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Multidrug-Resistant Uropathogenic *Escherichia coli* Typing by ERIC-PCR: A Genetic and Antibiogram Profiling in a Tertiary Care Hospital

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ABSTRACT

Introduction: Uropathogenic *Escherichia coli* (UPEC) is a leading cause of community-acquired and healthcare-associated infections, and antimicrobial resistance in UPEC poses significant challenges to managing these infections. This study aimed to investigate the molecular types of UPEC using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) and analyze their resistance patterns in a tertiary care setting. **Methods:** A cross-sectional study was conducted at a tertiary care hospital, where 65 consecutive *E. coli* isolates from urinary specimens were collected. Isolates were identified biochemically and confirmed by *16S rRNA* gene PCR. Antibiotic susceptibility testing was conducted following CLSI guidelines, and molecular typing was performed using ERIC-PCR. ERIC-PCR profiles were analyzed using PAST software version 4.0, generating a dendrogram to visualize similarity among ERIC types. Fisher's exact test was used to determine if specific ERIC types were significantly associated with particular antibiotic resistance profiles. **Results:** The results showed that 95% of the isolates were resistant to at least two antibiotics, with 92.3% being multidrug resistant (MDR). The highest resistance was observed against ampicillin, while no resistance was seen against colistin and tigecycline. The resistant isolates displayed 36 different antibiograms, indicating a significant degree of resistance variability. ERIC-PCR typing revealed 22 unique clusters at a similarity coefficient of approximately 70%, highlighting the genetic diversity of UPEC isolates in our setting. **Conclusion:** This study enhances the understanding of UPEC epidemiology in healthcare by revealing the molecular characteristics and resistance profiles of prevalent strains. The high occurrence of MDR UPEC and the absence of a correlation between ERIC types and antibiograms suggest adaptability and increased resistance. These results highlight the necessity for continuous surveillance to inform infection control measures and direct targeted interventions against the spread of MDR UPEC.

INTRODUCTION

While most *E. coli* strains are harmless commensals, certain pathotypes can cause disease. These include extraintestinal pathogenic *E. coli* (ExPEC), which causes infections outside the gut, and intestinal pathogenic *E. coli* (IPEC) or diarrheagenic *E. coli* (DEC), which primarily cause intestinal infections [1]. Uropathogenic *E. coli* (UPEC), a subgroup of extraintestinal pathogenic *E. coli* (ExPEC), is a highly diverse group of bacteria responsible for approximately 80-90% of community-acquired urinary tract infections (UTIs). UPEC strains harbor genomic pathogenic islands (PAIs), which are mobile genetic elements encoding key virulence factors that contribute to their pathogenic potential [2]. These virulence factors allow UPEC to evade the host immune system and persist in the urinary tract, leading to complicated and often recurrent or chronic infections [3]. The rapid emergence of antimicrobial resistance among UPEC isolates has significantly compromised treatment efficacy, owing to the limited availability of effective therapeutic options [4]. UPEC strains and their antibiotic resistance patterns vary significantly between healthcare facilities. This variation is likely driven by factors like antibiotic stewardship practices, prescribing habits, and duration of the study [5].

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High-resolution typing of *E. coli*, going beyond species-level identification, is crucial for tracing transmission pathways, identifying the sources of outbreaks, and understanding the clonal spread of multidrug-resistant strains [6]. The selection of a subtyping technique is contingent upon the laboratory's resources and expertise, as well as the specific research question and objectives of the study [7]. Conventional typing methods, such as serotyping or phage typing, although useful, are often time-consuming, labor-intensive, and may lack sufficient discriminatory power to distinguish between closely related isolates. In contrast, molecular typing approaches, which involve DNA analysis, offer higher resolution and improved discriminatory power, allowing for differentiation between closely related bacterial isolates by analyzing their genetic profiles [8]. Several DNA-based typing methods, including pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), DNA sequencing, ribotyping, amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP), are considered robust and reproducible, often exhibiting high discriminatory power. However, some techniques, such as PFGE, can be technically complex, require specialized equipment, and may have turnaround times of several days. While highly informative, methods like MLST and DNA sequencing often require significant bioinformatic analysis [9]. Alternatively, PCR-based typing methods, including arbitrary primed PCR and repetitive extragenic palindromic-PCR (rep-PCR), offer a rapid, cost-effective, and relatively straightforward approach compared to some of the previously mentioned methods. However, proper training and adherence to standardized protocols are essential for reliable results [10]. Rep-PCR employs oligonucleotide primers that target short, conserved repetitive sequences dispersed throughout the *E. coli* genome, enabling the amplification of intergenic regions [11]. This approach amplifies distinct genomic regions flanked by repetitive extragenic palindromic (rep) sequences, generating amplicon patterns that can differentiate between *E. coli* strains. Several rep-PCR-based methods, including REP-PCR, ERIC-PCR (using either ERIC1R and ERIC2 primers or ERIC 2 primers only), BOX-PCR, and (GTG)5-PCR, are widely employed for bacterial genotyping, facilitating strain discrimination [12, 13].

While rep-PCR methods offer advantages in terms of speed and cost-effectiveness, it is important to acknowledge their limitations. Compared to techniques like PFGE, MLST, and DNA sequencing, rep-PCR methods may exhibit lower typability, reproducibility, and discriminatory power in some contexts [9]. However, they remain valuable tools for bacterial typing, particularly when resource limitations or turnaround time are critical, and can provide sufficient resolution for specific research questions.

Among the aforementioned PCR-based methods, ERIC-PCR was selected for our study due to its wide use for typing various strains of *E. coli* globally, its cost-effectiveness, and relatively rapid turnaround time [14-17]. This study aimed to determine the prevalence of MDR UPEC, characterize the circulating ERIC-PCR types, and explore any potential associations between ERIC types and resistance profiles.

MATERIAL AND METHODS

This study was conducted in the Department of Microbiology over a period of ten months, from January 2022 to October 2022, and included the collection of isolates during the first two months and molecular testing during the rest of the eight months. The research protocol was reviewed and approved by the Institutional Ethical Committee (approval number: RP 086/2022), ensuring compliance with ethical standards and guidelines for human research.

Patient selection and sample collection. This study included patients of all age groups and both sexes who presented with clinical signs and symptoms suggestive of UTI (dysuria, frequency, urgency, and fever) and provided informed consent. In accordance with hospital policy, urine samples were collected prior to the initiation of antibiotic therapy. Patients already receiving antibiotics were advised to discontinue treatment for a minimum of five days before sample collection. Midstream urine samples were collected using the clean catch technique, which was explained to patients beforehand and supervised by a healthcare professional. For neonates and catheterized patients, however, urine samples were collected by attending physicians in sterile containers, following standard protocol. The clinical and demographic data collected included history of catheterization, sex, and patient location (outpatient or inpatient).

Urine samples were cultured on HiCrome UTI agar (HiMedia, Mumbai, India) and incubated at 37°C overnight. *E. coli* colonies, characterized by their purple, dome-shaped appearance on HiCrome UTI agar, were isolated and further processed. A colony count of $\geq 10^5$ CFU/ml was considered indicative of a significant infection (microbiologically confirmed UTI) [18].

Inclusion criteria. This study included consecutive urinary isolates of *E. coli* that were identified by standard biochemical tests like indole, methyl red, Voges-Proskauer, citrate, triple sugar iron, urease, and lysine decarboxylase [19].

Exclusion criteria. This study excluded uropathogens other than *E. coli*, repeat isolates from the same patient, and samples from patients who did not provide informed consent. These exclusion criteria ensured a focused investigation of *E. coli* isolates from unique patients who had provided consent for participation in the study.

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A total of 106 biochemically confirmed UPEC isolates were initially collected. However, only 65 isolates met the inclusion criteria and were selected for further analysis, including species-specific PCR confirmation, antimicrobial susceptibility testing, and ERIC-PCR typing. The remaining isolates were excluded due to being repeat isolates or lacking consent.

DNA Extraction. Genomic DNA was extracted from the 65 UPEC isolates using a modified boiling, chilling, and centrifugation method [8, 20]. Briefly, a single colony of *E. coli* from HiCrome UTI agar was inoculated into 5 mL of Luria-Bertani (LB) broth (HiMedia, Mumbai, India) and incubated at 37°C for 18 h. After brief vortexing, 1.5 mL of the incubated LB broth was transferred to a 2 mL microfuge tube and centrifuged at 10,000 rpm (10,864 × g) for 10 min using a Heraeus Megafuge 16R (Thermo Fisher Scientific Inc.). The supernatant was discarded, and the pellet was washed with 1 mL of RNase-free water and vortexed. The tube was then subjected to repeated cycles of boiling (10 min in a water bath) and freezing (10 min at -20°C) twice, followed by centrifugation using a fixed-angle rotor with a radius of 97 mm at 10,000 rpm (10,864 × g) for 5 min. The final supernatant was assessed for purity and concentration using a NanoDrop spectrophotometer (Multiskan Sky; Thermo Fisher). DNA samples with an A_{260}/A_{280} ratio of approximately 1.8 were considered pure and stored at -20°C for subsequent PCR analysis.

Species-specific PCR. To confirm the identity of the 65 presumptive UPEC isolates, a species-specific PCR assay targeting the *16S rRNA* gene was performed using the primer pairs described by Fattahi *et al*. (2013) [21]: P1 (RES) 5'-GGAAGAAGCTTGCTTCTTTGCTG-3' and P2 (FES) 5'-AGCCCGGGGATTTCACATCTGA-3'. The primers were synthesized by Sigma Aldrich Chemical Pvt. Ltd. and PCR amplification was carried out in an Applied Biosystems Thermocycler (Model-2720). The reaction mixture consisted of a 25 µL volume containing 4 µL of DNA template, 18.25 µL of distilled water, 2.5 µL of 10X buffer, 0.5 µL of 10 mM dNTPs, 0.25 µL of 10 µM of each primer, and 0.25 µL of 5 U/µL Taq polymerase. To prevent amplicon contamination, a three-room setup was employed, and sterile nuclease water was used as a negative control, which showed no amplification.

The thermocycler amplification settings consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 50 seconds, annealing at 56°C for 1 min, extension at 72°C for 2 min, and a final extension at 72°C for 7 min. The PCR products were then resolved by electrophoresis in a 1.5% agarose gel at 80 V for 30 min. The gel was stained with ethidium bromide and visualized using a UV gel documentation system (Alpha Innotech, USA). A 100bp DNA ladder from Thermo Fisher was used as a molecular weight marker to determine the size of the PCR products. The ATCC 25922 strain of *E. coli* served as a positive PCR control.

Antibiotic susceptibility testing (AST). Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (HiMedia, Pvt. Ltd, Mumbai, India) according to CLSI 2022 guidelines [22]. Colistin sulfate susceptibility was determined by microbroth dilution, also following CLSI guidelines. The testing encompassed eleven antimicrobial classes, including penicillins: ampicillin (10µg); cephalosporins: ceftriaxone (30µg), cefotaxime (30µg), and cefepime (30µg); aminoglycosides: amikacin (30µg) and gentamicin (10µg), carbapenems: imipenem (10µg), meropenem (10µg), and ertapenem (10µg); beta lactam-beta lactamase inhibitor combinations: piperacillin/tazobactam (100/10 µg), ampicillin/sulbactam (10/10µg), amoxicillin/clavulanic acid (20/10µg); quinolones: norfloxacin (10 µg), ciprofloxacin (5 µg), and levofloxacin (5 µg); folate pathway inhibitors: trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracyclines: tigecycline (15 µg), nitrofurans: nitrofurantoin (300 µg); and phosphonic acids: fosfomycin (200 µg), polymyxins: colistin (potency ≥ 15,000 IU/mg). *E. coli* ATCC 25922 served as a quality control strain for disc diffusion, while a *mcr-1*-positive clinical strain was used as a control for colistin MIC to ensure detection of this clinically significant resistance mechanism. The discs were purchased from HiMedia, Pvt. Ltd, Mumbai, India, while colistin was obtained from Sigma-Aldrich Chemical Pvt Ltd.

Molecular typing of UPEC isolates by ERIC-PCR. The 65 UPEC isolates were subjected to ERIC-PCR using the ERIC1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') primers (Sigma-Aldrich), as described by Versalovic *et al*. (1991) [11]. The ATCC 25922 strain of *E. coli* was used as a positive control to verify PCR efficiency and banding patterns [23]. A gradient PCR was performed in triplicate using DNA from the control strain to determine the optimal annealing temperature for ERIC-PCR. The annealing temperature gradient ranged from 45°C to 55°C. Reagent concentrations were identical to those used for species-specific PCR. The thermocycling conditions were standardized according to Ardakani *et al*. (2016) [7], consisting of initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing between 45-55°C for 35 seconds, extension at 72°C for 4 min, and final extension at 72°C for 5 minutes. The PCR products were separated by electrophoresis at 80 V for 45 min in 2.5% agarose gel stained with ethidium bromide. The band size was compared with a 100 bp DNA ladder (Thermo Fisher) as a molecular weight marker in a UV gel documentation system (Alpha Innotech, USA). An annealing temperature of 49°C was found to be optimal.

To evaluate the precision and reproducibility of our standardized method, the protocol was repeated in triplicate for ten clinical isolates using a conventional thermocycler (Applied Biosystems 2720). Visual

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comparison of the triplicate band patterns showed no discernible differences, indicating good reproducibility of the method.

Data analysis. Antibiotic resistance data were summarized as frequencies and percentages for each drug tested. No statistical comparisons were made between the data sets as the study was designed as an initial exploration of circulating ERIC types, and statistical comparisons of resistance data were beyond the scope of this study.

The ERIC-PCR products were analyzed using a gel documentation system, considering all visible bands. Band intensity variations among isolates were not considered significant. The sizes of the ERIC-PCR products were determined by comparison with a 100 bp DNA ladder (Thermo Fisher), serving as a molecular weight marker.

The ERIC-PCR fingerprints of the amplified DNA fragments of 65 isolates obtained from agarose gel electrophoresis were documented and analyzed. However, ERIC fingerprints of only 62 resistant isolates were converted into a binary matrix, where '1' indicated the presence and '0' indicated the absence of bands. Using the numerical taxonomy and multivariate analysis software package PAST (Version 4.0), cluster analysis was performed. The clustering correlation coefficients were calculated using the unweighted pair group method with arithmetic averages (UPGMA) and were based on the Dice similarity coefficient with a 1% position tolerance [14, 23]. Each isolate was considered an operational taxonomic unit (OTU). To facilitate interpretation and reduce the number of OTUs in the dendrogram, isolates exhibiting a similarity coefficient of at least 70% were categorized as a single isolate or ERIC type [14].

RESULTS

Over the two-month study period, 1419 urine samples were received for culture. Of these, 203 (14.3%) were culture-positive, out of which 175 (86.2%) yielded clinically significant bacterial growth and 28 (13.8%) yielded *Candida* spp. Of the 175 uropathogenic bacteria, 106 (60.6%) were UPEC, 24 (13.7 %) *Enterococcus* spp., 22 (12.6%) *Klebsiella* spp., 9 (5.1%) *Pseudomonas* spp., 4 (2.3%) *Acinetobacter* spp., 2 (1.1%) each of *Proteus* spp., and *Staphylococcus aureus* with 6 (3.4%) being other bacteria. Out of the 106 UPEC isolates initially identified, 65 met the inclusion criteria and were included in the subsequent analysis.

Demographic characteristics. Of the 65 UPEC isolates included in the study, 49 (75.4%) were obtained from outpatients, while 16 (24.6%) were from inpatients. The isolates were collected from 65 patients, of whom 37 (56.9%) were female and 28 (43.1%) were male (Table 1).

Table 1. Demographic characteristics of patients with UTI due to UPEC

Gender distribution of samples (n=65)	
Male 28 (43.1%)	Female 37 (56.9%)
Location distribution of sample (n=65)	
Outpatients 49 (75.4%)	Inpatients 16 (24.6%)

Table 2. Overall percentage resistance to tested antibiotics

S. No	Antibiotics tested (n=20)	Observed resistance in *E. coli* isolates (n=65) n (%)
1	Ampicillin	60 (92.3)
2	Ampicillin-Sulbactam	58 (89.2)
3	Cefotaxime	54 (83.1)
4	Levofloxacin	54 (83.1)
5	Norfloxacin	51 (78.5)
6	Ceftriaxone	49 (75.4)
7	Ciprofloxacin	45 (69.2)
8	Cefepime	44 (67.7)
9	Trimethoprim-Sulfamethoxazole	42 (64.6)
10	Amoxicillin- Clavulanic acid	41 (63.1)
11	Piperacillin-Tazobactam	40 (61.5)
12	Fosfomycin	30 (46.2)
13	Gentamicin	19 (29.2)
14	Nitrofurantoin	13 (20.0)
15	Meropenem	13 (20.0)
16	Amikacin	12 (18.5)
17	Imipenem	11 (16.9)
18	Ertapenem	11 (16.9)
19	Tigecycline	0 (0.0)
20	Colistin	0 (0.0)

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Antibiotic resistance profile of isolates. The 65 UPEC isolates were subjected to susceptibility testing against 20 antibiotics (Table 2). Isolates exhibiting resistance to three or more antibiotic classes were classified as multidrug-resistant (MDR) [24]. Overall, 62 (95.4%) of the isolates displayed resistance to at least two antibiotic classes, highlighting the high prevalence of multidrug resistance among UPEC in this setting. Antibiotic resistance rates varied widely (Table 2), ranging from 0% for colistin and tigecycline to 92.3% for ampicillin.

In contrast, two isolates (3.1%) displayed non-MDR profiles, and three isolates (4.6%) were pan-susceptible (susceptible to all 20 antibiotics tested). Among the 60 MDR isolates, 47 (78.3%) were derived from outpatients (OPD) and 13 (21.7%) from inpatients (IPD). The two non-MDR isolates were recovered from one outpatient and one inpatient, respectively.

The 62 resistant isolates exhibited remarkable diversity in their antibiotic resistance profiles, yielding 36 distinct antibiograms. Each unique antibiogram was considered a distinct antibiotype (Table 3). The most prevalent antibiotypes were antibiotype 15 (AMP-AMC-CIP-STX-CTX), antibiotype 14 (AMP-AMC-CIP-CTX-FOS), and antibiotype 23 (AMP-CIP-CTX-FOS), with 6 (9.67%), 4 (6.45%), and 4 (6.45%) isolates, respectively. Notably, 26 of the 36 antibiotypes (72.2%) were represented by a single isolate, indicating a high degree of strain diversity within this collection. Isolates belonging to antibiotype 1 displayed a broad resistance profile, with resistance to nine of the 11 antibiotic classes. All 60 MDR strains were distributed across 34 antibiotypes, highlighting the complexity of resistance patterns in this population.

Table 3. Observed antibiograms of resistant UPEC isolates.

Antibiotype	Isolate No.	Antibiotic resistance profile (representing nine antimicrobial classes)	No. (%)
1	ECO 41, ECO 81	AMP-AMC-GEN-CIP-SXT-CTX-NIT-FOS-IMP	2 (3.2)
2	ECO 90	AMP-AMC-GEN-CIP-SXT-CTX-NIT-FOS	1 (1.6)
3	ECO 71	AMP-AMC-GEN-CIP-SXT-CTX-NIT-IMP	1 (1.6)
4	ECO 110, ECO 145	AMP-AMC-GEN-CIP-SXT-CTX-FOS-IMP	2 (3.2)
5	ECO 94	AMP-GEN-CIP-SXT-CTX-NIT-FOS	1 (1.6)
6	ECO 53, ECO 97	AMP-AMC-CIP-SXT-CTX-FOS-IMP	2 (3.2)
7	ECO 39, ECO 70	AMP-AMC-GEN-CIP-SXT-CTX-IMP	2 (3.2)
8	ECO 38, ECO 60, ECO 86	AMP-AMC-CIP-SXT-CTX-FOS	3 (4.8)
9	ECO 116, ECO 120, ECO 134	AMP-AMC-GEN-CIP-SXT-CTX	3 (4.8)
10	ECO 17, ECO80	AMP-GEN-CIP-SXT-CTX-NIT	2 (3.2)
11	ECO 57	AMP-AMC-GEN-CIP-SXT-FOS	1 (1.6)
12	ECO 143	GEN-CIP-CTX-NIT-FOS-IMP	1 (1.6)
13	ECO 56	AMP-AMC-SXT-CTX-NIT-FOS	1 (1.6)
14	ECO 37, ECO 47, ECO 88, ECO 126	AMP-AMC-CIP-CTX-FOS	4 (6.4)
15	ECO 42, ECO 84, ECO 89, ECO 128, ECO 129, ECO 142	AMP-AMC-CIP-SXT-CTX	6 (9.7)
16	ECO 82	AMP-AMC-SXT-CTX-FOS	1 (1.6)
17	ECO 105	AMP-AMC-CTX-NIT-FOS	1 (1.6)
18	ECO 55	AMP-AMC-CIP-NIT-FOS	1 (1.6)
19	ECO 58	AMP-GEN-SXT-CTX-FOS	1 (1.6)
20	ECO 135	GEN-CIP-SXT-CTX-NIT	1 (1.6)
21	ECO 150	AMP-AMC-CIP-CTX-IMP	1 (1.6)
22	ECO 18, ECO 40	AMP-CIP-SXT-CTX	2 (3.2)
23	ECO 21, ECO 83, ECO 146, ECO 147	AMP-CIP-CTX-FOS	4 (6.4)
24	ECO 144	AMP-AMC-CIP-CTX	1 (1.6)
25	ECO 107, ECO 114	AMP-AMC-SXT-CTX	2 (3.2)

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26	ECO 54, ECO 106	AMP-SXT-CTX-FOS	2 (3.2)
27	ECO 62, ECO 108	AMP-AMC-CIP-SXT	2 (3.2)
28	ECO 109	AMP-AMC-CTX-FOS	1 (1.6)
29	ECO 131	AMP- GEN-SXT-CTX	1 (1.6)
30	ECO 61, ECO 115, ECO 122	AMP-SXT-CTX	3 (4.8)
31	ECO 121	AMP-CIP-CTX	1 (1.6)
32	ECO 130	AMP-AMC-SXT	1 (1.6)
33	ECO 65	AMP-AMC-FOS	1 (1.6)
34	ECO 52	AMP-NIT-FOS	1 (1.6)
35	ECO 99	AMP-CTX	1 (1.6)
36	ECO 79	AMP-AMC	1 (1.6)

AMP=Ampicillin, AMC=Amoxicillin/clavulanic acid, GEN=Gentamicin, CIP=Ciprofloxacin, SXT=Trimethoprim-sulfamethoxazole (Co-trimoxazole), CTX=Cefotaxime, NIT=Nitrofurantoin, FOS=Fosfomycin, IMP=Imipenem.

Genetic diversity of UPEC isolates revealed by ERIC-PCR. All 65 UPEC isolates yielded interpretable banding patterns using ERIC-PCR. All 65 isolates and the positive control exhibited the expected product size of 544 bp (acceptable range 540-550 bp) (Figure 1) for the 16S rRNA primer pair [21]. The ERIC-PCR analysis revealed a wide range of molecular weights, ranging from 110 to 2010 bp, indicating a high degree of genetic diversity among the isolates (Figure 2).

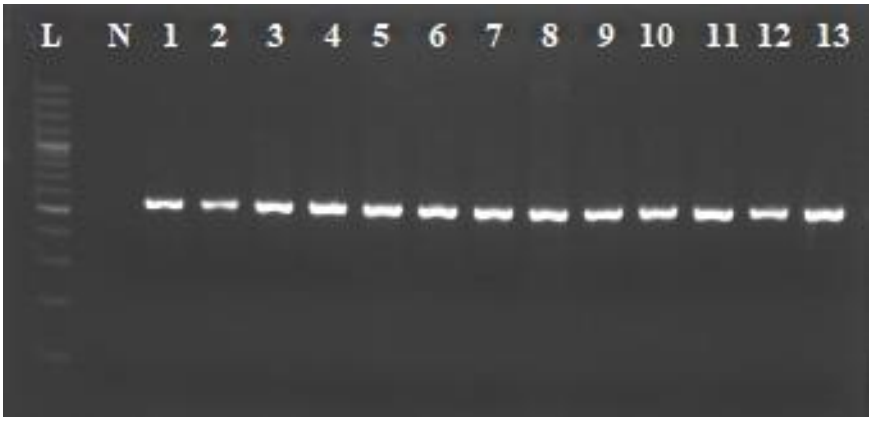

Fig. 1. Gel electrophoresis confirmation of *E. coli* by RES/FES-PCR. Lane L: 100 bp DNA ladder, Lane N: Negative control (Sterile nuclease free water), Lanes 1-13: *E. coli* isolates.

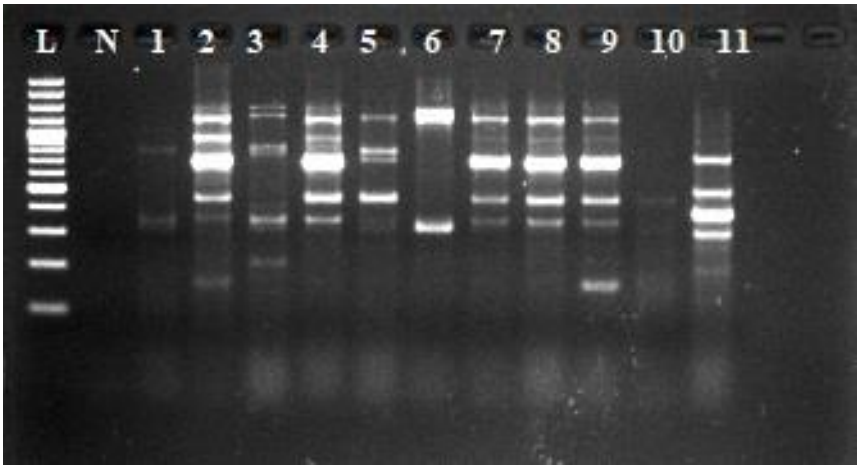

Fig. 2. ERIC-PCR profiles of *E. coli* isolates. Lane L: 100bp DNA ladder, N: Negative control (Sterile nuclease free water), Lanes 1-11 strains of *E. coli.*

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A dendrogram (Figure 3) was constructed for the 62 resistant UPEC isolates. At a similarity coefficient of 100%, representing identical banding patterns, 47 unique ERIC types (E1 to E47) were identified (Table 4). Notably, ERIC types 8, 16, 23, 25, 31, 34, 38, 39, 40, and 41 each comprised two isolates, while type E33 consisted of three isolates, and type E45 comprised four isolates with highly similar ERIC-PCR patterns. These 62 resistant isolates were further grouped into 22 clusters (I-XXII) at a similarity coefficient of 70% (cut-off) [14]. Cluster XX consisted of four isolates belonging to a single ERIC type, while clusters XIII and XV each comprised two isolates from a single ERIC type.

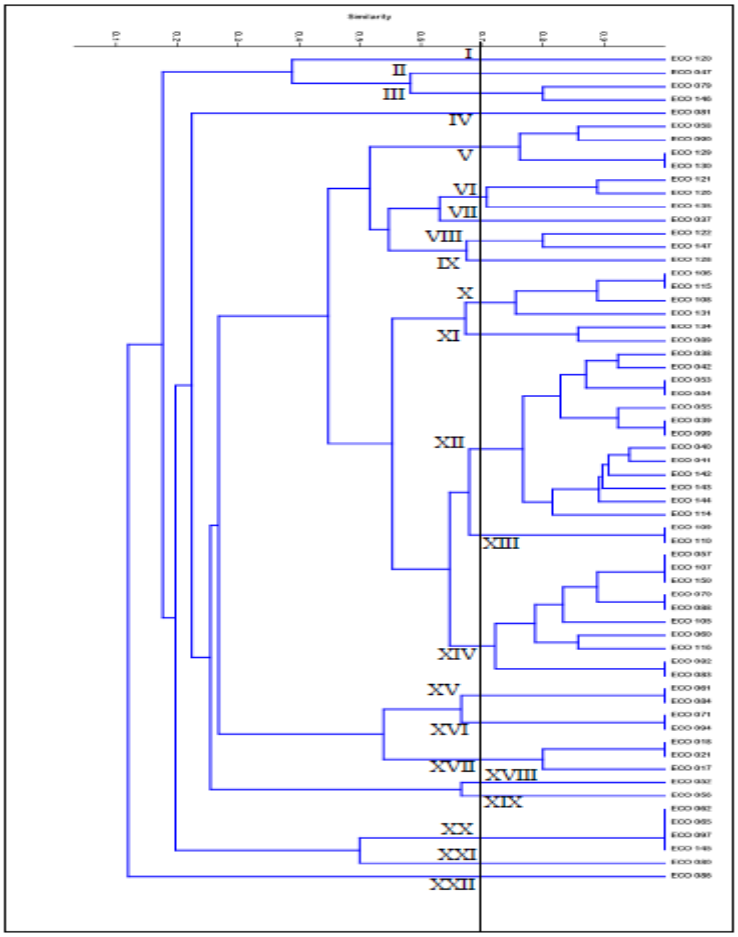

Fig 3. Dendrogram depicting the genetic relatedness of antibiotic-resistant UPEC isolates based on ERIC-PCR profiles.

Table 4. ERIC-PCR typing and characteristics of antibiotic-resistant UPEC isolates

No	ERIC Type	Isolate ID	Sex	Patient location	MDR Status
1	E1	ECO 120	Male	Outpatient	MDR
2	E2	ECO 047	Male	Outpatient	MDR
3	E3	ECO 079	Female	Outpatient	Non-MDR
4	E4	ECO 146	Female	Outpatient	MDR
5	E5	ECO 081	Female	Inpatient	MDR
6	E6	ECO 058	Female	Outpatient	MDR
7	E7	ECO 090	Female	Outpatient	MDR
8	E8	ECO 129	Female	Inpatient	MDR
9		ECO 130	Female	Outpatient	MDR
10	E9	ECO 121	Male	Outpatient	MDR
11	E10	ECO 126	Male	Outpatient	MDR
12	E11	ECO 135	Female	Inpatient	MDR
13	E12	ECO 037	Male	Outpatient	MDR
14	E13	ECO 122	Male	Outpatient	MDR
15	E14	ECO 147	Female	Outpatient	MDR
16	E15	ECO 128	Male	Outpatient	MDR
17	E16	ECO 106	Female	Outpatient	MDR

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18		ECO 115	Female	Outpatient	MDR
19	E17	ECO 108	Female	Outpatient	MDR
20	E18	ECO 131	Female	Outpatient	MDR
21	E19	ECO 134	Male	Outpatient	MDR
22	E20	ECO 089	Female	Inpatient	MDR
23	E21	ECO 038	Male	Outpatient	MDR
24	E22	ECO 042	Female	Outpatient	MDR
25	E23	ECO 053	Male	Inpatient	MDR
26		ECO 054	Female	Outpatient	MDR
27	E24	ECO 055	Female	Outpatient	MDR
28	E25	ECO 039	Male	Inpatient	MDR
29		ECO 099	Female	Inpatient	Non-MDR
30	E26	ECO 040	Male	Inpatient	MDR
31	E27	ECO 041	Male	Inpatient	MDR
32	E28	ECO 142	Female	Outpatient	MDR
33	E29	ECO 143	Female	Outpatient	MDR
34	E30	ECO 144	Male	Outpatient	MDR
35	E31	ECO 109	Female	Outpatient	MDR
36		ECO 110	Male	Outpatient	MDR
37	E32	ECO 114	Female	Outpatient	MDR
38		ECO 107	Male	Outpatient	MDR
39	E33	ECO 057	Female	Outpatient	MDR
40		ECO 150	Female	Outpatient	MDR
41	E34	ECO 070	Male	Inpatient	MDR
42		ECO 088	Female	Outpatient	MDR
43	E35	ECO 105	Female	Outpatient	MDR
44	E36	ECO 060	Male	Outpatient	MDR
45	E37	ECO 116	Male	Inpatient	MDR
46	E38	ECO 082	Female	Outpatient	MDR
47		ECO 083	Female	Outpatient	MDR
48	E39	ECO 061	Female	Inpatient	MDR
49		ECO 084	Female	Outpatient	MDR
50	E40	ECO 071	Male	Outpatient	MDR
51		ECO 094	Male	Outpatient	MDR
52	E41	ECO 018	Female	Outpatient	MDR
53		ECO 021	Female	Outpatient	MDR
54	E42	ECO 017	Male	Outpatient	MDR
55	E43	ECO 052	Female	Outpatient	MDR
56	E44	ECO 056	Male	Inpatient	MDR
57		ECO 062	Male	Outpatient	MDR
58	E45	ECO 065	Female	Inpatient	MDR
59		ECO 097	Male	Outpatient	MDR
60		ECO 145	Male	Outpatient	MDR
61	E46	ECO 080	Female	Outpatient	MDR
62	E47	ECO 086	Female	Outpatient	MDR

DISCUSSION

UTIs are among the most common bacterial infections, leading to a significant global healthcare cost burden [25, 26]. In this study, among the 1419 patients with suspected UTIs, 203(14.3%) had culture-confirmed UTIs. Kubone *et al*. (2020) reported a UTI prevalence of 19.6% [4] and suggested that reliance on clinical assessments alone may contribute to the overdiagnosis of UTIs, potentially leading to unnecessary antibiotic prescriptions. Overdiagnosis and the ensuing overuse of empirical antibiotics can have detrimental consequences, including the selection and spread of antibiotic-resistant bacteria, increased healthcare expenditures, and a greater economic strain on both healthcare systems and patients [27].

Consistent with numerous previous studies [4, 7, 27], our findings revealed a higher prevalence of UTIs among females. This well-established gender disparity is attributed to anatomical factors, such as a shorter urethra and its close proximity to the anus, which can facilitate bacterial entry into the urinary tract. Additionally, physiological factors like pregnancy, which can alter urinary flow and immune response, may also contribute to increased UTI susceptibility in females.

UPEC is the most common cause of UTIs, accounting for approximately 75% of uncomplicated cases and 65% of complicated cases [28]. In our study, UPEC was the most frequently isolated uropathogen in over 60% of culture-confirmed UTIs. This finding aligns with the prevalence of 67.1% reported in a study from South Lebanon [29]. However, it is important to note that UPEC prevalence can exhibit considerable variability across different geographical regions and populations. For instance, studies have reported rates ranging from 19% in North Lebanon [29] to 81.2% in South Africa [4]. These variations underscore the need to consider regional and demographic factors when evaluating the burden of UPEC and designing tailored strategies to mitigate its dissemination.

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The *16S rRNA* gene primers used in this study successfully amplified the target region in all *E. coli* isolates, consistent with previous reports demonstrating their specificity [21, 30, 31]. The *E. coli* ATCC 25922 strain, employed as a control in our study, has been extensively utilized as a reference strain in various molecular studies on *E. coli*, including ERIC-PCR analysis [23, 32]. This strain is well-characterized at both molecular and physiological levels, making it as an ideal control for molecular assays.

Our study found 100% concordance between routine biochemical tests and species-specific PCR for identifying *E. coli*. This high agreement supports the reliability of our biochemical testing workflow in this specific context. However, it is essential to acknowledge that variations in laboratory protocols and the potential for encountering atypical or emerging strains can influence identification accuracy. This highlights the ongoing need for careful validation of identification methods and the potential role of confirmatory molecular techniques in certain scenarios.

The selection of first-line antibiotics for treating UTIs is guided by several factors, including the susceptibility profile of the causative organisms, potential drug side effects, patient affordability, and the healthcare setting [4]. A comprehensive consideration of these factors is crucial for effective treatment outcomes and antimicrobial stewardship. Following the Indian Council of Medical Research (ICMR) standard treatment guidelines (STG) updated in 2021, the recommended first-line empiric antibiotics for the treatment of cystitis in India are nitrofurantoin, co-trimoxazole, and ciprofloxacin. For the management of acute pyelonephritis, the guidelines recommend piperacillin/tazobactam and ertapenem as the preferred initial therapeutic options [33].

In this study, we observed alarmingly high resistance rates to several first-line antibiotics recommended by the STG, posing a significant challenge to UTI management. Specifically, high resistance rates were observed for ciprofloxacin (69.2%), co-trimoxazole (64.6%), and piperacillin-tazobactam (61.5%), along with notable resistance to nitrofurantoin (20.0%) and imipenem/ertapenem (16.9%). Similar resistance patterns, with high rates against ciprofloxacin, co-trimoxazole, and β-lactam/β-lactamase inhibitor combinations**,** were reported by Alshaikh *et al*. (2024) in Egypt [15]. Among the 62 UPEC isolates in our study, significant co-resistance was observed in 44 (71%) ciprofloxacin-resistant isolates, exhibiting resistance to two to eight additional antibiotics (Table 3). This finding aligns with the observations made by Alshaikh *et al*. (2024) [15]. This cross-resistance could be attributed to the simultaneous carriage of resistance genes on mobile genetic elements. Moreover, acquiring fluoroquinolone resistance can induce efflux mechanisms, potentially contributing to cross-resistance to other antibiotic classes [15]. These findings underscore the critical need for continuous surveillance to monitor the spread of these highly resistant UPEC strains and to guide the development of targeted interventions.

The global emergence of MDR UPEC has become a pressing concern over the past few decades. The alarmingly high MDR rate of 92% among UPEC strains observed in our study is consistent with findings from other studies [15, 34]. A recent meta-analysis conducted by Bunduki *et al*. (2021) [30] analyzed 13 studies worldwide, encompassing 1888 isolates, and reported significantly high resistance rates to various antibiotic classes, including aminopenicillins (74.3%), sulfonamides (59.3%), quinolones (49.4%), and cephalosporins (38.8%). These consistent findings underscore the critical need for effective antimicrobial stewardship programs and the development of alternative treatment strategies to effectively address the challenge of MDR UPEC infections.

The high resistance rates to third-generation cephalosporins observed in our study are concerning and suggest the possible presence of extended-spectrum β-lactamase (ESBL)-producing *E. coli*. Further investigation, including ESBL testing, is warranted to confirm this and guide appropriate treatment strategies. While carbapenems are generally considered the treatment of choice for ESBL-producing UPEC isolates, our findings reveal a notable discrepancy in carbapenem susceptibility rates, ranging from 77% to 80%, which differs from the 100% susceptibility reported by Bunduki *et al*. (2021) [35], potentially highlighting regional variations in resistance patterns. Our findings are consistent with those of Jena et al. (2017) [10], who reported resistance rates of 17% to imipenem and 26.8% to meropenem in their study conducted in Bhubaneswar, India. This similarity raises the possibility of comparable antimicrobial resistance patterns in UPEC isolates from different regions. The alarmingly high prevalence of carbapenem resistance observed in our study is a pressing concern, emphasizing the importance of judicious carbapenem use. Restricting carbapenem use to extensively drug-resistant isolates with limited treatment options, as suggested by Sabat *et al*. (2000) [30], warrants further consideration to help preserve their efficacy. This approach will help preserve the efficacy of carbapenems as a last line of defense against multidrug-resistant UPEC infections.

Our findings, showing 100% susceptibility to colistin, are consistent with those of Jena *et al*. (2017) and Alshaikh *et al*. (2024) [10, 15], who also reported complete susceptibility to this antibiotic. Colistin and tigecycline, while potentially effective against multidrug-resistant uropathogenic *E. coli*, have significant limitations. Colistin is associated with serious side effects, particularly nephrotoxicity, while tigecycline exhibits poor tissue penetration, making it less suitable for urinary tract infections due to its low concentration in urine**.** Therefore, these antibiotics should be reserved as last-

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resort treatment options. Although our study found no colistin-resistant UPEC isolates, aligning with some previous reports from similar settings [10, 15], the emergence and spread of colistin resistance, particularly *mcr*-mediated resistance, pose a significant threat, and ongoing surveillance is crucial**.**

Although Doesschate *et al*. (2018) [36] reported generally low resistance rates to fosfomycin in many countries, our study found a concerningly high resistance rate (46.1%). This finding raises serious concerns about the use of fosfomycin as an empirical therapy for UTIs in our setting.

The high prevalence of MDR UPEC observed in our study is concerning and may be linked to the widespread and indiscriminate use of empiric antibiotics, including β-lactams, macrolides, cephalosporins, and fluoroquinolones, for UTIs and other bacterial infections, often without definitive diagnostic evidence of the causative agent. This practice has contributed to the rapid acquisition of high-level resistance in *E. coli*, a pathogen commonly acquired both in the community and healthcare settings. These findings underscore the urgent need for enhanced diagnostic capabilities to ensure appropriate antibiotic selection and robust antimicrobial stewardship programs to guide their judicious use. Further research is warranted to evaluate the impact of tailored prescribing practices, informed by local resistance patterns, on the prevalence of MDR UPEC.

Our study revealed a high degree of genetic diversity among MDR UPEC isolates, with 22 distinct ERIC clusters identified among 62 resistant isolates at a 70% cutoff value. This finding aligns with previous studies that have also documented significant heterogeneity among MDR UPEC isolates using ERIC-PCR. For instance, Jena *et al*. (2017) identified 14 unique ERIC clusters among their isolates**,** and Adwan *et al*. (2021) identified 11 unique ERIC clusters among 41 MDR *E. coli* isolates at a 60% cutoff value. Jena *et al*. (2017) included all clinical *E. coli* isolates, while Adwan *et al*. (2021) focused specifically on UPEC isolates in their study [10, 37].

However, several instances of identical or highly similar ERIC profiles were observed in our study, suggesting potential clonal dissemination. For example, four isolates (ECO 62, ECO 65, ECO 97, ECO 145) shared ERIC type E45; three isolates (ECO 57, ECO 107, ECO 150) shared type E33; and nine ERIC types (E8, E16, E23, E25, E31, E34, E38, E40, and E41) were each shared by two isolates. These findings suggest a greater degree of clonality among our UPEC isolates compared to some previous studies [10].

Ardakani *et al*. (2016) assessed the utility of ERIC-PCR for molecular typing of 98 UPEC isolates from hospitalized patients, revealing a diverse genotypic distribution among the strains [7]. Their analysis categorized the isolates into six distinct clusters (E1-E6) based on 70% similarity, with three isolates remaining non-typeable due to the absence of bands. While their findings highlight the discriminatory power of ERIC-PCR, our study demonstrated an even greater degree of genetic diversity among 62 UPEC isolates, which were grouped into 22 clusters, with all isolates successfully yielding typeable banding patterns**.** This difference in observed diversity could be attributed to several factors, including variations in the study populations, sample sizes, geographic locations, ERIC-PCR protocols, or local antibiotic usage patterns [7].

In a study by Ramazanzadeh *et al*. (2013), 230 *E. coli* isolates from various clinical samples were subjected to ERIC-PCR typing, yielding 205 distinct ERIC types, which were further grouped into 20 major clusters (C1-C20) [38]. The fingerprint patterns revealed complex banding profiles, with 6–15 bands per electrophoretic path, and PCR product sizes ranging from approximately 100 bp to 1400 bp. Clustering analysis categorized the 230 strains into 10 similar groups, with 187 (81.3%) isolates displaying unique profiles and 43 (18.7%) sharing similar patterns, suggesting common dissemination sources. While ERIC-PCR profiling did not reveal genetic relatedness among 187 *E. coli* strains, suggesting that most infections in their university hospital setting were not clonally spread, but rather resulted from independent acquisition, this finding should be interpreted with caution given the diverse nature of their clinical isolates. Our study, which focused specifically on UPEC isolates, might be more likely to identify clonal transmission events due to the specific niche and potential for common transmission routes within this group.

Our study utilized ERIC-PCR for molecular typing, a cost-effective and relatively straightforward approach. However, it is important to acknowledge that ERIC-PCR has inherent limitations, including reduced reproducibility, typeability, and discriminatory power, compared to gold-standard techniques such as pulsed-field gel electrophoresis (PFGE) or whole-genome sequencing (WGS). Consequently, our findings may represent an underestimation of the true genetic diversity of our isolates or may not fully capture potential clonal relationships. Despite these limitations, ERIC-PCR proved to be a valuable tool for our study, providing initial insights into the genetic relatedness of MDR UPEC isolates and laying the groundwork for future investigations using higher-resolution typing methods.

To the best of our knowledge, this study represents one of the first investigations in this region to characterize MDR UPEC isolates using a combined antibiotyping and molecular typing approach. Our findings highlight the significant presence of diverse MDR UPEC isolates among UTI cases in our tertiary care hospital setting, suggesting a complex relationship between genetic background and resistance profiles that could be influenced by horizontal gene transfer or other evolutionary processes**.** Future investigations employing higher-resolution typing methods, such as WGS, are

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warranted to gain a clearer understanding of clonality, evolutionary history, and transmission dynamics within this population. Our findings underscore the need for continuous surveillance of MDR UPEC using combined typing approaches to monitor the emergence and spread of resistance, guide infection control measures, and inform the development of effective antimicrobial stewardship programs tailored to the local epidemiology of UPEC.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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