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Diabetes Mellitus and Enhanced Vulnerability to *Escherichia coli* Catheter-Associated Urinary Tract Infections: Integrative Clinical and Molecular Analysis

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Abstract

Catheter-associated urinary tract infections (CAUTIs) represent a substantial clinical burden, particularly in diabetes Mellitus (DM) patients, with extended duration of catheterization. *Escherichia coli* remains most prevalent uropathogen, often exhibiting virulence factors, robust biofilm formation, and multidrug resistance (MDR). This study investigates antimicrobial resistance patterns, virulence gene profiles, and biofilm production of *E. coli* isolates from CAUTI patients with and without diabetes mellitus. A total of 260 CAUTI patients were enrolled in this study, comprising 130 diabetic (HbA1c > 6.5%) and 130 non-diabetic (HbA1c < 5.7%) individuals admitted to various wards of DHQ Hospital, Jhang, between January 2023 and January 2024. From 183 urine culture-positive urine samples 123 *E. coli* isolates were analyzed. Antimicrobial susceptibility testing was performed by disk diffusion, Molecular profiling and virulence genes were conducted via polymerase chain reaction (PCR), and biofilm quantification was assessed by microtiter plate method. MDR (89.7%) and XDR (19.2%) phenotypes were significantly more common in diabetic isolates with increased resistance to β-lactams, fluoroquinolones, carbapenems, and sulfonamides. The most prevalent genes were $bla_{\text{CTX-M}}$, bla_{NDM} and $bla_{\text{OXA-48}}$. Virulence genes (*fimH* (78%), *PapC* (50%), *FyuA* (45%), and *KpsMTII* (33%) associated with enhanced biofilm formation. Diabetes mellitus (DM) substantially exacerbates CAUTIs caused by *E. coli* through increased multidrug resistance, virulence genes prevalence and biofilm production emphasizing the need for targeted antimicrobial stewardship and stringent infection control strategies in diabetic populations.

Keywords: CAUTI, *E. coli*, diabetes, antibiotic resistance, virulence, biofilm

Introduction

Escherichia coli is the most common causative agent of catheter-associated urinary tract infections (CAUTIs), which rank among the most prevalent healthcare-associated infections globally (Latthe et al. 2024). Urinary tract infections account for approximately 40% of all nosocomial infections, with indwelling urinary catheters implicated in nearly 80% of these cases (CDC NHSN 2022). Among hospitalized adults, CAUTI incidence ranges from 3% to 7%, with catheter utilization occurring in 12% to 16% of hospital admissions (Mohamed et al. 2024). The global burden is significant, with an estimated 222 million urinary tract infections annually (Mohamed et al. 2024). Clinically, these infections typically present with symptoms such as fever, flank pain, and suprapubic tenderness (Kaur et al. 2021).

The presence of diabetes mellitus in patients with CAUTIs presents a serious clinical concern due to associated compromises in immune function. Notably, *E. coli* is responsible for approximately 70% of urinary tract infections in diabetic patients (Papp et al. 2023). Emerging evidence indicates that diabetes markedly increases the risk and severity of *E. coli* infections in catheterized individuals. Pathophysiological factors including hyperglycemia, impaired insulin signaling, and neutrophil dysfunction collectively establish a micro-

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environment that facilitates *E. coli* colonization, biofilm formation, and the development of antibiotic resistance. Biofilms offer protection against host immune defenses and antimicrobial agents, making CAUTIs particularly persistent and recalcitrant to treatment in diabetic patients. This phenomenon is described as a "triple threat" involving impaired tissue nutrition, suppressed immune function, and an increased likelihood of treatment failure caused by multidrug-resistant strains (Darwitz et al. 2024).

In diabetic CAUTI cases, biofilm-forming *E. coli* strains have been strongly associated with specific virulence genes, including *fimH*, *papC*, and *afa1* (Mohamed et al. 2024). Despite this understanding, a critical research gap persists, particularly within the context of Pakistan. While extant literature consistently identifies *E. coli* as a predominant CAUTI pathogen, few studies explicitly delineate diabetes mellitus as a distinct and significant risk factor for *E. coli*-associated CAUTI. Furthermore, epidemiological data are disproportionately derived from tertiary care centers, leading to a significant under-representation of community, regional, and rural populations. This gap underscores the need for focused studies in these settings. The objective of the present investigation is, therefore, to elucidate and compare the etiology of CAUTIs in these patient groups by analyzing the patterns of antibiotic resistance, biofilm formation, and associated risk factors in diabetic versus non-diabetic patients.

Experimental

Materials and Methods

Study design and sample collection. This cross-sectional study was conducted at DHQ Hospital Jhang between January 2023 and January 2024. The study cohort comprised adult catheterized patients (≥ 18 years) who developed symptoms of a urinary tract infection following an indwelling catheterization period of at least two day (Centers for Disease Control and Prevention 2021). In order to assess glycemic control among individuals with diabetes, HbA1c readings were obtained from patient medical records, with values exceeding 6.5% indicating diabetes and those below 5.7% suggesting non-diabetic status (American Diabetes Association Professional Practice Committee 2024). Exclusion criteria included individuals with malignancy, hydronephrosis, pyelonephritis, or a known catheter allergy. Demographic and clinical data were retrieved from patient medical records. Using strict aseptic techniques, approximately 3–5 ml of urine was aseptically collected from the catheter's sampling port via a sterile needle and syringe (Centers for Disease Control and Prevention 2017; Centers for Disease Control and Prevention 2021).

Ethical approval and informed consent. Ethical permission was secured from the Ethics Review Committee under letter Ref. No. GCUF/ERC/512-A. Prior to enrollment, written informed consent was obtained from all participants. All information was anonymized before analysis, and patient confidentiality was fully ensured.

Sample processing and bacterial identification. All urine samples were inoculated onto Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar using a calibrated 1µl loop and incubated aerobically at 37°C for 24 hours. Samples exhibiting significant bacteriuria (≥ 10^3 CFU/ml) were selected for further processing. Presumptive *E. coli* isolates were sub-cultured on Eosin Methylene Blue (EMB) agar to obtain characteristic metallic green colonies (Tankeshwar 2023). Initial identification was based on Gram stain morphology, revealing pink-stained Gram-negative rods. Biochemical confirmation was performed using the API 20E kit according to the manufacturer's instructions, which included Triple Sugar Iron (TSI) and indole tests.

Molecular confirmation of bacterial isolates. Genomic DNA was extracted from pure bacterial cultures using the GF-1 Bacterial DNA Extraction Kit (Vivantis® Technologies, Malaysia) in accordance with the manufacturer's protocol. The confirmed *E. coli* isolates were subjected to polymerase chain reaction (PCR) targeting the species-specific *uidA* gene (Mujahid et al. 2024). Amplification was performed using a Thermo Fisher Scientific ready-to-use PCR master mix on an Optimus 96G thermal cycler. The resulting PCR amplicons were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide, run at 90 volts for 60 minutes, and visualized under ultraviolet light.

Antimicrobial susceptibility testing. Antibiotic susceptibility of all confirmed *E. coli* isolates was determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Bacterial suspensions were standardized to a 0.5 McFarland standard and inoculated onto plates. Commercially available Oxoid™ antibiotic discs (Thermo Scientific, UK) were applied, including: Cefotaxime (CTX, 30 µg), Ceftriaxone (CRO,

30 µg), Amikacin (AK, 30 µg), Gentamicin (CN, 10 µg), Amoxicillin-clavulanic acid (AMC, 30 µg), Imipenem (IPM, 10 µg), Meropenem (MEM, 10 µg), Levofloxacin (LEV, 5 µg), Ciprofloxacin (CIP, 5 µg), Doxycycline (DO, 30 µg), Trimethoprim-sulfamethoxazole (SXT, 25 µg), Nitrofurantoin (F, 100 µg), and Tigecycline (TGC, 15 µg). Following incubation at 37°C for 24 hours, zones of inhibition were measured and interpreted according to CLSI 2024 guidelines. Tigecycline results were interpreted using breakpoints defined by the U.S. Food and Drug Administration (FDA). Isolates were categorized as susceptible, intermediate, or resistant.

The minimum inhibitory concentrations (MICs) of key antimicrobial agents were determined using the broth microdilution technique in 96-well microtiter plates. Serial two-fold dilutions of each drug were prepared in Mueller-Hinton broth. Each well was inoculated with 100 µl of a bacterial suspension, yielding a final concentration of approximately 5×10^5 CFU/ml. Plates were incubated at 37°C for 16–20 hours. Bacterial growth was assessed visually and confirmed by measuring the optical density at 600 nm using a spectrophotometer. *Pseudomonas aeruginosa* ATCC 27853 was used as quality control.

Identification of resistant genes. The genomic basis of antimicrobial resistance was characterized through the comprehensive screening of all *E. coli* isolates for an extensive array of resistance determinants via polymerase chain reaction (PCR).The amplification process included an initial denaturation at 95°C for 5 minutes, succeeded by 35 cycles including denaturation at 95°C for 30 seconds, annealing at (Primer-specific annealing temperatures were taken from previously published studies) for 60 seconds, and extension at 72°C for 1 minute, resulting in a final extension at 72°C for 5 minutes (Mujahid et al. 2024). Our investigation targeted major β-lactamase genes, including bla_{TEM}, bla_{SHV}, and the broadly prevalent $bla_{\text{CTX-M}}$ group; isolates positive for $\text{bla}_{\text{CTX-M}}$ were subsequently sequenced to identify specific variants ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, and $bla_{\text{CTX-M-15}}$). Given the rising threat of carbapenem resistance, we also screened for a panel of carbapenemase genes, encompassing bla_{IMP}, bla_{NDM}, bla_{VIM}, bla_{GIM}, bla_{SIM}, bla_{SPM}, bla_{KPC}, and $bla_{\text{OXA-48}}$. Furthermore, the study evaluated genes conferring resistance to other drug classes, such as tetracyclines (*tet*A, *tet*B), sulfonamides (*sul*1, *sul*2), and quinolones, with a focus on plasmid-mediated quinolone resistance (PMQR) determinants (*qnr*A, *qnr*B, *qnr*S, *qep*A). Finally, we assessed the presence of aminoglycoside-modifying enzymes (*aac(6′)-Ib, aph(3”)-Ib, ant(2′′)-Ia*) and 16S methyltransferases (*arm*A, *rmt*A-F), which confer high-level aminoglycoside resistance. All primers used were adopted from previously published study (Mujahid et al. 2024).

Identification of virulence factors. The pathogenic potential of the *E. coli* isolates was evaluated by profiling a suite of virulence genes critical for uropathogenesis. Using PCR, we identified genetic determinants encoding key mechanisms for host colonization and infection. This included adhesins (*fimH*, *papC*, *papG*), which facilitate binding to the uroepithelium; siderophores (*fyuA*, *iutA*), which are essential for iron acquisition in the nutrient-limited host environment; and the hemolysin toxin (*hlyA*), which promotes tissue invasion and injury. Additionally, the *kpsMTII* gene, responsible for group II capsule synthesis and conferring serum resistance, was detected. The full set of primers utilized for virulence gene amplification were sourced from a previously published study (Mujahid et al. 2024).

Microtiter plate assay for biofilm detection. The biofilm-forming capacity of all *E. coli* isolates was quantitatively assessed using a standard microtiter plate assay. Briefly, bacterial cultures were grown in tryptic soy broth and incubated statically at 37°C for 24 hours. After incubation, non-adherent cells were removed by washing, and the adherent biofilms were fixed and stained with a 0.1% crystal violet solution. The bound dye was subsequently solubilized with acetic acid, and the optical density (OD) of each well was measured spectrophotometrically at a wavelength of 570 nm. The critical value for biofilm formation (ODc) was defined as the mean OD of the negative control wells plus three times their standard deviation (Kırmusaoğlu 2019). The biofilm production for each isolate was calculated as $\text{OD}_{\text{isolate}} = (\text{mean OD}_{\text{isolate}}) - \text{ODc}$. Based on this value, isolates were classified into four categories: non-biofilm producer, weak, moderate, or strong biofilm producer. To ensure experimental accuracy, all assays were performed in triplicate, and *Pseudomonas aeruginosa* ATCC 27853 was included as a positive control strain and for negative control sterile media is used.

Statistical analysis. All data were compiled in Microsoft Office Excel and analyzed using IBM SPSS Statistics version 25 (SPSS Inc., USA). Categorical variables, presented as frequencies and percentages, were compared between diabetic and non-diabetic groups

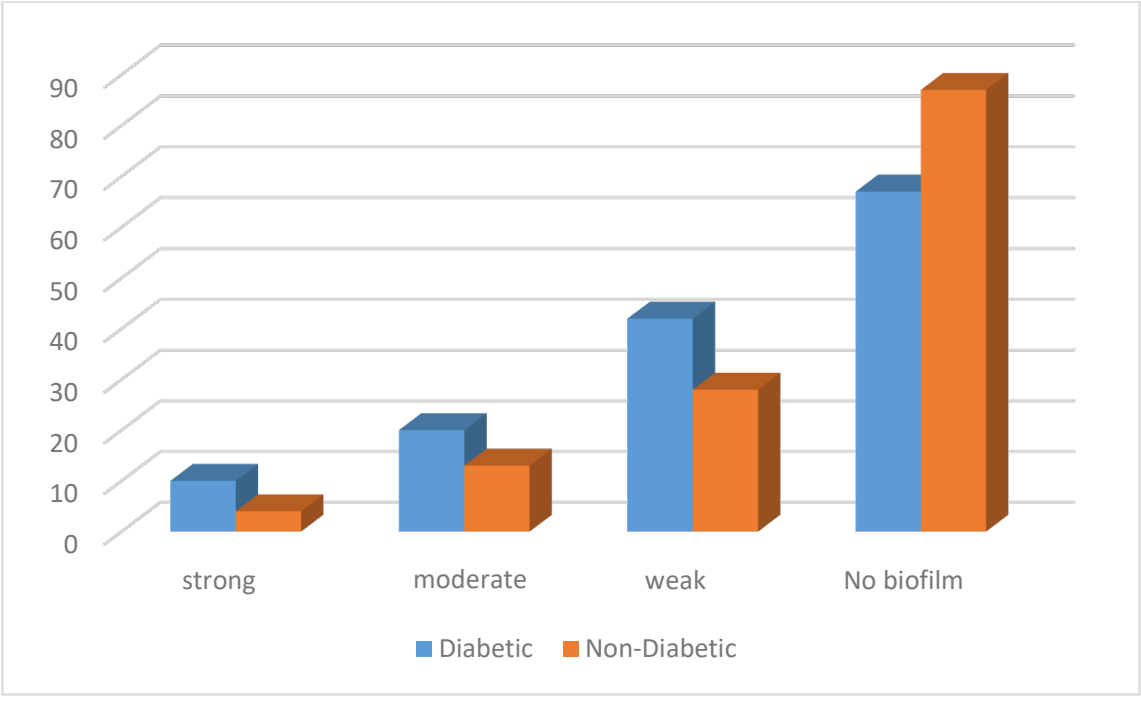

Fig. 1. Comparison of biofilm production in diabetic and non-diabetic CAUTI associated *E. coli* isolates

using the Chi-square test or Fisher's exact test, as appropriate. A p-value of < 0.05 was considered indicative of statistical significance. To account for the increased risk of Type I error due to multiple comparisons as a consequence of employing the chi-square test across numerous variables, a Bonferroni correction was applied, thereby adjusting the threshold for statistical significance to a more stringent $p < 0.004$ for these analyses.

Results

Demographic data and clinical information. This study analyzed 260 patients diagnosed with catheter-associated urinary tract infections (CAUTIs), comprising 130 diabetic (HbA1c > 6.5) and 130 non-diabetic (HbA1c < 5.7) individuals. A two-sample comparison of proportions (two-sided $\alpha = 0.05$), giving us ≥ 80% power to identify absolute risk differences of 10–13%. From these, a total of 123 *Escherichia coli* isolates were recovered for further analysis. The cohort consisted of 136 (52.3%) females and 124 (47.7%) males, with a mean age of 53.8 years (range: 20–96 years). Diabetes was more prevalent among female patients, with 80 (58.8%) diabetic females compared to 56 (41.2%) non-diabetic females. In contrast, non-diabetic status was more common among males, with 74 (59.7%) non-diabetic males compared to 50 (40.3%) diabetic males (Fig. 2).

Among 183 culture-positive CAUTI cases, *E. coli* was the predominant pathogen, accounting for 123 isolates (67.2%). Other identified pathogens included *Klebsiella pneumoniae* (28, 15.3%), *Pseudomonas aeruginosa* (15, 8.1%), *Enterococcus* spp. (10, 5.4%), and *Proteus* spp. (7, 3.8%). The distribution of CAUTI cases across hospital wards was as follows: the medical ward accounted for the majority of cases (40.0%), followed by the surgical ward (24.8%), the emergency room (15.0%), and the gynecological department (11.5%). The intensive care unit (ICU) had the lowest incidence rate (7.7%) (Table I).

A statistically significant association was observed between catheter material and CAUTI incidence ($p = 0.0026$), with latex catheters conferring a significantly higher risk of infection compared to other materials. This finding suggests that silicone or antimicrobial-impregnated catheters may be associated with a lower risk. No other risk factors, including duration of hospital stay, catheterization time, or frequency of urine drainage, demonstrated a significant correlation with CAUTI incidence in this cohort (Table I).

Antibiotic resistance. *E. coli* isolates from catheter-associated urinary tract infections exhibited high

Table I
List of Risk Factors associated with CAUTI

Variables	Number	Percentage (%)	*p*-Value
Hospital ward			
Medical ward	104	40.0	0.0035
Surgical ward	67	25.8	
Emergency	39	15.0	
Gynecology	30	11.5	
Intensive care unit	20	7.7	
Gender			
Female	136	52.3	<0.00001
Male	124	47.7	
Diabetic status			
Diabetic	130	50.0	<0.0001
Non-diabetic	130	50.0	
Duration of hospital stay (Days)			
1 (0-3 Days)	26	10.0	0.718
2 (4-6 days)	174	66.9	
3 (7-9 Days)	40	15.4	
4 (10 or Greater)	20	7.7	
Duration of catheterization (Days)			
1 (0-3 Days)	149	57.3	0.533
2 (4-6 days)	68	26.2	
3 (7-9 Days)	29	11.2	
4 (10 or Greater)	14	5.4	
Types of catheters			
Latex	157	60.4	0.002
Silicon	87	33.5	
Anti-microbial	16	6.2	
Urine drainage in hours			
10 h	25	9.5	0.959
12 h	71	26.9	
24 h	164	62.1	
Prior use of antibiotics			
Ceftriaxone	242	93.1	0.137
Meropenem	18	6.9	
Biofilm microplate			
No biofilm	154	59.20	0.001
Weak	59	22.60	
Moderate	33	12.60	
Strong	14	5.38	
Microorganisms			
E. coli	123.0	67.2	0.001
Klebsiella pneumoniae	28.0	15.3	
Pseudomonas aeruginosa	15.0	8.1	
Enterococcus spp.	10.0	5.4	
Proteus Spp.	7.0	3.8	

Table II
Association in Between Biofilm and the Presence of Virulence Genes in *Escherichia coli* CAUTI isolates

Virulence Genes	Positive biofilm (n = 67)	Negative biofilm (n = 56)	*p*-Value
Fim-H	67 (100.0%)	29 (51.7%)	0.0001
Pap-C	45 (67.1%)	17 (30.3%)	0.0002
Fyu-A	42 (62.6%)	13 (23.2%)	0.0001
Hyl-A	11 (16.4%)	7 (12.5%)	0.5274
Iut-A	29 (43.2%)	19 (33.9%)	0.34
KpsMTII	30 (44.7%)	11(19.6%)	0.0001
Pap-G	35 (52.2%)	9 (16.0%)	0.0001

and varying degrees of antibiotic resistance. Among the 123 isolates, a substantial proportion demonstrated multidrug resistance, with 97 (78.8%) classified as MDR (resistant to at least one agent in three or more antimicrobial categories) and 20 (16.2%) classified as XDR (resistant to at least one agent in all but two or fewer antimicrobial categories). A significantly higher prevalence of these phenotypes was observed in isolates from diabetic patients (MDR: 89.7%; XDR: 19.2%; n=78) compared to those from non-diabetic patients (MDR: 60.0%; XDR: 11.1%; n=45), indicating a heightened risk of treatment failure in the diabetic cohort.

Resistance to amoxicillin-clavulanic acid was markedly higher in diabetic isolates (88.4%) than in non-diabetic isolates (42.2%), suggesting a greater prevalence of ESBL-producing strains that compromise the efficacy of β-lactam/β-lactamase inhibitor combinations. Universal resistance (100%) to the third-generation cephalosporins ceftriaxone and cefotaxime was observed in isolates from diabetic patients, strongly indicating widespread ESBL production. A similarly high, though non-universal, resistance pattern to these cephalosporins was confirmed in non-diabetic isolates.

High resistance rates were also evident against fluoroquinolones. Resistance to ciprofloxacin was detected in 83.3% of diabetic isolates and 73.3% of non-diabetic isolates, yielding an overall resistance rate of 79.6%. A comparable pattern was observed for levofloxacin, affecting 87.1% of diabetic and 77.7% of non-diabetic isolates, with a total resistance rate of 83.7%. No statistically significant difference in fluoroquinolone resistance was found between the two groups.

Notably, carbapenem resistance was significantly more prevalent in diabetic isolates. Resistance to imi-

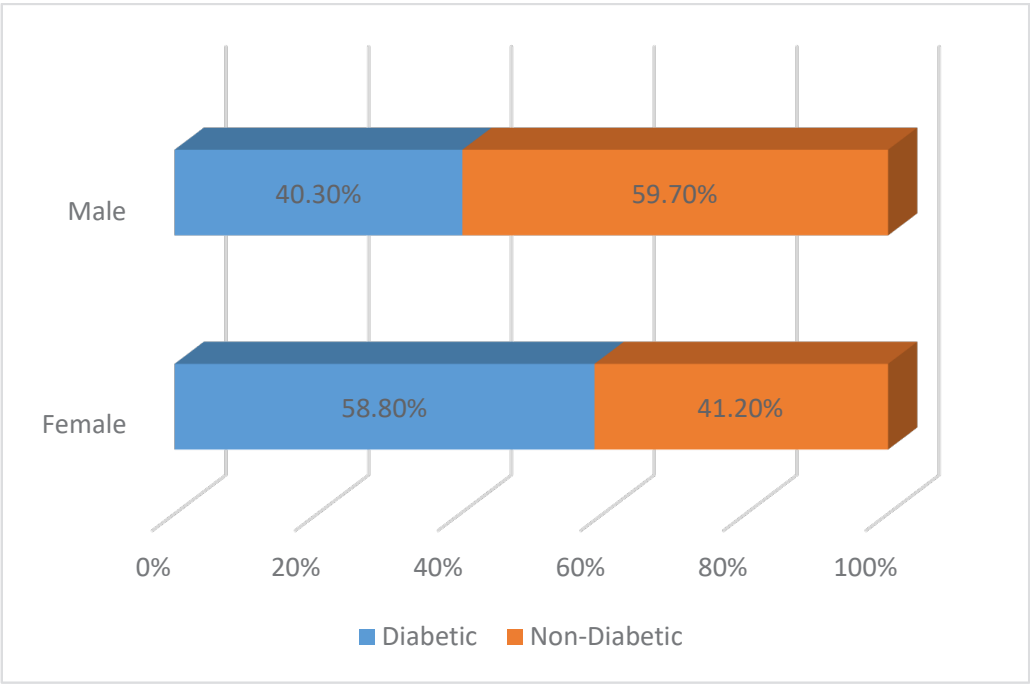

Fig. 2. Gender-wise distribution of diabetic and non-diabetic status

penem was found in 58.9% of diabetic isolates compared to 15.5% of non-diabetic isolates. A nearly identical trend was observed for meropenem, with 53.8% of diabetic and 13.3% of non-diabetic isolates showing resistance. Resistance to trimethoprim-sulfamethoxazole was identified in 75.6% of diabetic and 60.0% of non-diabetic isolates, resulting in an overall resistance rate of 69.9%. A detailed comparison of antibiotic resistance between groups is provided in Table III.

In contrast, all isolates remained susceptible to colistin and nitrofurantoin, with nearly no resistance detected in either group. The distribution of Minimum Inhibitory Concentrations (MICs) for various antibiotics is illustrated in Table IV.

Identification of resistance genes. Molecular analysis revealed a significantly higher prevalence of antimicrobial resistance genes among isolates from diabetic patients compared to non-diabetic patients. The CTX-M gene was identified in 77 (98.7%) isolates from diabetic patients and 13 (28.8%) from non-diabetic patients, yielding an overall prevalence of 73.1% (90/123). This difference was statistically significant ($p = 0.001$), indicating a strong association between diabetes status and the presence of the CTX-M gene.

The $bla_{\text{CTX-M-15}}$ variant was the most prevalent ESBL gene, detected in 71 isolates (57.7%) overall. It was found in 78.2% (61/78) of diabetic isolates compared to 22.2% (10/45) of non-diabetic isolates. The bla_{TEM} and bla_{SHV} genes were detected in 82 (66.6%) and 59 (47.9%) of all isolates, respectively. Both bla_{TEM} and $bla_{\text{CTX-M-15}}$ were significantly more prevalent in the diabetic group (92.3% and 67.9%, respectively; $p = 0.001$), underscoring the concern regarding ESBL-mediated resistance. Among carbapenemase genes, bla_{NDM} was prevalent in 60.2% of diabetic isolates compared to 6.6% of non-diabetic isolates. The $bla_{\text{OXA-48}}$ gene was identified exclusively in the diabetic group, with a prevalence of 15.3%.

The aminoglycoside modifying enzyme gene *aac(6')-Ib* was highly prevalent in diabetic CAUTI isolates (73.0%) compared to non-diabetic isolates (20.0%), further corroborating the high incidence of ESBL-producing strains in the diabetic cohort. Other resistance genes, including *aph(3")-Ib*, *ant(2")-Ia*, and the 16S methyltransferase genes *rmtB* and *armA*, were more frequently observed in diabetic isolates (23.0%, 11.5%, 12.8%, and 16.6%, respectively), though these differences were not statistically significant ($p = 0.604$).

Analysis of plasmid-mediated quinolone resistance (PMQR) genes showed that *qepA* was present in 51.2% of isolates (63/123), while *qnrA* and *qnrB* were detected in 10.5% (13/123) and 70.7% (87/123) of isolates, respectively. The *qnrS* gene was not identified in any isolate. Sulfonamide resistance genes were common, with

Table III

Comparison of antimicrobial resistance pattern in *E. coli* CAUTI isolates from diabetic versus non-diabetic patients.

Antibiotic	Diabetic (n = 78)	Non-diabetic (n = 45)	Total (n = 123)	*p*-Value
Amoxicillin-clavulanic acid	69 (88.4%)	19 (42.2%)	88 (71.5%)	<0.001*
Cefotaxime	78 (100%)	45 (100%)	123 (100%)	-
Ceftriaxone	78 (100%)	45 (100%)	123 (100%)	-
Imipenem	46 (58.9%)	7 (15.5%)	53 (43.0%)	<0.001*
Meropenem	42 (53.8%)	6 (13.3%)	48 (39.0 %)	<0.001*
Amikacin	56 (71.7%)	20 (44.4%)	76 (61.7%)	0.004
Gentamicin	61 (78.2%)	19 (42.2%)	80 (65.0%)	<0.001*
Ciprofloxacin	65 (83.3%)	33 (73.3%)	98 (79.6%)	0.191
Levofloxacin	68 (87.1%)	35 (77.7%)	103 (83.7%)	0.231
Tri-methoprim sulphamethoxazole	59 (75.6%)	27 (60.0%)	86 (69.9%)	0.091
Nitrofurantoin	9 (11.5%)	4 (8.8%)	13 (10.5%)	0.742
Doxycycline	56 (71.7%)	15 (33.3%)	71 (57.7%)	<0.001*
Colistin	0	0	0	-
Tigecycline	0	0	0	-

* – *p*-values < 0.004 was considered statistically significant after Bonferroni correction

Table IV
MIC distribution in CAUTI associated *Escherichia coli* isolates

Antibiotics	Number of isolates with MIC (µg/ml)											
	≤ 0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**
Cefotaxime	NT	NT	-	-	-	39	30	26	12	9	5	2
Ceftriaxone	NT	NT	-	-	-	40	27	21	16	10	8	1
Imipenem	NT	NT	36	27	7	9	14	8	19	1	1	1
Meropenem	NT	NT	34	31	10	14	10	8	12	2	1	1
Amikacin	NT	NT	9	8	19	6	6	25	15	20	13	3
Gentamicin	NT	NT	17	12	9	5	24	18	10	6	22	-
Doxycycline	NT	NT	25	13	9	5	0	15	20	30	5	1
Ciprofloxacin	15	10	-	25	16	10	18	5	15	9	NT	NT
Colistin	12	38	52	21	-	-	-	-	-	-	NT	NT
Tigecycline	28	44	35	16	-	-	-	-	-	-	NT	NT

Values represent the number of isolates at each MIC. Interpretation of MICs was performed according to CLSI guidelines (2025).
NT – not tested

sul1 and *sul2* identified in 65.0% (80/123) and 65.8% (81/123) of all isolates, respectively, a finding that was statistically significant (p=0.001). For tetracycline resistance, the *tetB* gene was the most prevalent, identified in 54.4% of isolates (67/123), while *tetA* was observed in 21.1% (26/123). The incidence of all antimicrobial resistance genes demonstrated significant variation between the two patient groups ($p < 0.05$), with a detailed distribution provided in Table SI.

Detection of virulence gene. The distribution of virulence genes among the 123 *E. coli* isolates was characterized. The *fimH* gene was the most prevalent, detected in 96 isolates (78.0%). The *papC* and *papG* genes demonstrated moderate prevalence, identified in 62 (50.4%) and 44 (35.8%) isolates, respectively. The iron acquisition genes *iutA* and *fyuA* were present in 48 (39.0%) and 55 (44.7%) isolates. The capsular synthesis gene *kpsMTII* was found in 41 isolates (33.3%), while the hemolysin gene *hlyA* was the least prevalent, detected in only 18 isolates (14.6%) (Fig. 3).

A strong correlation was observed between biofilm-forming capability and the presence of key virulence genes. Among the 67 biofilm-forming isolates, the prevalence of virulence genes was significantly higher: *fimH* (100%), *papC* (67.1%), *fyuA* (62.6%), *papG* (52.2%), and *kpsMTII* (44.7%). In contrast, the presence of *hlyA* and *iutA* showed no significant association with biofilm formation status ($p = 0.527$ and $p = 0.34$, respectively) (Table II).

Biofilm formation. Isolates derived from diabetic patients demonstrated a significantly greater propensity for biofilm formation. Among diabetic CAUTI isolates, 19 (24.4%) were classified as moderate biofilm producers and 6 (7.7%) as strong producers. In contrast, non-diabetic isolates showed lower rates, with 11 (24.4%) and 4 (8.9%) isolates classified as moderate and strong biofilm producers, respectively. This indicates an enhanced capacity for pathogenic persistence in isolates from individuals with diabetes. Conversely, the non-biofilm producer category was substantially more prevalent among non-diabetic isolates (72.2%) compared to diabetic isolates (55.9%) (Fig. 1).

Discussion

Catheter-associated urinary tract infections (CAUTIs) caused by *Escherichia coli* represent a significant clinical challenge, particularly among hospitalized patients with diabetes mellitus (DM). Our findings underscore a strong association between DM and an increased risk of these infections, a vulnerability largely attributed to the diabetic physiological state. Hyperglycemia-induced glycosuria provides a nutrient-rich environment that promotes bacterial proliferation, while concomitant immune dysfunction facilitates enhanced bacterial adherence and biofilm formation on catheter surfaces (Flores-Mireles et al. 2015). While previous studies have often reported on general *E. coli* resistance patterns, a critical gap exists in the comparative profiling of isolates from diabetic versus non-diabetic

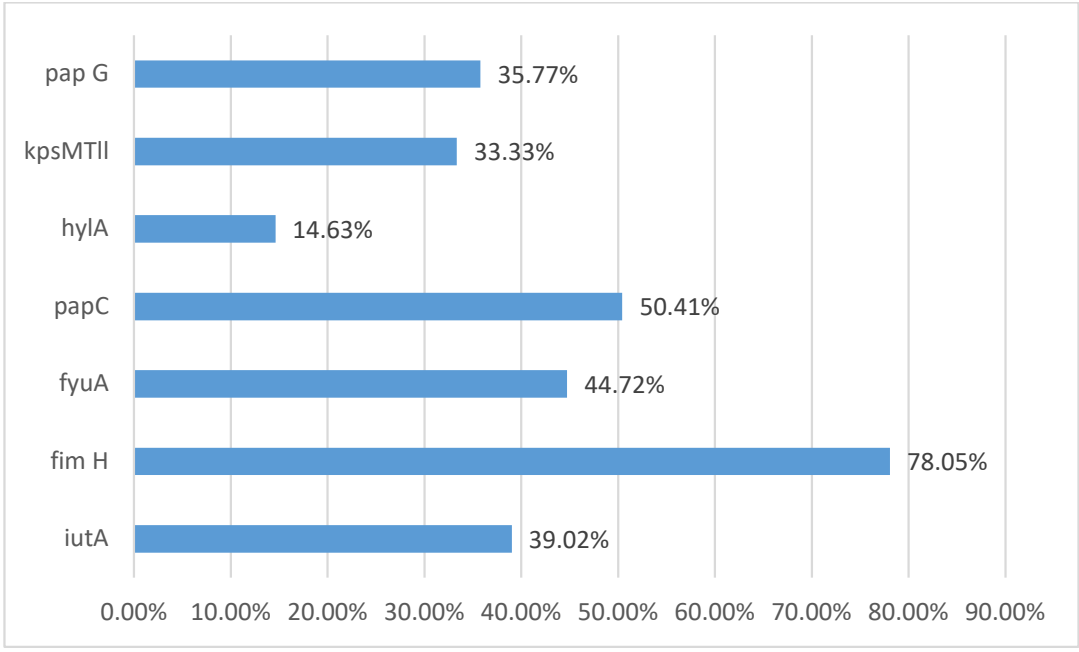

Fig. 3. Frequency of Virulence factors in CAUTI associated *E. coli* isolates.

patients, specifically concerning biofilm capacity and virulence gene expression in the context of CAUTIs. Our study addresses this gap by integrating clinical patient data with molecular characterization of isolates, revealing distinct pathogenic signatures in diabetic CAUTI cases.

The clinical burden for diabetic patients is substantially heightened, as evidenced by our cohort in which a history of diabetes was present in approximately 50% of CAUTI patients. This population contends with an elevated susceptibility to multidrug-resistant (MDR) infections, often experience delayed healing, and typically endures prolonged catheterization periods. Despite stringent adherence to infection control protocols, these intrinsic risk factors perpetuate a high incidence of CAUTIs. Furthermore, the demographic profile of our study population, which was 52.3% female with a mean age of 53.8 years, aligns with established epidemiological trends. The higher representation of women may be linked to well-documented anatomical and hormonal factors that predispose them to UTIs (Letica-Kriegel et al. 2019).

The majority of patients in our cohort were admitted to medical and surgical wards, a finding consistent with established research indicating that prolonged hospitalization and surgical procedures increase exposure to indwelling devices (Saint et al. 2016). A statistically significant association between the use of latex catheters and a higher incidence of CAUTI ($p = 0.0026$) raises concerns regarding material safety. Compared to silicone or antimicrobial-coated alternatives, latex may promote greater bacterial adherence and biofilm formation, underscoring the potential benefit of transitioning to safer materials (Ricardo et al. 2020).

Contrary to the established paradigm that prolonged catheterization duration increases the risk of infection via ascending bacterial colonization (Tambyah et al. 2000), our study observed no significant correlation between CAUTI rates and either catheterization time or frequency of urine drainage. This discrepancy may suggest that modern catheter care protocols including closed drainage systems and stringent aseptic insertion techniques have effectively mitigated risks historically associated with longer catheter use (Hooton et al. 2010). Furthermore, prior antibiotic use did not significantly correlate with CAUTI incidence in our analysis. The predictive utility of prior antibiotics is likely diminished by widespread indiscriminate use and the extensive baseline resistance prevalent in diabetic populations (Ventola 2015).

This inherent resistance is reflected molecularly in our findings. Isolates from diabetic patients exhibited a significantly higher prevalence of multiple resistance genes, indicating a more complex resistance phenotype likely driven by host-specific factors such as glycosuria, immunosuppression, and extended catheterization. Notably, two sulfonamide resistance determinants (sul1 and sul2) were substantially more

common in diabetic isolates compared to non-diabetic CAUTI isolates ($p = 0.001$ and $p = 0.003$, respectively). This pattern may be attributed to integron-mediated acquisition of resistance cassettes, a mechanism frequently documented in chronic diabetic infections characterized by recurrent antibiotic exposure (Akash et al. 2020). Although sulfonamide use has declined, resistance remains a persistent clinical concern. Our observation of persistent sul2 gene expression aligns with prior studies that reported over 69.9% resistance to sulfonamides among urinary *E. coli* isolates, attributing the dominance of sul2 to its association with mobile genetic elements that facilitate horizontal gene transfer. (Bean et al. 2005). This underscores the enduring clinical significance and environmental persistence of these resistance determinants, particularly within the vulnerable demographic of catheterized diabetic patients.

Quinolone resistance was predominantly mediated by *qnrB*, which was identified in 97.4% of diabetic isolates compared to 24.4% of non-diabetic isolates ($p = 0.001$); *qnrA* and *qepA* contributed minimally. These plasmid-mediated genes encode protective proteins that prevent quinolone binding to DNA gyrase, thereby conferring high-level fluoroquinolone resistance. This is consistent with recent reports from South Asia (Bilal et al. 2021) The association between diabetes mellitus and multidrug non-susceptibility in *E. coli* and *Klebsiella pneumoniae* urinary tract infections was highlighted by Akash et al. (2020), whose emphasis on the frequent identification of *qnr* genes and ESBLs in immunocompromised diabetic patients aligns with our findings in CAUTI isolates (Akash et al. 2020).

Furthermore, tetracycline resistance was significantly more prevalent in our diabetic CAUTI isolates, with *tetB* detected in 76.9% of diabetic strains versus 15.9% of non-diabetic strains ($p = 0.02$). This finding is consistent with the previous work that reported that *tetA* and *tetB* are widely distributed among uropathogenic *E. coli* and are often located on mobile genetic elements co-harboring ESBL and sulfonamide resistance genes (Poirel et al. 2018). The increased frequency of these genes in our multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates is likely explained by co-selection pressure from frequent antibiotic exposure in diabetic patients.

The high prevalence of the ESBL genes $bla_{\text{CTX-M}}$ (98.7%), bla_{TEM} (92.3%), and bla_{SHV} (67.9%) in our diabetic CAUTI isolates reflects the endemicity of ESBL-producing *E. coli* in Pakistani healthcare settings. The β-lactamases encoded by these genes hydrolyze third-generation cephalosporins, rendering conventional empirical therapies ineffective. Our results align with a recent study from Pakistan that reported a similar distribution of ESBL genes in uropathogenic *E. coli* from non-catheterized UTI patients, implying that ESBL-mediated resistance is widespread across both community and hospital settings (Ashraf et al. 2025).

Notably, we identified a significant increase in carbapenem resistance among diabetic CAUTI isolates ($p < 0.001$), strongly associated with the presence of bla_{NDM} (60.2%). The $bla_{\text{OXA-48}}$ gene was observed in 15.3% of cases. The non-significant association of $bla_{\text{OXA-48}}$ in our data may be explained by its tendency to confer low-level resistance to carbapenems, which can often evade phenotypic detection (Boyd et al. 2022). The prevalence of bla_{NDM} is consistent with regional reports on its dissemination (Kumarasamy et al. 2010), and suggests co-resistance with other β-lactams, likely facilitated by the co-expression of ESBLs and AmpC enzymes (Boxtel et al. 2017).

Our investigation revealed a significantly higher prevalence of aminoglycoside resistance in diabetic CAUTI isolates. The resistance gene *aac(6')-Ib* was identified in 73.0% of diabetic isolates compared to only 9.2% of non-diabetic isolates. This gene encodes an aminoglycoside acetyltransferase that modifies and inactivates antibiotics such as amikacin and gentamicin, a mechanism that can directly lead to therapeutic failure in catheterized patients. The clinical relevance of this genomic finding is corroborated by the work (Nouraldein Mohammed Hamad et al. 2020), that demonstrated elevated aminoglycoside resistance in *E. coli* from community-acquired UTIs, suggesting this is a widespread and persistent challenge.

Furthermore, our data indicates that *aac(6')-Ib* frequently co-occurs with ESBL genes, particularly $bla_{\text{CTX-M}}$ and bla_{TEM}, suggesting plasmid-mediated co-selection of resistance traits. The convergence of genotypic and phenotypic resistance patterns across studies underscores the critical need for genetic analysis to guide targeted antimicrobial therapy, especially in diabetic patients with indwelling catheters who are highly vulnerable to chronic and multidrug-resistant infections.

Virulotyping of the CAUTI isolates revealed a high prevalence of key virulence genes, including *fimH* (78%), *papC* (50%), *fyuA* (45%), and *kpsMTII* (33%). Expression of these genes was markedly elevated in biofilm-positive isolates ($p < 0.001$). While *fimH* was ubiquitously present in biofilm-producing strains,

papG, *fyuA*, and *kpsMTII* also exhibited significant correlations with biofilm formation. Conversely, *hlyA* and *iutA* showed no significant association ($p > 0.05$). These results underscore the pivotal role of adhesins (e.g., *fimH*, *papC*) and capsule synthesis genes (e.g., *kpsMTII*) in fostering persistent colonization and enhancing antimicrobial resistance, a conclusion that aligns with the previous studies (Baldiris-Avila et al. 2020; Ramírez Castillo et al. 2023).

A significantly higher prevalence of biofilm formation was observed in isolates from diabetic patients (47.6%) compared to non-diabetics (34.0%). This disparity is likely attributable to poor glycemic control, which creates a conducive environment for bacterial colonization and growth by promoting the development of a robust biofilm matrix and enhancing pathogen resistance (Tankeshwar 2023). Isolates from patients exhibiting elevated HbA1c levels (> 6.5%) showed a marked increase in biofilm production and multidrug resistance, indicating a potential correlation between inadequate glycemic control and heightened virulence. Although the availability of patient-level HbA1c data was restricted, this observation is consistent with earlier research suggesting that chronic hyperglycemia undermines host defenses and promotes biofilm-associated infections (Shahsavari et al. 2024) The clinical consequences of this are substantial; the enhanced resilience and resistance of biofilm-forming uropathogens in diabetic patients present a significant therapeutic challenge, necessitating the use of focused antimicrobial therapy.

Despite its contributions, this study has several limitations. As it was conducted at a single healthcare facility, the generalizability of our findings to other populations or healthcare settings may be limited. Regional disparities in antimicrobial stewardship measures, catheterization techniques, and demographics of patients may affect bacterial profiles and resistance patterns. Although the sample size was sufficient for statistical analysis, a larger cohort might better capture the full genetic and phenotypic diversity of *E. coli* isolates across different patient demographics and geographic regions. Furthermore, although HbA1c data was obtained from patient medical records, the timing of the measurements was inadequate, and longitudinal glycemic variations were not recorded. Moreover, while our molecular profiling identified key resistance and virulence genes, the study did not measure gene expression levels or investigated underlying regulatory mechanisms, which could provide deeper insights into pathogenic behavior.

Conclusions

Our findings reveal a fundamental divergence in the pathogenesis of CAUTIs between diabetic and non-diabetic patients, driven by distinct molecular profiles in causative *E. coli* isolates. Diabetic CAUTIs are characterized by a significantly elevated burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, high-frequency resistance genes ($bla_{\text{CTX-M-15}}$, bla_{TEM}, bla_{NDM}), and an enhanced capacity for biofilm formation. The strong association of specific virulence factors with biofilm production further underscores a heightened and persistent pathogenic state in the diabetic host environment. These results argue that a one-size-fits-all approach to CAUTI management is inadequate. Instead, the findings mandate a paradigm shift towards host-specific diagnostic and therapeutic strategies. Effectively combating these resilient infections in the diabetic population will require integrating rapid molecular diagnostics for resistance and virulence profiling into clinical practice to guide targeted, early intervention. Furthermore, our data highlight the critical urgency for enhanced antimicrobial stewardship programs specifically designed to address the unique selective pressures in this vulnerable cohort. Future research integrating longitudinal patient tracking with transcriptomic and proteomic analyses is essential to elucidate the regulatory mechanisms driving this heightened pathogenicity and to inform the development of novel therapeutic interventions to curb the spread of resistant infections.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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