
Amikacin	CA	NT	NT	4	21	7	0	0	2	1	2	0	0	1	16
	HA	NT	NT	7	50	20	0	0	3	6	1	3	1	1	32
	Total	NT	NT	11	71	27	-	-	5	7	3	3	1	1	32
Gentamicin	CA	NT	NT	20	7	0	0	0	4	4	0	2	0	0.5	32
	HA	NT	NT	50	15	0	0	0	12	9	2	3	0	0.5	32
	Total	NT	NT	70	22	-	-	-	16	13	2	5	-	0.5	32
Doxycycline	CA	NT	NT	3	10	3	0	0	0	7	7	7	0	32	128
	HA	NT	NT	5	21	6	0	0	0	22	24	13	0	32	128
	Total	NT	NT	8	31	9	-	-	-	29	31	20	-	32	128
Ciprofloxacin	CA	5	7	0	0	0	6	13	6	0	0	NT	NT	8	16
	HA	9	17	0	0	0	18	24	20	3	0	NT	NT	8	16
	Total	14	24	-	-	-	24	37	26	3	-	NT	NT	8	16
Colistin	CA	31	6	0	0	0	0	0	0	0	0	NT	NT	0.125	0.25
	HA	81	9	1	0	0	0	0	0	0	0	NT	NT	0.125	0.25
	Total	112	15	1	-	-	-	-	-	-	-	NT	NT	0.125	0.25
Tigecycline	CA	34	3	0	0	0	0	0	0	0	0	NT	NT	0.125	0.125
	HA	88	3	0	0	0	0	0	0	0	0	NT	NT	0.125	0.125
	Total	122	6	-	-	-	-	-	-	-	-	NT	NT	0.125	0.125

*The number corresponds to an MIC ≤0.5.

Table 3: Distribution of Antimicrobial Resistance Determinants and Virulence Factors Genes Among *K. pneumonia*

Genes	Community Acquired Isolates (*n*=37) *n*(28.9%)	Hospital Acquired Isolates (*n*=91) *n*(71.1%)	Total Isolates (*n*=128) *n*(%17.8)	P-value
		AMR genes		
bla_{SHV}	7(18.9)	36(39.6)	43(33.6)	0.024998
bla_{TEM}	7(18.9)	30(33)	37(28.9)	0.111979
bla_{CTXM}	5(13.5)	24(26.4)	29(22.7)	0.115111
bla_{CTXM-1}	-	3(3.3)	3(2.3)	0.263733
$bla_{CTXM-15}$	5(13.5)	21(23.1)	26(20.3)	0.222792
bla_{OXA-48}	2(5.4)	5(5.5)	7(5.5)	0.983965
bla_{NDM}	1(2.7)	9(9.9)	10(7.8)	0.169568
aac(6')-Ib	4(10.8)	9(9.9)	13(10.2)	0.875778
ant(2'')-Ia	6(16.2)	15(16.5)	21(16.4)	0.970470
*arm*A	1(2.7)	5(5.5)	6(4.7)	0.498142
*tet*A	12(32.4)	29(31.9)	41(32)	0.950541
*tet*B	20(54.1)	59(64.8)	79(61.7)	0.255300
*qnr*A	11(29.7)	34(37.4)	45(35.2)	0.412262
*qnr*B	5(13.5)	20(22.0)	25(19.5)	0.273488
*qnr*S	10(27.0)	17(18.7)	27(21.1)	0.294098
*sul*1	14(37.8)	43(47.3)	57(44.5)	0.331262
*sul*2	6(16.2)	21(23.1)	27(21.1)	0.388419
		Virulence factors genes		
*fim*H	11(29.7)	30(33)	41(32)	0.883
*rmp*A	7(18.9)	13(14.3)	20(15.6)	0.700
*mrk*D	9(24.3)	25(27.5)	34(26.6)	0.885
*ycf*M	11(29.7)	29(31.9)	40(31.3)	0.979

Table 4: Distribution of Antimicrobial Resistance Genotypes and Virulence Factors among *K. pneumoniae* Isolates

Source	Total Isolates	MIC (µg/mL) Range										AMR genotypes (number of isolates)	Virulence Factors (number of isolates)
		CTX	CRO	IPM	MEM	AK	CN	DO	CIP	CT	TGC		
HA-UTI	91	0.25-64	0.5-64	0.25-32	0.5-32	0.5-256	0.5-128	0.5-128	0.125-32	0.125-0.25	0.125-0.25	bla_{SHV}(36), bla_{TEM}(30), bla_{CTXM-1}(3), $bla_{CTXM-15}$(21), bla_{OXA-48}(5), bla_{NDM}(9), *aac(6')-Ib*(9), *ant(2'')-Ia*(15), *arm*A(5), *tet*A(29), *tet*B(59), *qnr*A(34), *qnr*B(20), *qnr*S(17), *sul1*(43), *sul*2(21)	*fim*H(30), *rmp*A(13), *mrk*D(25), *ycf*M(29)
CA-UTI	37	0.5-64	0.5-64	0.25-4	0.25-8	0.5-64	0.5-128	0.5-128	0.125-16	0.125-0.25	0.125-0.25	bla_{SHV}(7), bla_{TEM}(7), $bla_{CTXM-15}$(5), bla_{OXA-48}(2), bla_{NDM}(1), *aac(6')-Ib*(4), *ant(2'')-Ia*(6), *arm*A(1), *tet*A(12), *tet*B(20), *qnr*A(11), *qnr*B(5), *qnr*S(10), *sul*1(14), *sul*2(6)	*fim*H(11), *rmp*A(7), *mrk*D(9), *ycf*M(11)

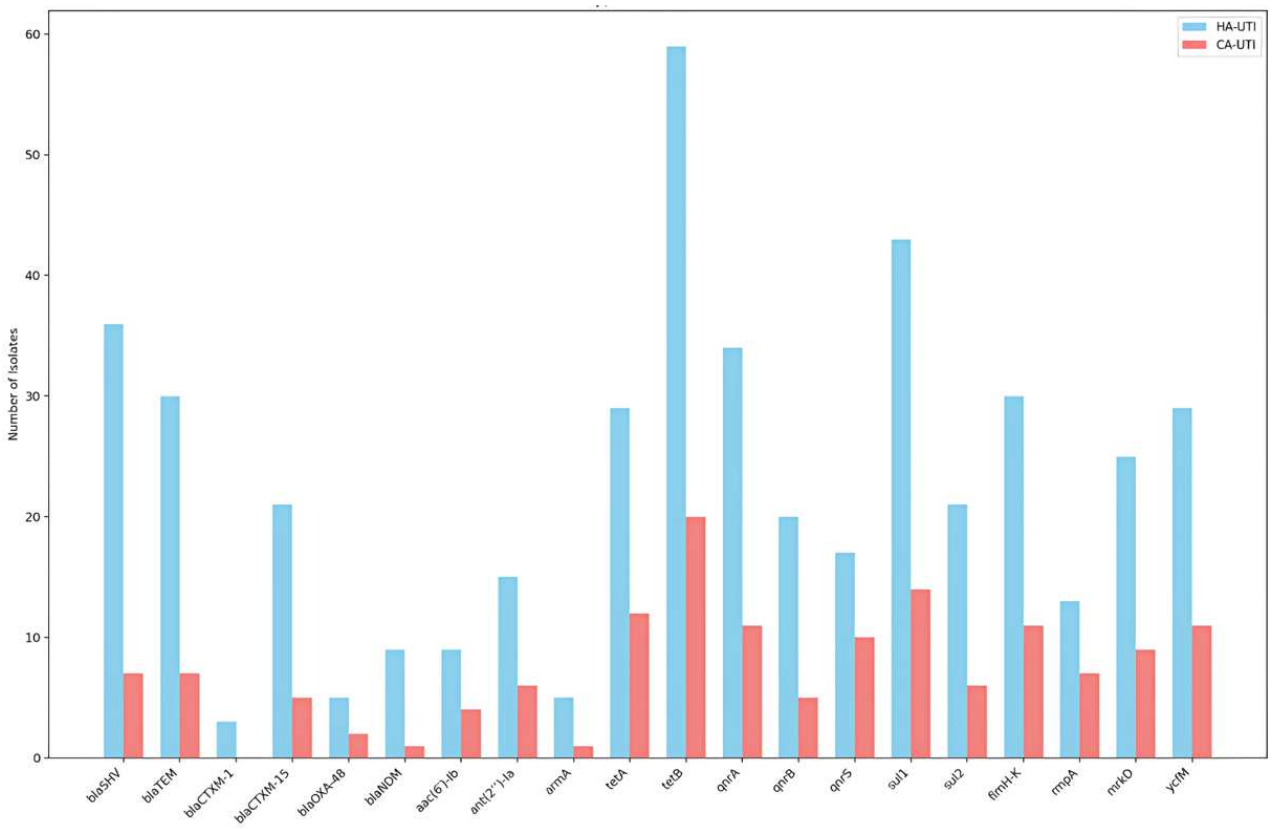

Fig. 3: Comparison of AMR genes and virulence factor genes between VA and HA isolates

Tetracycline resistance genes *tetA* (32.4% vs. 31.9%, p=0.950541) and *tetB* (54.1% vs. 64.8%, p=0.255300) were commonly detected, with a slightly higher prevalence in HA-UTI isolates. Quinolone resistance determinants *qnrA* (29.7% vs. 37.4%, p=0.412262), *qnrB* (13.5% vs. 22.0%, p=0.273488), and *qnrS* (27.0% vs. 18.7%, p=0.294098) showed no statistically significant difference between the two groups. Sulfonamide resistance genes *sul1* (37.8% vs. 47.3%, p=0.331262) and *sul2* (16.2% vs. 23.1%, p=0.388419) were also frequently detected but did not show significant variation between CA-UTI and HA-UTI isolates. All isolates were found negative for *rmtA, rmtB, rmtC, rmtD, rmtE, rmtF* and *qepA* genes.

The distribution of virulence factor genes among *Klebsiella pneumoniae* isolates from CA-UTI and HA-UTI was analyzed. The most prevalent virulence factor gene was *fimH*, detected in 32.0% of isolates, followed by *ycfM* (31.2%), *mrkD* (26.6%), and *rmpA* (15.6%). The chi-square test showed no statistically significant differences in the prevalence of these virulence factor genes between CA-UTI and HA-UTI isolates which suggest that the distribution of virulence factors is relatively similar between CA and HA acquired *K. pneumoniae* strains in UTI cases.

AMR Genotypes

The analysis of *K. pneumoniae* isolates from HA-UTI and CA-UTI cases demonstrated differences in AMR genotypes and virulence factor distribution. HA-UTI isolates displayed elevated MIC ranges for key antimicrobials, including amikacin (0.5-256µg/mL vs. 0.5–64 µg/mL in CA-UTI), meropenem (0.5-32µg/mL vs. 0.25–8 µg/mL), and ciprofloxacin (0.125–32 µg/mL vs. 0.125–16 µg/mL). ESBL genes, particularly $bla_{\text{CTXM-15}}$ (HA-UTI: 21 isolates; CA-UTI: 5 isolates), and carbapenemase genes (bla_{NDM}: 9 vs. 1; bla_{OXA-48}: 5 vs. 2) were more prevalent in HA-UTI. Tetracycline resistance gene *tetB* (59 vs. 20) and fluoroquinolone resistance determinants (*qnrA*: 34 vs. 11; *qnrB*: 20 vs. 5) dominated HA-UTI isolates, while sulfonamide resistance gene *sul1* was common in both (HA-UTI: 43; CA-UTI: 14). Aminoglycoside-modifying enzymes (*aac(6')-Ib*, *ant(2")-Ia*) and the 16S rRNA methylase *armA* were also prevalent in HA-UTI. Virulence factors linked to adhesion (*fimH*) and biofilm formation (*mrkD*) were significantly more frequent in HA-UTI (table 4).

DISCUSSION

This study highlights significant differences in the prevalence and antimicrobial resistance (AMR) profiles of *K. pneumoniae* between community-acquired (CA-UTI) and hospital-acquired urinary tract infections (HA-UTI). The higher UTI positivity rate in hospital settings (33.3% vs. 20.1% in CA-UTIs) aligns with global trends attributing this disparity to selective antibiotic pressure and frequent exposure to resistant pathogens in healthcare environments

(Asmare *et al.*, 2024; Ku *et al.*, 2023; Shields *et al.*, 2021). The elevated UTI prevalence in females (60.9%) is consistent with global trends that is likely due to the anatomical, hormonal, and reproductive factors that heighten susceptibility of UTI (Abu Aleinein & Salem Sokhn, 2024).

Of particular concern is the dominance of multidrug resistance (MDR) in *K. pneumoniae* among HA-UTIs isolates. The HA-UTI isolates exhibited elevated resistance to beta-lactams (e.g., third-generation cephalosporins: 61.7%), fluoroquinolones (70.3%), and carbapenems (12.5%), reflecting the global challenge of AMR in clinical settings (Ameshe *et al.*, 2022; Mukubwa *et al.*, 2023). These resistance rates correlate with intensive antibiotic use in hospitals, where invasive procedures and prolonged therapy drive resistance evolution. For instance, our carbapenem resistance rates align with regional studies from Pakistan (Bilal *et al.*, 2021), but remain lower than reports from Saudi Arabia (20%) (Hafiz *et al.*, 2023), likely reflecting differences in stewardship practices. Notably, universal susceptibility to colistin and tigecycline mirrors global findings (Andrade *et al.*, 2020; Wang *et al.*, 2022), underscoring their retained efficacy as last-line therapies.

Resistance gene profiling revealed a high prevalence of $bla_{\text{CTX-M-15}}$ (96.1%) and bla_{SHV} (87%) among ESBL-producing isolates which is consistent with previous reports from Thailand (Chaisaeng *et al.*, 2024) and Pakistan (Bilal *et al*., 2021). The $bla_{\text{CTX-M}}$ gene confers resistance to extended-spectrum beta-lactam antibiotics, primarily 3rd and 4th generation cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime) and monobactams (e.g., aztreonam) (Rasheed *et al*., 2020). The literature indicates that $bla_{\text{CTX-M-15}}$ is one of the most prevalent ESBL genes worldwide, particularly in *Klebsiella pneumoniae*. Several studies have reported a high prevalence of this gene in clinical isolates. For instance, a study conducted in a Ghanaian hospital found that 98.4% (62/63) of ESBL-producing *K. pneumoniae* isolates carried $bla_{\text{CTX-M-15}}$ (Agyekum *et al.*, 2016). Similarly, research from Portugal reported that 91.7% (11/12) of ESBL-producing *K. pneumoniae* isolates harbored this gene (Carvalho *et al.*, 2021). These findings highlight the widespread distribution of $bla_{\text{CTX-M-15}}$ in *K. pneumoniae* underscoring its clinical and epidemiological significance.

Carbapenem resistance in *K. pneumoniae* isolates from this study was primarily mediated by carbapenemase genes, notably bla_{NDM} and $bla_{\text{OXA-48}}$. While these genes dominated in our study, carbapenem resistance in *K. pneumoniae* is broadly associated with diverse genetic mechanisms. For instance, globally disseminated genes such as bla_{KPC}, bla_{VIM}, and bla_{IMP} are also critical drivers of resistance (Budia-Silva *et al.*, 2024; Pourgholi *et al.*, 2022; Veloso *et al.*, 2023), highlighting the genetic variability underlying carbapenemase dissemination. Additionally, non-enzymatic mechanisms may contribute to resistance, such as mutations in porin proteins (e.g., OmpK35 and OmpK36). Reduced expression or loss of these porins can limit carbapenem uptake, conferring resistance even in isolates lacking carbapenemase genes (Budia-Silva *et al*., 2024; Veloso *et al*., 2023). This underscores the multifactorial nature of carbapenem resistance, necessitating comprehensive surveillance of both enzymatic and non-enzymatic pathways. These findings emphasize the need for region-specific surveillance, as resistance patterns are influenced by local antibiotic use and infection control measures.

Virulence profiling identified *fimH* (100%) and *mrkD* (96.3%) as key adhesins in both HA-UTI and CA-UTI isolates, critical for biofilm formation and urinary tract colonization. Similar distributions have been reported in hypervirulent strains of *K. pneumoniae* (Yadav *et al.*, 2023), highlighting their role in persistent infections and treatment challenges. The *fimH* gene encodes the tip adhesin of type 1 fimbriae, which mediates bacterial attachment to mannosylated host receptors, such as uroepithelial cells. This mechanism is essential for initial urinary tract colonization, as it enables surface adherence and promotes bacterial aggregation. This process is critical for both biofilm formation and immune evasion. Similarly, *mrkD*, a core component of type 3 fimbriae facilitates biofilm formation on abiotic surfaces (e.g., catheters and medical devices) by binding extracellular matrix proteins like collagen. It also stabilizes biofilms in host tissues, enhancing bacterial persistence by shielding pathogens from antibiotics and immune defenses (Clegg & Murphy, 2016; Krawczyk *et al.*, 2022). The synergistic activity of *fimH* and *mrkD* enables *K. pneumoniae* to establish infections through initial epithelial attachment and subsequent biofilm maturation. These biofilms pose significant treatment hurdles, as their protective matrix reduces antimicrobial efficacy and complicates host immune clearance. Notably, the co-occurrence of these adhesins with MDR phenotypes in clinical isolates highlights the dual threat of *K. pneumoniae* in UTIs: virulence factors driven pathogenicity coupled with escalating antibiotic resistance.

Our study has several important limitations that should be considered when interpreting the results. The cross-sectional design precludes analysis of temporal trends in both antimicrobial resistance patterns and virulence gene distribution. While resistance and virulence genes were characterized, whole-genome sequencing could provide deeper insights into genetic mechanisms. These limitations highlight opportunities for future research, particularly longitudinal studies that incorporate WGS analysis and comprehensive clinical data collection, to better elucidate the relationships between genetic determinants and clinical outcomes in *K. pneumoniae* infections.

CONCLUSIONS

The AMR patterns and virulence profiles of *K. pneumoniae* in this study underscore an urgent need for context-specific strategies to curb the dual threat of drug resistance and pathogenicity. Our findings highlight the importance of tailored stewardship programs differentiating between hospital and community settings. Moreover, integrated molecular surveillance need to be incorporated into national AMR action plans to preempt resistance gene dissemination. Since ESBL and carbapenemase genes are more prevalent in hospitalized isolates, rapid diagnosis should be prioritized to guide antimicrobial therapy and carbapenem use should be restricted. In contrast, the narrow spectrum antimicrobial agents should be used in community settings to preserve last-line antibiotics.

An important insight is the conjunction of virulence and resistance in HA-UTI isolates, suggesting that biofilm-forming MDR clones may colonize the healthcare environments to establish endemicity. To address this, infection prevention should be strengthened through routine molecular typing of outbreak-associated strains and environmental screening in high-risk wards. Additionally, the therapeutic innovations targeting biofilm disruption such as adjuvant therapies with anti-adhesion agents or phage cocktails should be explored.

Although, the effectiveness of colistin and tigecycline is reassuring, but careful monitoring is needed as resistance to these drugs is increasingly reported worldwide. To turn these findings into action, we recommend setting up regional AMR hubs to collect resistance data, ensure consistent testing, and share treatment guidelines tailored to each area. The collaborative efforts between clinicians, microbiologists and public health agencies are essential to align stewardship with the genetic epidemiology of *K. pneumoniae* in Pakistan as well as globally.

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