

Characterization of Microbiome Incidence and Antibacterial Resistance Profiles in Urinary Tract Infected Patients

Abstract :

Urinary tract infections (UTIs) represent one of the most common bacterial infections worldwide and are increasingly complicated by the emergence of multidrug-resistant (MDR) uropathogens. The present cross-sectional study aimed to investigate the incidence, microbial diversity, and antimicrobial resistance profiles of uropathogens isolated from clinically diagnosed UTI patients belonging to different high-risk groups. A total of 385 clean-catch midstream urine samples were collected and processed using standard microbiological procedures. Samples were cultured on MacConkey agar and Blood agar, yielding 770 primary culture plates. Based on significant bacteriuria ($\geq 10^5$ CFU/mL), 121 morphologically distinct bacterial isolates were recovered and subjected to detailed cultural, biochemical, molecular, and antibiotic susceptibility analyses. Cultural and morphological characterization revealed a clear predominance of Gram-negative rods, with lactose-fermenting and non-fermenting bacteria widely distributed. Biochemical profiling identified major uropathogens including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*. Antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method demonstrated variable resistance patterns, with fluoroquinolones and imipenem showing relatively higher efficacy, while several β -lactams, macrolides, and glycopeptides exhibited substantial resistance. Multiple Antibiotic Resistance (MAR) index analysis indicated that a majority of tested isolates possessed MAR values ≥ 0.30 , highlighting widespread multidrug resistance. Molecular identification of representative isolates by 16S rRNA gene sequencing confirmed phenotypic identification with $\geq 99\%$ sequence similarity.

Keywords: Urinary tract infection (UTI), uropathogens, antimicrobial resistance, biochemical tests, 16S rRNA, Kirby–Bauer, multidrug resistance.

1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections affecting individuals of all age groups and represent a significant cause of morbidity, healthcare burden, and antibiotic use worldwide. Females are disproportionately affected due to anatomical and

physiological factors, while children, diabetic patients, and pregnant women constitute high-risk groups because of immune, metabolic, and hormonal influences that favor bacterial colonization and persistence (Foxman, 2014; Geerlings, 2008; Delzell&Lefevre, 2000). *Escherichia coli* remains the predominant uropathogen, accounting for the majority of community-acquired UTIs, followed by *Klebsiellapneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*, all of which display varying virulence and resistance profiles (Terlizzi *et al.*, 2017). Conventional diagnosis relies on urine culture, colony morphology, Gram staining, and biochemical testing, while molecular tools such as 16S rRNA gene sequencing enhance species-level identification, particularly for atypical or biochemically ambiguous isolates (Cheesbrough, 2005; Sambrook& Russell, 2001). The increasing prevalence of multidrug-resistant (MDR) uropathogens, driven largely by indiscriminate antibiotic use, poses a major challenge to effective UTI management and limits empirical treatment options (Tamma *et al.*, 2021). In this context, the present study was undertaken to evaluate the incidence, diversity, and antimicrobial susceptibility patterns of uropathogens isolated from 385 clinically diagnosed UTI patients belonging to four high-risk populations children, adults, diabetic patients, and pregnant women using integrated cultural, biochemical, molecular, and antibiotic susceptibility analyses to support evidence-based therapy and strengthened antimicrobial stewardship.

2. Materials and Methods

2.1 Study Design and Population

The present cross-sectional study was conducted to evaluate the incidence, microbial diversity, and antimicrobial resistance profiles of uropathogens isolated from clinically diagnosed urinary tract infection (UTI) patients. A total of 385 clean-catch midstream urine samples were collected from four clinically relevant populations, namely children, adults, diabetic patients, and pregnant women. Patients presenting with symptoms suggestive of UTIs, including dysuria, urinary urgency, frequency, flank pain, fever, or cloudy and foul-smelling urine, were included in the study. Demographic and clinical information such as age, sex, diabetic status, and pregnancy status was recorded prior to sample collection. Samples yielding bacterial counts of $\geq 10^5$ CFU/mL were considered positive for significant bacteriuria. Ethical approval was obtained from the institutional ethics committee, and written informed consent was secured from all participants or their legal guardians before enrolment.

2.2 Sample Collection and Transportation

Clean-catch midstream urine samples (approximately 20 mL) were aseptically collected from 385 clinically diagnosed UTI patients in sterile, screw-capped urine containers following standard clinical and microbiological guidelines. Prior to collection, participants were provided with clear instructions regarding proper perineal cleansing and midstream urine collection to minimize contamination from peri-urethral flora (Leslie *et al.*, 2024). Each sample was labeled with a unique identification code along with relevant patient details. The collected urine specimens were transported to the microbiology laboratory within 2 hours of collection in insulated ice boxes maintained at 4 °C to prevent bacterial overgrowth. In cases where immediate transport was not feasible, samples were refrigerated at 4 °C (without freezing) and processed within a maximum of 8 hours to ensure microbial integrity and diagnostic reliability.

2.3 Microbiological Processing

Microbiological processing of all urine samples was carried out using standard quantitative culture techniques to ensure accurate recovery of uropathogens. Upon arrival at the laboratory, each urine sample was gently mixed to homogenize the microbial load. A sterile calibrated 10 µL loop was used to inoculate urine onto two culture media MacConkey agar (385 plates) and Blood agar (385 plates) following the semi-quantitative streaking method recommended for routine UTI diagnosis.

MacConkey agar was used for selective isolation of Gram-negative enteric bacteria and for differentiating lactose fermenters from non-lactose fermenters, while Blood agar supported the growth of fastidious organisms and enabled observation of hemolytic patterns. All inoculations were performed inside a laminar airflow cabinet to maintain aseptic conditions and avoid contamination. Inoculated plates were incubated aerobically at 37 °C for 24–48 hours.

After incubation, plates were examined for bacterial growth and colony-forming units (CFU/mL) were calculated. Plates showing $\geq 10^5$ CFU/mL were interpreted as significant bacteriuria. Based on plate counts, 135 MacConkey agar plates were countable (35.06%), while 250 plates were classified as Too Many to Count (TMTC) (64.94%). Similarly, 159 Blood agar plates were countable (41.30%), whereas 226 plates were TMTC (58.70%), reflecting high bacterial loads in several samples.

Table1. Plate distribution on different culture media

Medium	Total plates inoculated	Countable plates (%)	TMTC plates (%)
MacConkey agar	385	35.06	64.94
Blood agar	385	41.30	58.70
Total (both media)	770	38.18	61.82

93

94 From the countable plates, distinct well-isolated colonies were selected for further processing. A
95 total of 59 isolates were recovered from MacConkey agar (16.4%) and 62 isolates from Blood
96 agar (17.2%), yielding 121 primary isolates. These isolates were subcultured onto nutrient agar
97 slants and incubated at 37 °C for 24 hours to obtain pure cultures. The purified isolates were
98 preserved at 4 °C for subsequent cultural, biochemical, molecular, and antibiotic susceptibility
99 analyses.

100 2.4 Cultural and Morphological Identification

101 Cultural and morphological identification of the isolated bacteria was performed as an essential
102 preliminary step before biochemical and molecular characterization. All 121 purified isolates
103 (59 from MacConkey agar and 62 from Blood agar) were examined on their primary culture
104 plates, followed by assessment on nutrient agar to confirm colony consistency. Colony
105 characteristics, including size, shape, texture, elevation, margin, opacity, pigmentation, lactose
106 fermentation, hemolysis, and swarming behavior, were recorded in accordance with the
107 guidelines of *Bergey's Manual of Systematic Bacteriology* (Krieg & Holt, 1984). On MacConkey
108 agar, lactose-fermenting organisms produced pink to red colonies, indicative of acid production
109 from lactose, whereas non-lactose fermenters appeared pale, colorless, or translucent. Many
110 isolates such as MACS29, MACS42, MACS55, and MACS166 showed mucoid, smooth, or
111 yellowish colonies, suggestive of *Klebsiella* or *Enterobacter* species. Several isolates displayed
112 typical non-lactose-fermenting flat or opaque colonies, commonly associated with *Proteus*,
113 *Pseudomonas*, and other Gram-negative rods. Swarming motility characteristic of *Proteus spp.*
114 was observed in isolates such as MACS32, MACS42, MACS61, and MACS94.

115 On Blood agar, colony morphology and hemolysis patterns were assessed. β -hemolysis
116 (complete clearing) indicated potential *Staphylococcus aureus* or *Hemolytic Streptococcus*,
117 whereas α -hemolysis (greenish discoloration) suggested *Enterococcus* or viridans group
118 streptococci. Non-hemolytic (γ -hemolysis) colonies were also documented. Gram-positive

119 cocci formed creamy white, convex colonies arranged in clusters or chains, characteristic of
120 *Staphylococcus* and *Enterococcus spp.*, respectively.

121 Isolates recovered on MacConkey agar were designated with the prefix “MAC”, while those
122 obtained on Blood agar were designated with the prefix “BA”.

123 Gram staining was performed on all isolates to classify them as Gram-positive or Gram-
124 negative and to determine morphology (rods, cocci, or coccobacilli). Smears were stained using
125 crystal violet, Gram’s iodine, alcohol decolorizer, and safranin counterstain, after which
126 microscopic examination under oil immersion was used to confirm cell shape and arrangement.
127 These combined cultural and morphological assessments provided foundational differentiation
128 of isolates and guided further biochemical identification.

129 **2.5 Biochemical Characterization**

130 Biochemical characterization was performed on all 121 purified isolates to determine their
131 metabolic profiles and support accurate species identification, following the standard procedures of
132 Bergey’s Manual (Krieg & Holt, 1984) and Aneja (2003). Each isolate was subjected to a
133 comprehensive series of biochemical tests, including carbohydrate fermentation (glucose, lactose,
134 sucrose, and mannitol) to assess acid and gas production patterns essential for differentiating enteric
135 bacteria. Indole, Methyl Red (MR), and Voges–Proskauer (VP) tests were performed to evaluate
136 tryptophanase activity and fermentation pathways, enabling clear distinction between MR-positive
137 organisms such as *Escherichia coli* and VP-positive organisms such as *Klebsiella* and *Enterobacter*
138 species. Citrate utilization on Simmon’s citrate agar was used to identify citrate-positive taxa, while
139 urease testing and hydrogen sulfide (H₂S) production on urea agar and SIM medium aided in
140 identifying characteristic urease- and sulfur-producing organisms, particularly *Proteus* species.
141 Additional tests including nitrate reduction, catalase, and oxidase assays supported differentiation of
142 enteric bacteria, staphylococci, enterococci, and non-fermenters such as *Pseudomonas aeruginosa*.
143 Motility was assessed using motility agar, while ONPG, aesculin hydrolysis, and arginine hydrolysis
144 tests provided further discriminatory power for identifying β -galactosidase activity, bile-aesculin
145 hydrolysis typical of *Enterococcus* species, and amino acid metabolism patterns. Together, these
146 biochemical profiles allowed accurate classification of isolates into major uropathogenic groups,
147 including *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, *Enterococcus spp.*, and
148 *Staphylococcus spp.*, forming the foundation for molecular and antibiotic susceptibility analysis
149 (Samanta et al., 2020).

2.6 Antibiotic Susceptibility Testing

Antibiotic susceptibility patterns of all confirmed isolates were assessed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, following the standardized guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Fresh 24-hour cultures were prepared in sterile saline and adjusted to 0.5 McFarland turbidity, equivalent to approximately 1.5×10^8 CFU/mL. Using a sterile cotton swab, the standardized inoculum was uniformly spread over the surface of Mueller–Hinton agar plates to ensure the formation of a confluent lawn. Commercial antibiotic discs representing commonly used therapeutic agents were aseptically placed onto the agar surface at appropriate distances to avoid overlap of inhibition zones (Kibre and Abera 2014). The antibiotic panel included fluoroquinolones (ciprofloxacin, ofloxacin), β -lactams (ampicillin, penicillin G, oxacillin, piperacillin), cephalosporins (cefuroxime), aminoglycosides (gentamicin), macrolides (erythromycin), glycopeptides (vancomycin), carbapenems (imipenem), chloramphenicol, and trimethoprim sulfamethoxazole (McCarty *et al.*, 1999). Inoculated plates were incubated at 37°C for 18-24 hours, after which the diameters of inhibition zones were measured in millimeters using a digital caliper. Results were interpreted as Sensitive, Intermediate, or Resistant according to NCCLS/CLSI breakpoint criteria (Leslie *et al.*, 2024). To ensure reliability, each batch included quality control strains (Beyene and Tsegaye 2011). The susceptibility profiles obtained guided determination of multidrug-resistant isolates and provided critical insights into therapeutic effectiveness against uropathogens.

2.7 Molecular Identification

Molecular characterization was performed on twenty representative isolates selected based on distinct cultural and biochemical profiles to confirm species-level identification. Genomic DNA was extracted using the Phenol:Chloroform:Isoamyl Alcohol (PCI) method described by Sambrook and Russell (2001). Briefly, overnight-grown bacterial cultures were harvested and lysed using TE buffer, SDS, and Proteinase K, followed by PCI extraction to remove proteins and cellular debris. DNA was precipitated with ice-cold ethanol and sodium acetate, washed with 70% ethanol, air-dried, and resuspended in TE buffer. The purity and concentration of genomic DNA were assessed spectrophotometrically at 260/280 nm. Amplification of the 16S rRNA gene was carried out using universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'),

targeting an expected ~1500 bp region. PCR mixtures contained template DNA, PCR buffer, MgCl₂, dNTPs, forward and reverse primers, and Taq DNA polymerase, and were subjected to standardized thermocycling conditions comprising initial denaturation, 35 cycles of denaturation–annealing–extension, and a final extension step. Amplified products were visualized on a 1.5% agarose gel under UV illumination, and successful bands were purified for Sanger sequencing. The resulting sequences were edited and compared using the NCBI BLASTN database to determine taxonomic identity based on ≥97% similarity and low E-value. Phylogenetic relationships among isolates were further assessed using MEGA software to validate evolutionary placement (Kuboneet *al.*,2020).This molecular approach provided definitive confirmation of phenotypic identifications and ensured accurate classification of dominant uropathogens.

2.8 Statistical Analyses

Quantitative data from antibiotic susceptibility testing, including mean zones of inhibition, as well as molecular characterization data were analyzed using one-way analysis of variance (ANOVA) to compare mean values among antibiotics and predefined molecular groups (Shaw *et al.*, 2014), assuming a normal (Gaussian) distribution. All statistical analyses were performed using GraphPad Prism software, and results were expressed as mean ± standard deviation. Statistical significance was defined at $P \leq 0.05$.

3.Result

3.1 Cultural and Morphological Identification

A total of 385 urine samples were cultured on both MacConkey agar and Blood agar, generating 770 primary culture plates. On MacConkey agar, 37.5% plates produced countable colonies while 62.5% were recorded as Too Many to Count (TMTC), yielding 16.4% distinct isolates. Blood agar showed 44.2% countable plates and 55.8% TMTC plates, from which 17.2% isolates were purified, resulting in a total of 121 morphologically distinct bacterial isolatesfor downstream analysis. Colony appearances varied widely, reflecting the microbial diversity among UTI-positive patients. Gram-negative rods, both lactose fermenters and nonfermenters, were predominant. Many isolates (e.g., MACS29, MACS42, MACS55, MACS166, BAS8) exhibited mucoid, milky, yellowish, or orange colonies characteristic of enteric bacteria such as *Escherichia coli*, *Klebsiellapneumoniae*, and *Enterobacter* spp. Several isolates (including BAS4, BAS86, BAS89, BAS121, BAS236, BAS274,

BAS372, MACS32, MACS42, MACS61, MACS94, MACS114, and MACS294) showed pronounced swarming motility on Blood agar, strongly indicative of *Proteus* species. Gram-positive isolates were fewer and typically formed small, opaque to creamy colonies: *Staphylococcus* spp. (e.g., BAS69, BAS100, BAS111, MACS101, MACS351, MACS358) produced smooth, convex golden or white colonies arranged in clusters, whereas *Enterococcus* spp. (e.g., BAS125, BAS148, BAS159, BAS162, MACS92, MACS108, MACS138) formed translucent colonies with umbonate elevations, characteristic of chain-forming cocci. Collectively, the cultural and morphological findings demonstrated a clear predominance of Gram-negative uropathogens with significant representation of *Proteus*, *E. coli*, *Klebsiella*, *Staphylococcus*, and *Enterococcus* species.

3.2 Biochemical Characterization

Biochemical profiling of the 121 isolates revealed distinct metabolic patterns that enabled clear differentiation of major uropathogenic groups. A substantial proportion of Gram-negative rods exhibited characteristic H₂S production, urease positivity, nitrate reduction, catalase activity, and strong swarming motility, features consistent with *Proteus mirabilis* and *Proteus vulgaris*; representatives of this group included BAS4, BA70A, BAS86, BAS89, BAS121, BAS140, BAS236, BAS274, BAS372, MACS32, MACS42, MACS61, MACS94, and MACS114. Several isolates displayed the classic *Escherichia coli* biochemical signature: Indole positive, MR positive, VP negative, ONPG positive, Oxidase negative, motile, and lactose-fermenting such as BAS23, BAS63, BAS81, BAS238, BAS248, BAS266, MACS10, MACS28, MACS33, MACS37, and MACS95. Non-motile, mucoid Gram-negative rods that were Indole negative and Citrate positive, including MACS29, MACS33, and BAS12, were identified as belonging to the *Klebsiella/Enterobacter* group, supported by their carbohydrate fermentation profiles. Gram-positive cocci showing Catalase positivity and arranged in clusters, including BAS34, BAS51, BAS100, BAS111, MACS101, MACS281, and MACS351, were classified as *Staphylococcus* spp., whereas Catalase-negative, Esculin-positive isolates forming chains such as BAS125, BAS148, BAS159, BAS162, BAS191, MACS92, MACS108, and MACS138 were identified as *Enterococcus* spp. Overall, the biochemical profiles confirmed that the predominant uropathogens in the study population included *Proteus*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Enterococcus* species, reflecting the diverse metabolic capabilities of urinary pathogens.

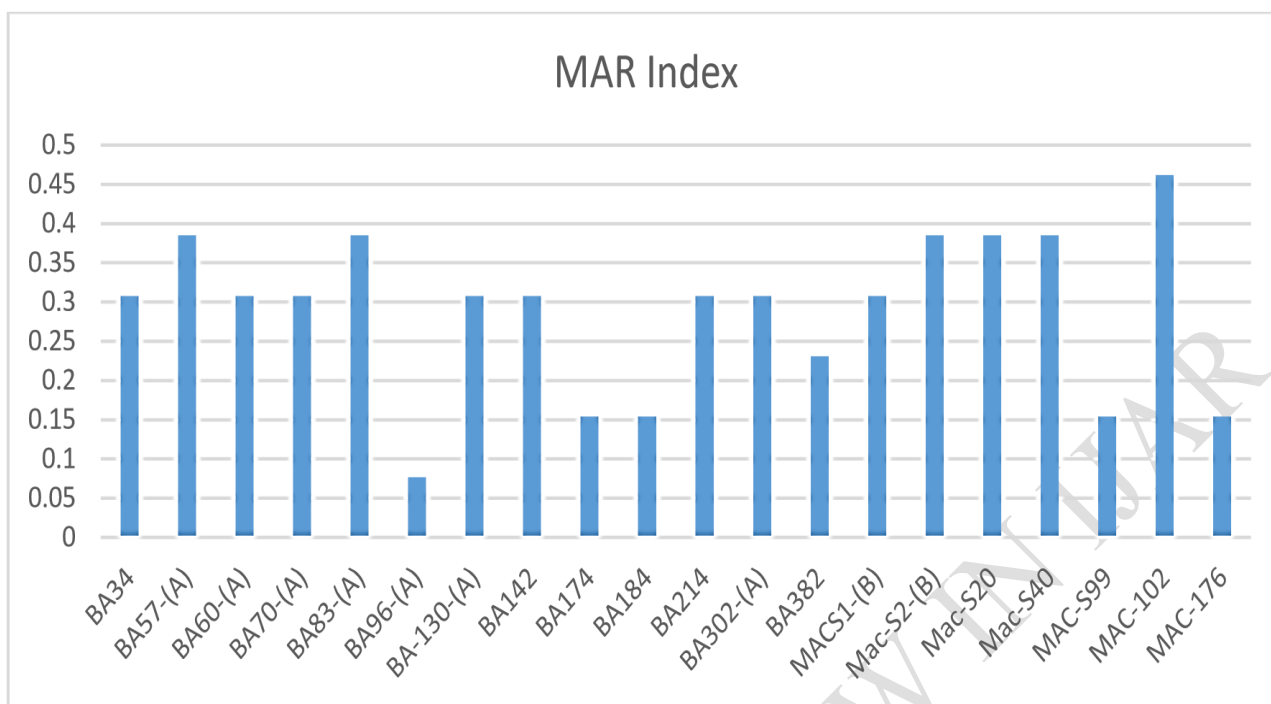
3.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the twenty representative uropathogenic isolates revealed marked heterogeneity in resistance profiles across both Gram-negative and Grampositive organisms. Fluoroquinolones (ciprofloxacin, ofloxacin) and the carbapenemimipenem showed the most consistent inhibitory activity, whereas macrolides (erythromycin), several narrow-spectrum β -lactams (penicillin G, oxacillin, some penicillins), vancomycin and certain aminoglycosides displayed frequent resistance. To quantify multidrug exposure, Multiple Antibiotic Resistance (MAR) indices were calculated for each isolate using resistance to 13 tested antibiotics. Table 2 summarizes the MAR index and the specific antibiotics to which each isolate was resistant. High MAR values identify isolates with resistance to multiple drug classes and flag potential high-risk sources of contamination or prior heavy antibiotic exposure.

Table:2. Multiple Antibiotic Resistance (MAR) index profile of 20 uropathogenic isolates based on resistance to 13 tested antibiotics

S. No.	Isolate Code	Antibiotics Showing Resistance	MAR Index
1	BA34	Penicillin G, Oxacillin, Piperacillin, Chloramphenicol	0.308
2	BA57-(A)	Cefuroxime, Penicillin G, Chloramphenicol, Gentamicin, Vancomycin	0.385
3	BA60-(A)	Ciprofloxacin, Cefuroxime, Piperacillin, Chloramphenicol	0.308
4	BA70-(A)	Ciprofloxacin, Piperacillin, Gentamicin, Vancomycin	0.308
5	BA83-(A)	Erythromycin, Piperacillin, Gentamicin, Vancomycin, Imipenem	0.385
6	BA96-(A)	Ofloxacin	0.077
7	BA-130-(A)	Oxacillin, Chloramphenicol, Gentamicin, Ofloxacin	0.308
8	BA142	Ciprofloxacin, Cefuroxime, Piperacillin,	0.308

		Chloramphenicol	
9	BA174	Chloramphenicol, Ofloxacin	0.154
10	BA184	Ampicillin, Chloramphenicol	0.154
11	BA214	Gentamicin, Imipenem, Trimethoprim–sulfamethoxazole, Ofloxacin	0.308
12	BA302-(A)	Ciprofloxacin, Penicillin G, Chloramphenicol, Gentamicin	0.308
13	BA382	Ciprofloxacin, Oxacillin, Gentamicin	0.231
14	MACS1-(B)	Erythromycin, Penicillin G, Oxacillin, Vancomycin	0.308
15	Mac-S2-(B)	Penicillin G, Chloramphenicol, Gentamicin, Vancomycin, Ofloxacin	0.385
16	Mac-S20	Piperacillin, Chloramphenicol, Gentamicin, Trimethoprim–sulfamethoxazole, Ofloxacin	0.385
17	Mac-S40	Erythromycin, Penicillin G, Gentamicin, Vancomycin, Trimethoprim–sulfamethoxazole	0.385
18	MAC-S99	Ampicillin, Cefuroxime	0.154
19	MAC-102	Ampicillin, Oxacillin, Gentamicin, Vancomycin, Imipenem, Ofloxacin	0.462
20	MAC-176	Piperacillin, Gentamicin	0.154



255

256 The MAR-index distribution highlights concerning resistance trends: one isolate (MAC-102) shows
 257 the highest MAR index (0.462), indicating resistance to six antibiotic agents, while the lowest MAR
 258 (0.077) was recorded for BA96-(A). Notably, 14 of 20 isolates (70%) have $MAR \geq 0.30$, reflecting
 259 widespread multi-drug resistance among the tested uropathogens. Isolates with $MAR \geq 0.30$
 260 predominantly carried resistance to combinations of penicillins, chloramphenicol, gentamicin,
 261 vancomycin and fluoroquinolones, suggesting exposure to multiple antibiotic classes and
 262 acquisition of mobile resistance determinants. These findings underscore the need for routine
 263 culture-guided therapy, restriction of empirical use of broad-spectrum agents when unnecessary, and
 264 strengthened antimicrobial stewardship and infection-control measures to limit dissemination of
 265 high-MAR strains. Continued phenotypic surveillance combined with molecular investigation of
 266 resistance mechanisms is recommended to inform local empiric therapy and containment strategies.

267 3.4Molecular Identification

268 To confirm the taxonomic identity of the selected uropathogenic isolates, molecular characterization
 269 was carried out using 16S rRNA gene sequencing. A total of twenty representative isolates were
 270 chosen based on distinct cultural, morphological, and biochemical profiles. The amplified ~1500 bp
 271 16S rRNA gene fragments were sequenced and the obtained nucleotide sequences were compared
 272 with reference sequences available in the NCBI GenBank database using the BLASTN algorithm.
 273 Species-level identification was assigned based on the highest sequence similarity, low E-value, and

274 consistent clustering with reference strains in phylogenetic analysis. This molecular approach
 275 provided highresolution confirmation of phenotypic identification and minimized ambiguities
 276 arising from overlapping biochemical characteristics among closely related taxa.

277 **Table 3:** Molecular Identification of Uropathogenic Isolates Based on 16S rRNA Gene Analysis

S.No.	Sample Code	Closest Species	Accession No.	% Identity
1	BA174	<i>Escherichia coli</i>	KTZGNZDT016	99.62%
2	BA57A	<i>Klebsiellapneumoniae</i>	KTXV3AUE014	99.84%
3	BA302A	<i>Proteus mirabilis</i>	KTZB64ZR016	99.71%
4	BA70A	<i>Proteus vulgaris</i>	KTZCHEHA016	99.56%
5	BA83A	<i>Klebsiellapneumoniae</i>	KU09MAVN014	99.48%
6	BA382	<i>Klebsiellapneumoniae</i>	KTZFDSGS014	99.67%
7	BA130A	<i>Proteus mirabilis</i>	KU0G6YPK014	99.73%
8	BA34	<i>Staphylococcus aureus</i>	KU0S66VF014	99.12%
9	BA184	<i>Escherichia coli</i>	KU15X4PB016	99.58%
10	BA142	<i>Escherichia coli</i>	KU1A80EF014	99.64%
11	BA214	<i>Enterococcus faecalis</i>	KU1FXWE3014	99.21%
12	BA60A	<i>Proteus mirabilis</i>	KU1R5KNR014	99.69%
13	BA96A	<i>Pseudomonas aeruginosa</i>	KU1XWZDH014	99.05%
14	MACS176	<i>Enterococcus faecalis</i>	KU24KS8N016	99.18%
15	MACS1A	<i>Staphylococcus aureus</i>	KU25K209014	99.09%
16	MACS2B	<i>Klebsiellapneumoniae</i>	KU2CSPE3016	99.72%
17	MACS40	<i>Proteus vulgaris</i>	KU2E1TP6016	99.44%
18	MACS102	<i>Escherichia coli</i>	KU2P9E41014	99.61%
19	MACS20	<i>Enterococcus faecalis</i>	KU2R5EPC014	99.14%
20	MACS99	<i>Klebsiellapneumoniae</i>	KU333K3A016	99.76%

278

279 The molecular identification results demonstrated a high degree of sequence similarity ($\geq 99\%$)
 280 between the query isolates and their closest reference strains, confirming accurate species-level

assignment. The majority of isolates clustered within Gram-negative Enterobacterales, with *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus* species being the most frequently identified pathogens. Specifically, *E. coli* isolates exhibited sequence identities ranging from 99.58–99.64%, while *Klebsiellapneumoniae* showed identities between 99.48–99.84%, indicating close genetic relatedness to clinically established uropathogenic strains.

Proteus species, including *Proteus mirabilis* and *Proteus vulgaris*, formed distinct phylogenetic clades with identity values exceeding 99.4%, consistent with their characteristic biochemical and swarming motility patterns observed earlier. Gram-positive isolates such as *Staphylococcus aureus* and *Enterococcus faecalis* also showed strong genetic concordance (99.09–99.21%) with reference sequences, validating their identification. Additionally, one isolate was confirmed as *Pseudomonas aeruginosa* with 99.05% identity, supporting its role as an opportunistic uropathogen.

3.5 Statistical Analyses

One-way analysis of variance (ANOVA) indicated a statistically significant variation in antibacterial activity among the tested antibiotics, as reflected by differences in mean zones of inhibition ($F(12, 234) = 2.246, P = 0.0106$). This result demonstrates that the efficacy of antibiotics against the tested bacterial isolates differed significantly.

Similarly, one-way ANOVA performed on molecular characterization data revealed a highly significant difference among the predefined bacterial groups (A–H) ($F(7, 17) = 8968, P < 0.0001$), indicating marked variation in molecular profiles among the groups. The high coefficient of determination ($R^2 = 0.9997$) indicates that the observed variability was predominantly explained by differences among the bacterial groups. These findings confirm the strong discriminatory capability of molecular characterization in differentiating bacterial isolates.

4. Discussion

The present study provides a comprehensive microbiological, molecular, and statistical evaluation of uropathogens isolated from 385 clinically diagnosed UTI patients, revealing a high burden of Gram-negative and multidrug-resistant (MDR) bacteria. Culture-based processing generated 770 primary culture plates, with a substantial proportion classified as Too Many to Count (TMTc), particularly on MacConkey agar (64.94%) and Blood agar (58.70%). Such high bacterial loads strongly indicate active infections rather than contamination, reflecting the clinical severity of UTIs in the studied population.

A total of 121 morphologically distinct isolates were recovered from countable plates, enabling detailed phenotypic and molecular analyses. Cultural and morphological characterization demonstrated a clear predominance of Gram-negative rods, including lactose-fermenting and nonfermenting organisms. The frequent observation of characteristic colony morphology and swarming motility highlighted the prominence of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* species, which are well-recognized uropathogens. Gram-positive cocci such as *Staphylococcus* and *Enterococcus* species were less prevalent but remain clinically relevant due to their documented resistance potential.

Antibiotic susceptibility testing revealed marked variability in antibacterial responses across the tested antibiotics. One-way analysis of variance (ANOVA) confirmed that the differences in mean zones of inhibition among antibiotics were statistically significant ($F(12, 234) = 2.246$, $P = 0.0106$), demonstrating that antibiotic efficacy varied significantly against the tested isolates. This statistical evidence supports the observed phenotypic resistance patterns, where fluoroquinolones and imipenem exhibited comparatively higher activity, whereas macrolides, penicillin derivatives, vancomycin, and several β -lactams showed limited effectiveness. Furthermore, the Multiple Antibiotic Resistance (MAR) index analysis revealed that 70% of the tested isolates had MAR values ≥ 0.30 , indicating widespread exposure to multiple antibiotic classes and a high prevalence of MDR strains.

Molecular characterization using 16S rRNA gene sequencing provided definitive confirmation of phenotypic identification, with $\geq 99\%$ sequence similarity to reference strains. Statistical evaluation of molecular characterization data using one-way ANOVA further demonstrated highly significant differences among predefined bacterial groups (A–H) ($F(7, 17) = 8968$, $P < 0.0001$; $R^2 = 0.9997$). This strong statistical separation highlights the discriminatory power of molecular tools in differentiating uropathogens at the species level and validates the robustness of the combined phenotypic–genotypic approach used in this study.

4. Conclusion

The present study provides a comprehensive and integrated evaluation of the incidence, diversity, and antimicrobial resistance patterns of uropathogens isolated from clinically diagnosed urinary tract infection patients, representing multiple high-risk population groups. Culture-based processing using MacConkey agar and Blood agar revealed high bacterial burdens in a substantial proportion of samples, as evidenced by the predominance of plates classified as Too Many to Count, indicating

active infections rather than contamination. From countable plates, 121 morphologically distinct bacterial isolates were recovered and subjected to detailed cultural, biochemical, molecular, and antimicrobial susceptibility characterization, generating a robust and representative dataset. The results demonstrated a clear predominance of Gram-negative uropathogens, particularly *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus* species, consistent with their well-established role as major etiological agents of UTIs, while Gram-positive pathogens such as *Staphylococcus aureus* and *Enterococcus faecalis* were also identified and remain clinically relevant due to their resistance potential. Biochemical profiling enabled reliable phenotypic differentiation of isolates, and molecular identification using 16S rRNA gene sequencing confirmed species-level identity with high sequence similarity, showing strong concordance between phenotypic and genotypic approaches. Antibiotic susceptibility testing revealed marked variability in antibacterial efficacy, with fluoroquinolones and imipenem demonstrating comparatively higher activity, whereas several commonly used antibiotics exhibited substantial resistance. Statistical analysis confirmed significant differences in antibiotic efficacy and highlighted the widespread occurrence of multidrug resistance, as reflected by elevated Multiple Antibiotic Resistance indices in the majority of tested isolates. Collectively, these findings underscore the limitations of empirical therapy and emphasize the urgent need for routine culture-guided diagnosis, susceptibility-based antibiotic selection, continuous surveillance of resistance trends, and strengthened antimicrobial stewardship programs to improve treatment outcomes and curb the spread of multidrug-resistant uropathogens in urinary tract infection management.

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