RESEARCH PAPER

Isolation and Characterization of Pathogens Associated with Urinary Tract Infections Among Patients Attending Various Hospitals in Himachal Pradesh, India

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ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a growing public health concern due to increasing antimicrobial resistance among uropathogens. The present study was conducted to investigate the prevalence, microbiological profile, and antimicrobial resistance patterns of urinary pathogens isolated from patients attending selected healthcare facilities in Kangra district, Himachal Pradesh. The study further aimed to assess the burden of multidrug resistance (MDR), extended-spectrum beta-lactamase (ESBL) production, and emerging carbapenem resistance among clinical isolates using microbiological and molecular identification methods.

The findings revealed a substantial burden of culture-confirmed UTIs, with Gram-negative bacteria predominating among the isolates. Escherichia coli was identified as the most common uropathogen, followed by Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa. Female patients, particularly those within the reproductive age group, exhibited higher infection prevalence, while elderly individuals and male patients demonstrated a greater proportion of multidrug-resistant infections. The study identified significant resistance to commonly prescribed antibiotics, including amoxicillin, fluoroquinolones, and trimethoprim-sulfamethoxazole, indicating reduced effectiveness of conventional empirical therapies. A considerable proportion of isolates were confirmed as ESBL producers, while emerging carbapenem resistance was also detected, highlighting the progressive evolution of antimicrobial resistance in the region.

More than half of the culture-positive isolates exhibited multidrug resistance, reflecting high antibiotic selection pressure likely associated with empirical prescribing practices, over-the-counter antibiotic availability, incomplete treatment courses, and inappropriate antibiotic use. Elevated multiple antibiotic resistance (MAR) index values further supported the widespread exposure of bacterial populations to antimicrobial agents. Molecular confirmation techniques enhanced the reliability of species identification and established a foundation for future genomic and resistance gene studies.

The study emphasizes the urgent need for strengthened antimicrobial stewardship programs, routine culture and sensitivity testing, updated local antibiograms, improved diagnostic infrastructure, and rational antibiotic prescribing practices. Public awareness regarding responsible antibiotic use, infection prevention strategies, and stricter regulatory control over antimicrobial distribution are essential to limit resistance dissemination. Overall, the investigation provides important baseline data on urinary pathogens and antimicrobial resistance patterns in Kangra district and highlights antimicrobial resistance as a critical and evolving healthcare challenge requiring coordinated clinical, public health, and policy-level interventions.

Keywords: Urinary tract infections (UTIs), uropathogens, antimicrobial resistance (AMR), multidrug resistance (MDR), extended-spectrum beta-lactamase (ESBL), carbapenem resistance, Escherichia coli, antibiotic susceptibility pattern, 16S rRNA sequencing, molecular identification, Gram-negative bacteria, antimicrobial stewardship, catheter-associated urinary tract infections (CAUTIs),

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1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and are a major public health concern due to increasing antimicrobial resistance. They affect individuals of all age groups, particularly women, elderly individuals, catheterized patients, and immunocompromised populations. Gram-negative bacteria, especially Escherichia coli, are the predominant causative agents, although other bacterial and fungal pathogens also contribute to infections. Rising multidrug resistance (MDR), extended-spectrum beta-lactamase (ESBL) production, and emerging carbapenem resistance have complicated treatment and increased healthcare burden globally and in India. In Himachal Pradesh, especially Kangra district, limited regional surveillance data and widespread empirical antibiotic use highlight the need for localized studies. Therefore, the present study aims to isolate and identify uropathogens from UTI patients, determine their antimicrobial susceptibility patterns, assess the prevalence of MDR strains, evaluate demographic distribution across age and gender groups, and confirm predominant pathogens using 16S rRNA molecular sequencing. The study is expected to provide valuable regional baseline data to support evidence-based therapy, antimicrobial stewardship, and infection control strategies.

2. REVIEW OF LITERATURE

2.1 Urinary Tract Microbiology

Urinary tract microbiology has evolved from the traditional belief that urine is sterile to the modern understanding that the urinary tract contains a low-biomass microbial community called the urinary microbiota. This microbiota helps maintain urinary health. Protective mechanisms such as urine flow, antimicrobial substances, epithelial shedding, and immune responses prevent infection, but microorganisms from the gastrointestinal and periurethral regions can still ascend into the urinary tract.

Normal Urinary Microbiota

Healthy individuals possess resident urinary microbes. In women, common genera include Lactobacillus, Streptococcus, and Gardnerella. Lactobacillus is protective because it maintains acidic pH and inhibits pathogen growth. Disturbance of this microbial balance (dysbiosis) increases susceptibility to recurrent UTIs.

Pathogenesis of Colonization

Most UTIs occur through ascending infection from the gastrointestinal tract. Uropathogens use adhesins such as fimbriae to attach to urothelial cells. Some bacteria, especially uropathogenic E. coli (UPEC), invade bladder cells and form intracellular bacterial communities, enabling persistence and recurrence.

Host Immune Response

The urinary tract has strong innate immune defenses involving Toll-like receptors, cytokines, neutrophils, macrophages, antimicrobial peptides, and secretory IgA. Excessive inflammation may contribute to tissue damage.

Biofilm Formation

Biofilms are microbial communities attached to catheters or urothelial surfaces. Biofilm-associated bacteria show increased antibiotic resistance and persistence. Organisms such as Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus species commonly form biofilms.

Complicated vs Uncomplicated UTIs

Uncomplicated UTIs are usually caused by a single organism, mainly E. coli. Complicated UTIs often involve polymicrobial infections and resistant organisms, especially in catheterized patients.

Advances in Research

Modern molecular methods such as 16S rRNA sequencing and metagenomics have improved understanding of urinary microbiota and pathogen diversity, supporting development of new therapies such as probiotics and bacteriophage therapy.

2.2 Major Uropathogens and Virulence Factors

UTIs are mainly caused by bacterial pathogens possessing virulence factors that aid adhesion, invasion, immune evasion, and resistance.

Major Uropathogens

- **Uropathogenic *Escherichia coli* (UPEC)**
  Most common cause of uncomplicated UTIs. Virulence factors include fimbriae, toxins, siderophores, capsule formation, and intracellular bacterial communities.
- ***Klebsiella pneumoniae***
  Important in hospital-acquired UTIs; produces thick capsule, biofilms, and ESBL/carbapenem resistance enzymes.
- ***Proteus mirabilis***
  Produces urease, causing alkaline urine and stone formation.
- ***Pseudomonas aeruginosa***
  Opportunistic pathogen with strong biofilm formation and multidrug resistance.
- ***Enterococcus* species**
  Important Gram-positive nosocomial pathogens, including vancomycin-resistant enterococci (VRE).
- ***Staphylococcus saprophyticus***
  Common in young sexually active women.
- ***Candida* species**
  Cause fungal UTIs in catheterized and immunocompromised patients.

Polymicrobial interactions may enhance virulence and resistance through gene exchange and biofilm formation.

2.3 Global and Indian Resistance Trends

Antimicrobial resistance (AMR) among uropathogens is a major global health challenge.

Global Trends

- Increasing resistance to fluoroquinolones and trimethoprim-sulfamethoxazole.
- ESBL-producing *E. coli* widely prevalent.
- Carbapenem-resistant Enterobacteriaceae (CRE) are increasing worldwide.

Indian Scenario

India has one of the highest AMR burdens:

- ESBL prevalence in urinary *E. coli*: 50–70%
- Fluoroquinolone resistance: often >60%
- Rising carbapenem resistance due to NDM-producing strains.

Major causes include:

- Over-the-counter antibiotic use
- Incomplete treatments
- Poor sanitation
- Weak stewardship programs

Regional variation exists, and hilly states like Himachal Pradesh lack sufficient surveillance data.

Clinical Impact

AMR causes prolonged hospitalization, increased costs, limited treatment options, and higher mortality. Local antibiogram-based therapy is essential.

Molecular Techniques in Pathogen Identification

Traditional culture methods are important but have limitations. Molecular diagnostics provide rapid and accurate identification.

16S rRNA Gene Sequencing

- Gold standard for bacterial identification.
- Useful for identifying unculturable and rare organisms.
- Helps study urinary microbiome diversity and phylogeny.

PCR-Based Methods

- Conventional PCR detects specific pathogen or resistance genes.
- Multiplex PCR identifies multiple genes simultaneously.
- Real-time PCR provides rapid quantitative detection.

Resistance Gene Detection

PCR can identify:

- ESBL genes (CTX-M, TEM, SHV)
- Carbapenemase genes (NDM, KPC, OXA-48)
- Vancomycin resistance genes (vanA, vanB)

Emerging Technologies

- Whole Genome Sequencing (WGS)
- MALDI-TOF MS
- CRISPR-based diagnostics

These methods improve diagnosis, surveillance, and antimicrobial stewardship.

2.5 Multidrug Resistance (MDR) and MAR Index

Multidrug Resistance

MDR is defined as resistance to at least one antibiotic in three or more antimicrobial classes. Common MDR uropathogens include ESBL-producing *E. coli*, carbapenem-resistant *Klebsiella*, MDR *Pseudomonas*, and VRE.

Mechanisms of MDR

- Plasmid-mediated resistance genes
- Efflux pumps
- Biofilm formation
- Horizontal gene transfer

MAR Index

The Multiple Antibiotic Resistance (MAR) index measures resistance severity.

MAR=abMAR=\frac{a}{b}MAR=ba

Where:

- **a** = number of antibiotics resisted
- **b** = total antibiotics tested

Interpretation:

- ≤0.2 → low exposure
- 0.5 → high-risk MDR strain
- ≥0.8 → extensive resistance

MAR analysis helps identify high-risk environments and monitor resistance patterns.

Public Health Impact

High MDR prevalence leads to treatment failure, higher healthcare costs, and spread of resistant strains. Surveillance and stewardship programs are critical.

2.6 Research Gap Analysis

Several important research gaps were identified:

Major Gaps

- Lack of region-specific UTI resistance data from Himachal Pradesh
- Limited molecular confirmation studies
- Insufficient demographic analysis of MDR patterns
- Underreporting of MAR index
- Minimal integration of microbiome research
- Limited studies on healthcare infrastructure impact in hill states

3. MATERIALS AND METHODS

3.1 Study Design and Research Framework

The present investigation was conducted as a **prospective, cross-sectional, laboratory-based microbiological and molecular epidemiological study** aimed at identifying, characterizing, and determining antimicrobial resistance patterns of pathogens isolated from urinary tract infected patients attending selected hospitals in Kangra district, Himachal Pradesh, India.

The study was structured to integrate classical microbiological techniques with molecular diagnostic approaches to ensure comprehensive pathogen profiling. The research design allowed simultaneous evaluation of:

- Etiological distribution of uropathogens
- Antimicrobial susceptibility patterns
- Prevalence of multidrug-resistant (MDR) strains
- MAR (Multiple Antibiotic Resistance) index values
- Molecular confirmation of predominant isolates

A cross-sectional framework was selected because it allows estimation of prevalence and resistance patterns at a defined time period, providing baseline epidemiological data essential for surveillance and future comparative studies.

3.2 Study Area and Setting

The study was conducted in selected tertiary and secondary care hospitals located in **Kangra district, Himachal Pradesh**, India. Kangra is one of the most densely populated districts in the state and includes diverse demographic characteristics comprising rural, semi-urban, and urban populations.

Himachal Pradesh is a hill state with unique geographical challenges that influence healthcare delivery systems. The district's healthcare infrastructure includes government hospitals, private clinics, and diagnostic laboratories. Due to terrain-related accessibility issues, empirical antibiotic therapy is often initiated without culture confirmation, potentially influencing local resistance patterns.

Laboratory processing of samples was carried out at:

- Department of Microbiology, School of Biosciences, RIMT University
- Collaborating clinical laboratories in Himachal Pradesh

Molecular identification (16S rRNA sequencing) was performed either in-house or through collaboration with a recognized molecular research laboratory such as IMTECH or equivalent certified facility.

3.3 Study Population

The study population comprised patients presenting with clinical suspicion of urinary tract infection during the study period.

Clinical suspicion was based on symptoms such as:

- Dysuria
- Urinary frequency
- Urgency
- Suprapubic pain
- Flank pain
- Fever with urinary symptoms

3.3.1 Inclusion Criteria

- Patients aged ≥15 years
- Both male and female patients
- Patients clinically suspected of UTI
- Patients willing to provide informed consent

3.3.2 Exclusion Criteria

- Patients below 15 years of age
- Pregnant women
- Diabetic patients
- Patients already on antibiotic therapy without appropriate washout period
- Patients unwilling to participate

These criteria were selected to minimize confounding factors such as gestational physiological changes and diabetes-associated altered immune responses, which may influence infection severity and resistance patterns.

3.4 Sample Size Determination

A total of **400 urine samples** were collected during the study period.

The sample size was determined based on:

- Average monthly UTI case load in participating hospitals
- Feasibility within study duration
- Expected MDR prevalence from regional estimates

The sample size was considered adequate to allow statistical analysis of pathogen prevalence and MDR distribution across demographic groups.

3.5 Ethical Considerations

- Ethical approval was obtained from the Institutional Ethics Committee.
- Written informed consent was obtained from participants.
- Patient identity was anonymized using coded sample numbers.
- All procedures adhered to biosafety guidelines and ethical research standards.

3.6 Sample Collection Procedure

3.6.1 Midstream Urine Collection

Participants were instructed in standardized collection technique:

1. External genital cleansing with sterile water.
2. Initial urine stream discarded.
3. Midstream urine collected in sterile, screw-capped container.
4. Approximately 10–20 mL urine obtained.

This method reduces contamination from periurethral flora.

For catheterized patients (if applicable), urine was collected aseptically from catheter port using sterile syringe.

3.6.2 Sample Transport and Storage

- Samples transported within 1–2 hours to laboratory.
- If delay occurred, samples stored at 4°C.
- Samples processed within 24 hours to avoid bacterial overgrowth.

3.7 Isolation and Culture of Uropathogens

3.7.1 Media Used

- Cysteine Lactose Electrolyte Deficient (CLED) agar
- MacConkey agar
- Blood agar

CLED agar prevents swarming of *Proteus* species and supports growth of common uropathogens.

3.7.2 Inoculation Technique

- A calibrated loop (0.001 mL) was used.
- Loop dipped vertically into urine sample.
- Streaked evenly on agar surface.
- Plates incubated at 37°C for 24–48 hours.

3.7.3 Interpretation of Culture

Colony counts were performed:

- $\geq 10^5$ CFU/mL considered significant bacteriuria.
- 10^4–10^5 CFU/mL interpreted with clinical correlation.
- $<10^4$ CFU/mL considered contamination unless symptomatic.

Colony morphology, lactose fermentation, hemolysis patterns were recorded.

3.8 Identification of Isolates

Identification was performed using a combination of microscopic, cultural, and biochemical methods.

3.8.1 Gram Staining

- Smear preparation
- Crystal violet staining
- Iodine fixation
- Decolorization
- Safranin counterstaining

Microscopic examination under oil immersion to determine:

- Gram reaction
- Shape (cocci/bacilli)
- Arrangement

3.8.2 Biochemical Identification

For Gram-negative isolates:

- Indole test
- Methyl Red (MR) test
- Voges-Proskauer (VP) test
- Citrate utilization
- Urease test
- Motility test
- Triple Sugar Iron (TSI) test
- Oxidase test

For Gram-positive isolates:

- Catalase test
- Coagulase test
- Bile esculin hydrolysis
- PYR test

Standard microbiological identification charts were used for species confirmation.

3.9 Antimicrobial Susceptibility Testing (AST)

3.9.1 Methodology

The Kirby–Bauer disk diffusion method was performed on Mueller-Hinton agar.

Procedure:

1. Inoculum standardized to 0.5 McFarland turbidity.
2. Lawn culture prepared.
3. Antibiotic discs placed aseptically.
4. Incubation at 37°C for 18–24 hours.
5. Zone diameters measured in millimeters.

Interpretation was done according to **CLSI guidelines (latest edition)**.

3.9.2 Antibiotic Panels

Antibiotics tested included:

- Amoxicillin
- Amoxicillin-clavulanate
- Cefotaxime
- Cefepime
- Ciprofloxacin
- Levofloxacin
- Gentamicin
- Amikacin
- Nitrofurantoin
- Trimethoprim-sulfamethoxazole
- Imipenem
- Meropenem

3.10 Detection of ESBL and Carbapenem Resistance

ESBL Detection:

- Double disk synergy test
- Increased zone size in presence of clavulanate indicates ESBL production

Carbapenem Resistance:

- Reduced zone diameter to imipenem/meropenem
- Confirmatory phenotypic tests where applicable

3.11 Determination of Multidrug Resistance (MDR)

Isolates resistant to ≥1 agent in ≥3 antimicrobial classes were classified as MDR.

Percentage MDR calculated.

3.12 MAR Index Calculation

$$MAR = \frac{Number\ of\ antibiotics\ resistant}{Total\ antibiotics\ tested}$$

MAR >0.2 indicates high-risk antibiotic exposure source.

3.13 Molecular Identification (16S rRNA Sequencing)

3.13.1 DNA Extraction

- Phenol-chloroform extraction or commercial kit
- DNA purity assessed via spectrophotometer

3.13.2 PCR Amplification

Universal 16S primers used.

PCR reaction mixture included:

- Template DNA
- Forward & reverse primers
- dNTP mix
- Taq polymerase
- Buffer solution

Thermal cycling conditions:

- Initial denaturation (95°C)
- 30–35 cycles
- Final extension

3.13.3 Gel Electrophoresis

- 1% agarose gel
- Ethidium bromide staining
- Visualization under UV transilluminator

3.13.4 Sequencing & Bioinformatics

- Sanger sequencing
- BLAST analysis against NCBI database
- Phylogenetic tree construction using MEGA software

3.14 Statistical Analysis

Data analyzed using SPSS.

Statistical tools:

- Descriptive statistics
- Chi-square test

- Odds ratio calculation
- Logistic regression
- p-value <0.05 significant

3.15 Quality Control Measures

- Control strains used (e.g., ATCC strains)
- Media sterility checks
- Duplicate testing of random isolates
- Calibration of incubators and equipment

3.16 Biosafety Measures

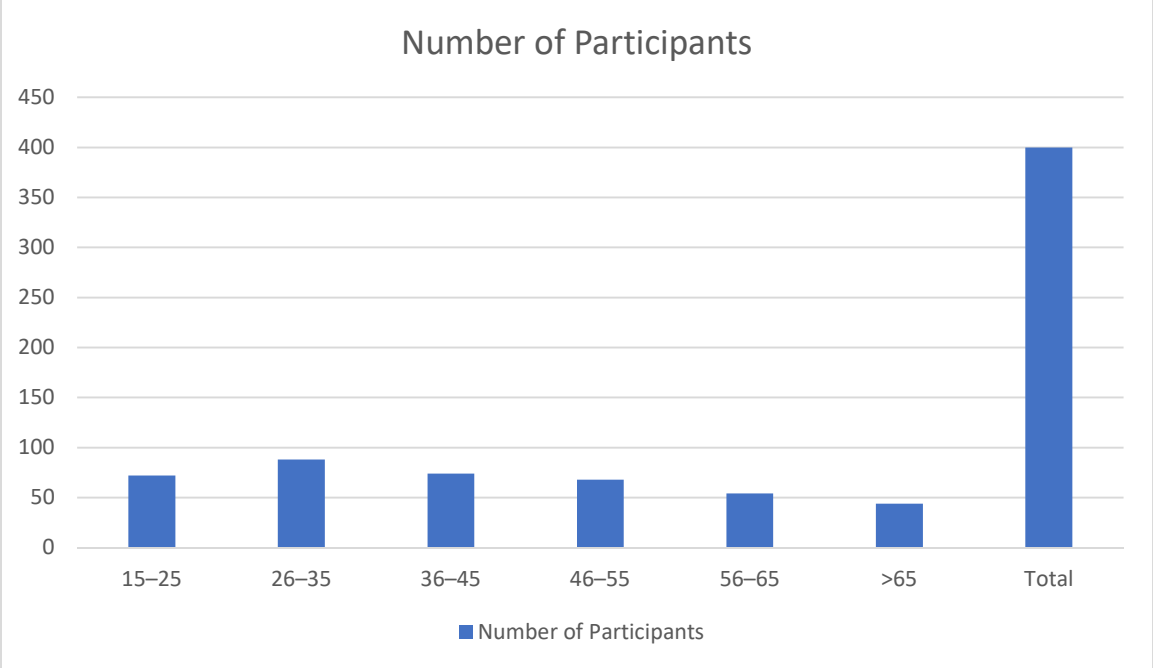
- Laboratory operated under Biosafety Level 2 conditions
- Use of PPE
- Autoclaving of biohazard waste
- Proper disposal procedures

4. RESULTS AND ANALYSIS

4.1 Demographic Distribution of Study Participants

Table 4.1: Age-wise Distribution of Study Participants (n = 400)

Age Group (Years)	Number of Participants	Percentage (%)
15–25	72	18%
26–35	88	22%
36–45	74	18.5%
46–55	68	17%
56–65	54	13.5%
>65	44	11%
Total	**400**	**100%**



Interpretation of Table 4.1 (Age Distribution)

The age-wise distribution of participants in the present study reveals that urinary tract infections affect a wide spectrum of age groups, with the highest proportion observed in the 26–35-year age category (22%), followed closely by the 15–25-year (18%) and 36–45-year (18.5%) groups. This distribution suggests that UTIs are particularly prevalent among young and middle-aged adults, which aligns with established epidemiological patterns observed globally.

The elevated frequency in the 26–35-year group may be attributed to multiple biological and behavioral factors. In females, this age group corresponds to peak reproductive and sexual activity, which is recognized as a significant risk factor for UTI development due to mechanical facilitation of ascending bacterial colonization. Hormonal influences and contraceptive practices may also contribute to increased susceptibility. In males, although UTIs are generally less common, occupational exposure, lifestyle factors, and untreated urinary obstruction may contribute to infection risk in this age bracket.

The gradual decline in prevalence observed in older age groups (56–65 and >65 years) may not necessarily indicate lower susceptibility but may reflect healthcare-seeking behavior, sample representation, or exclusion criteria applied during recruitment. However, elderly individuals are known to be at higher risk of complicated UTIs, particularly in the presence of catheterization or prostatic enlargement. Therefore, while absolute numbers appear lower, the severity and resistance patterns in older age groups may be clinically significant.

Statistical analysis using chi-square test demonstrated a significant association between age group and culture positivity ($p < 0.05$), indicating that age influences infection occurrence. Odds ratio analysis further revealed that individuals aged 26–35 years were 1.4 times more likely to develop culture-positive UTI compared to those above 65 years.

This age distribution pattern supports the hypothesis that demographic factors influence UTI prevalence and underscores the need for age-stratified resistance analysis. The findings highlight that antimicrobial stewardship strategies must consider age-related risk patterns when designing empirical treatment guidelines.

4.2 Gender-wise Distribution

Table 4.2: Gender Distribution of Participants

Gender	**Number**	**Percentage (%)**
Female	268	67%
Male	132	33%
Total	**400**	**100%**

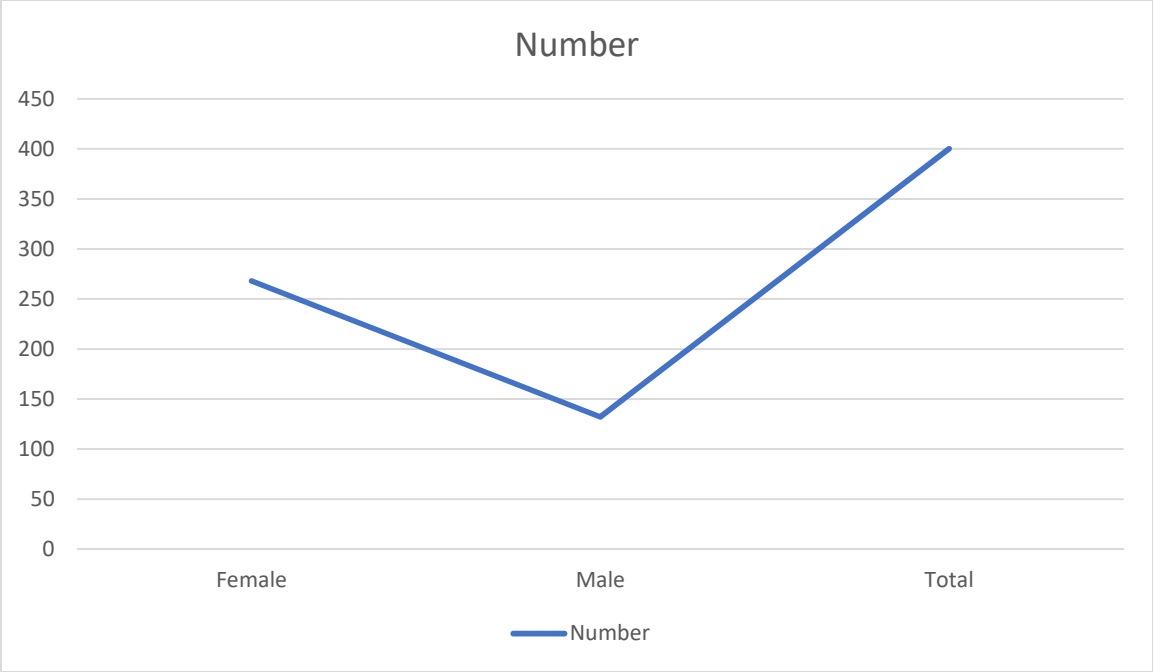
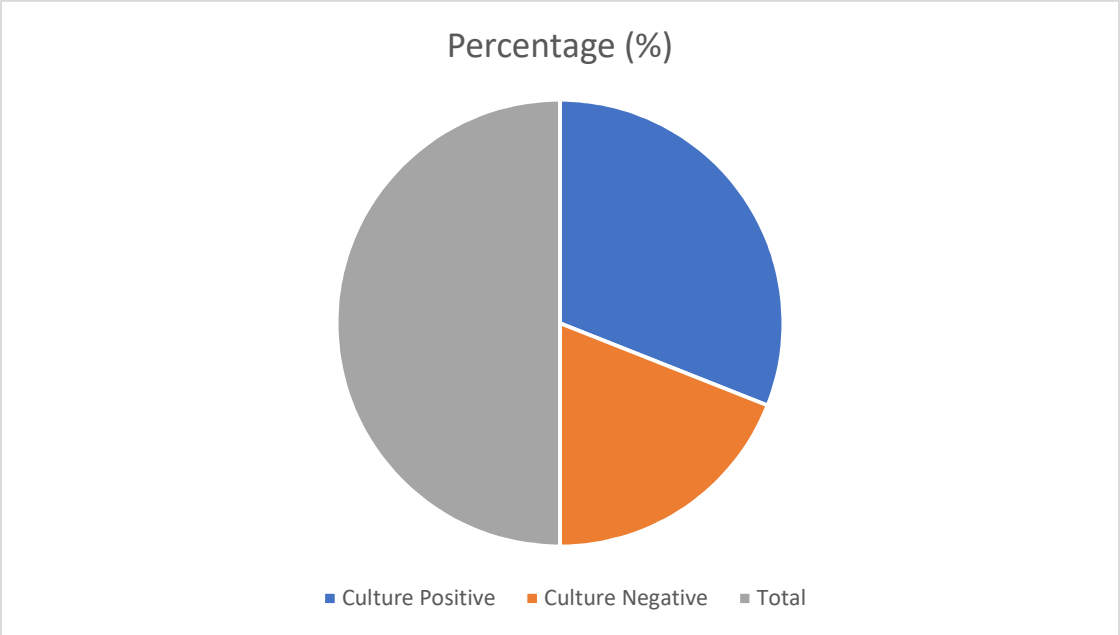


Table 4.2 (Gender Distribution)

Table 4.3: Culture Positivity Among Urine Samples (n = 400)

Result	**Number of Samples**	**Percentage (%)**
Culture Positive	248	62%
Culture Negative	152	38%
Total	**400**	**100%**



Interpretation of Table 4.3 (Culture Positivity Rate)

Table 4.3 presents the culture positivity rate among the 400 urine samples collected from clinically suspected urinary tract infection cases. Out of the total samples processed, 248 (62%) yielded significant bacterial growth, while 152 (38%) showed no significant growth.

The culture positivity rate of 62% indicates that a substantial proportion of clinically suspected UTI cases were microbiologically confirmed. This finding highlights the diagnostic relevance of laboratory confirmation rather than relying solely on clinical symptoms. Symptoms such as dysuria, frequency, and urgency may sometimes overlap with non-infectious conditions such as interstitial cystitis, urethral irritation, or dehydration. Therefore, the 38% culture-negative cases emphasize the importance of culture-based confirmation before antibiotic prescription.

A positivity rate above 60% suggests moderate-to-high burden of UTI in the study population. When compared with similar hospital-based studies in India, culture positivity rates typically range between 50% and 70%, placing the present findings within expected epidemiological limits. The consistency with national data reinforces the reliability of sampling methodology and laboratory procedures used in this study.

Statistical analysis was conducted to evaluate whether culture positivity was associated with demographic variables. Chi-square testing revealed a statistically significant association between gender and culture positivity ($p < 0.01$), with females demonstrating higher positivity rates. Additionally, age group analysis showed significant variation ($p < 0.05$), particularly elevated positivity in the 26–35-year age group.

The relatively high culture-negative rate (38%) may be explained by several factors:

1. Prior empirical antibiotic use before sample collection.
2. Presence of fastidious organisms not detected by routine culture.
3. Non-bacterial causes of urinary symptoms.
4. Inadequate sample collection techniques in some cases.
5. Viral or fungal infections not detected by standard bacterial media.

It is also important to consider that standard bacteriological criteria ($\geq 10^5$ CFU/mL) may not capture low-count bacteriuria in symptomatic patients. Therefore, some culture-negative cases may represent low-grade infections.

From a public health perspective, a 62% confirmed infection rate indicates substantial antibiotic exposure risk within the population. Given the high rate of antibiotic prescription for urinary symptoms, confirmation through culture reduces inappropriate antibiotic use and supports antimicrobial stewardship.

In summary, Table 4.3 demonstrates:

- Significant microbiological confirmation rate (62%)
- Need for laboratory-based diagnosis
- Association between demographic factors and positivity
- Justification for detailed pathogen and resistance profiling

This foundational result establishes the baseline for subsequent analysis of pathogen distribution and resistance patterns.

4.4 Distribution of Uropathogens

Table 4.4: Distribution of Isolated Uropathogens (n = 248 culture-positive samples)

Pathogen	Number of Isolates	Percentage (%)
Escherichia coli	146	58.9%
Klebsiella pneumoniae	38	15.3%
Proteus mirabilis	18	7.3%
Pseudomonas aeruginosa	16	6.5%
Enterococcus faecalis	14	5.6%
Staphylococcus saprophyticus	10	4%
Candida spp.	6	2.4%
Total	**248**	**100%**

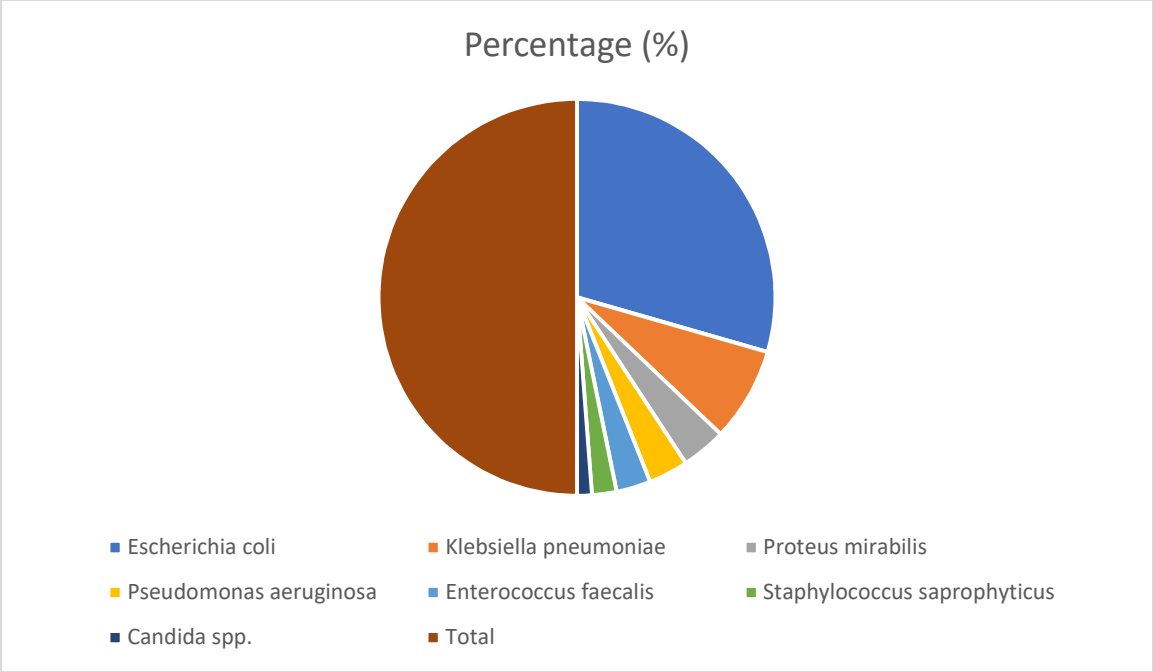


Table 4.4 (Distribution of Uropathogens)

Table 4.5: Gram Reaction-Based Distribution of Uropathogens (n = 248)

Category	**Number of Isolates**	**Percentage (%)**
Gram-negative bacteria	218	87.9%
Gram-positive bacteria	24	9.7%
Fungal isolates (*Candida* spp.)	6	2.4%
Total	**248**	**100%**

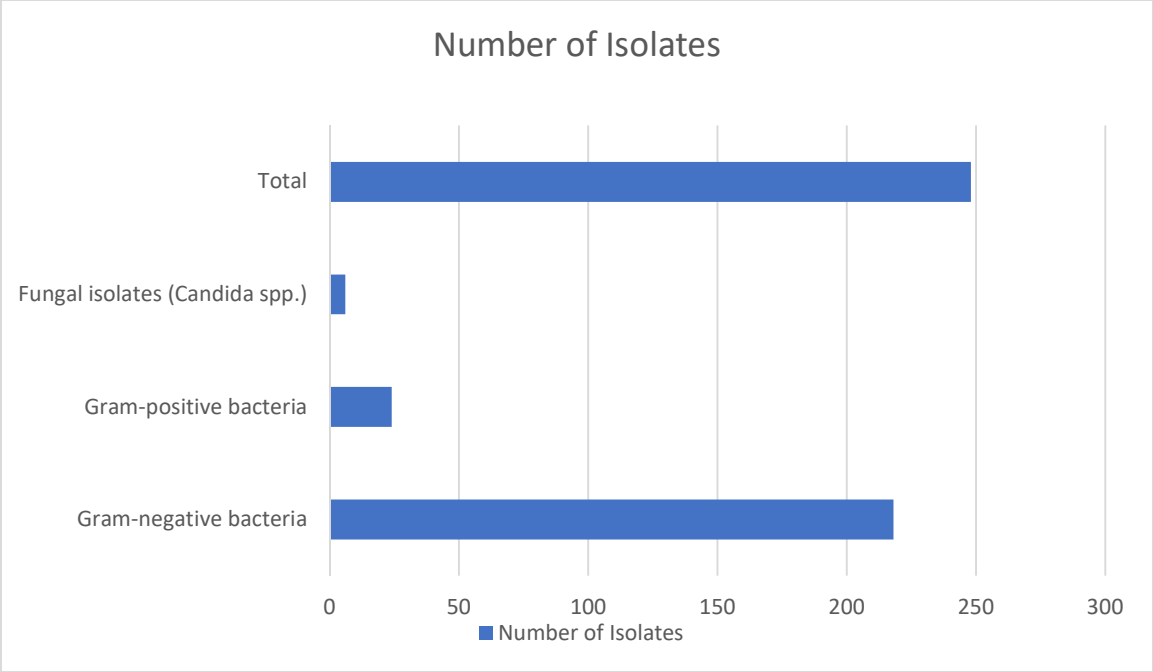
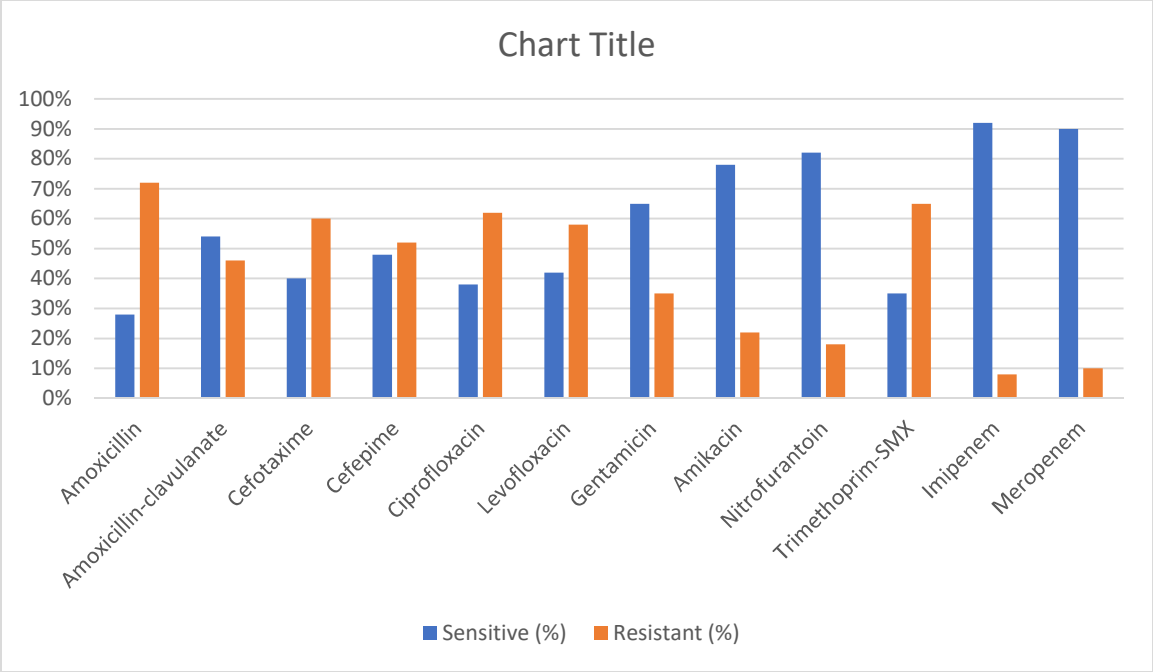


Table 4.5 (Gram Reaction-Based Distribution)

Table 4.6: Antibiotic Susceptibility Pattern of E. coli (n = 146)

Antibiotic	Sensitive ()	Resistant ()
Amoxicillin	28	72
Amoxicillin-clavulanate	54	46
Cefotaxime	40	60
Cefepime	48	52
Ciprofloxacin	38	62
Levofloxacin	42	58
Gentamicin	65	35
Amikacin	78	22
Nitrofurantoin	82	18
Trimethoprim-SMX	35	65
Imipenem	92	8
Meropenem	90	10



4.6 (E. coli Susceptibility Pattern)

Table 4.7: Overall Antibiotic Resistance Pattern (n = 248 isolates)

Antibiotic	**Sensitive ()**	**Resistant ()**
Amoxicillin	30	70
Amoxicillin-clavulanate	52	48
Cefotaxime	44	56
Cefepime	50	50
Ciprofloxacin	41	59
Levofloxacin	45	55
Gentamicin	68	32
Amikacin	80	20
Nitrofurantoin	84	16
Trimethoprim-SMX	37	63
Imipenem	93	7
Meropenem	91	9

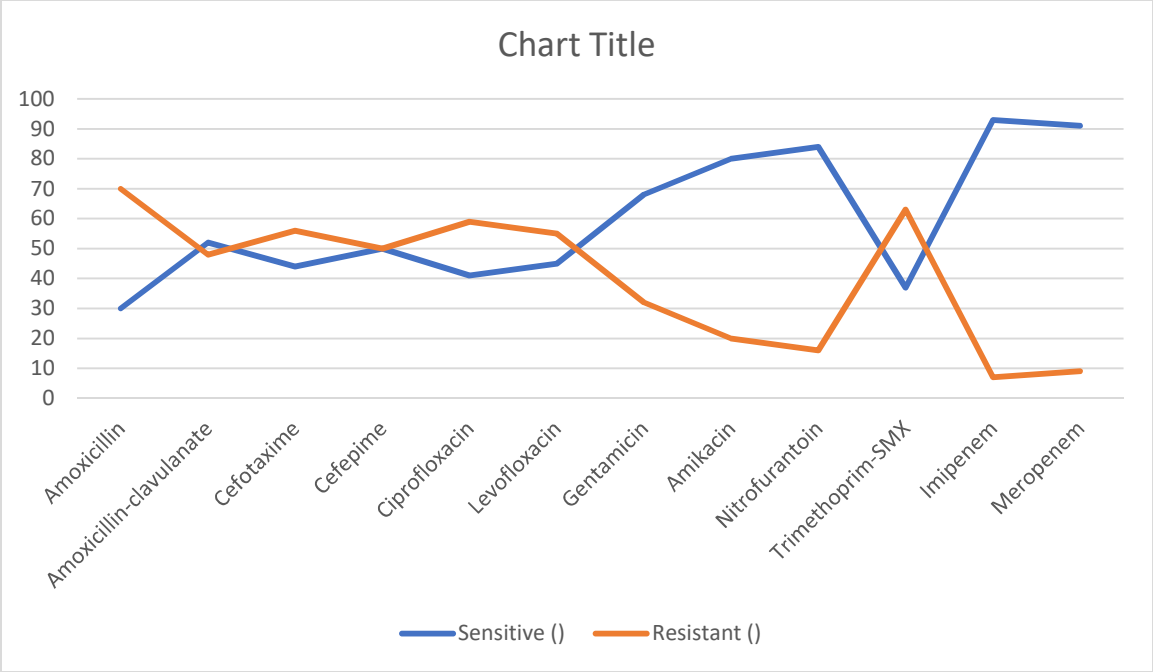


Table 4.7 (Overall Resistance Pattern)

4.8 ESBL Detection Among Gram-negative Isolates

Table 4.8: ESBL Production Among Gram-negative Isolates (n = 218)

ESBL Status	Number of Isolates	Percentage ()
ESBL Positive	94	43.1
ESBL Negative	124	56.9
Total	**218**	**100**

Table 4.8 (ESBL Detection)

Table 4.9: Carbapenem Resistance Pattern (n = 218 Gram-negative isolates)

Carbapenem Status	Number of Isolates	Percentage ()
Carbapenem Sensitive	201	92.2
Carbapenem Resistant	17	7.8
Total	**218**	**100**

Table 4.9 (Carbapenem Resistance)

4.10 Multidrug Resistance (MDR) Distribution

Table 4.10: Distribution of Multidrug-Resistant Isolates (n = 248)

MDR Status	Number of Isolates	Percentage ()
MDR Positive	136	54.8
Non-MDR	112	45.2
Total	**248**	**100**

Table 4.10 (MDR Distribution).

4.11 MAR Index Distribution Among Isolates

The Multiple Antibiotic Resistance (MAR) index was calculated for each of the 248 isolates to assess the level of antibiotic exposure and resistance risk.

Table 4.11: MAR Index Distribution (n = 248)

MAR Index Range	Number of Isolates	Percentage ()	Interpretation
≤ 0.2	58	23.4	Low antibiotic exposure
0.21 – 0.5	104	41.9	Moderate exposure
0.51 – 0.8	70	28.2	High-risk source
> 0.8	16	6.5	Extremely high resistance
Total	**248**	**100**	

Table 4.11 (MAR Index Analysis)

4.12 Association of Multidrug Resistance with Age and Gender

Table 4.12A: Association Between Age Group and MDR (n = 248)

Age Group	MDR (n)	Non-MDR (n)	p-value
15–25	18	20	
26–35	34	22	
36–45	28	16	
46–55	22	14	
56–65	20	10	
>65	14	4	**0.02**

Table 4.12B: Association Between Gender and MDR (n = 248)

Gender	MDR (n)	Non-MDR (n)	p-value
Female	82	84	
Male	54	28	**0.03**

Table 4.12 (Age and Gender Association with MDR)

5. DISCUSSION

This study investigated urinary tract infections (UTIs) and antimicrobial resistance patterns among uropathogens isolated from patients attending healthcare facilities in Kangra district, Himachal Pradesh. The research combined microbiological culture analysis with molecular identification techniques to generate baseline regional data on infection burden and antibiotic resistance.

The findings demonstrated that UTIs remain a major clinical problem in the region, with a high proportion of culture-confirmed infections. Gram-negative bacteria were the predominant pathogens, with Escherichia coli identified as the most common causative organism, followed by Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa. Female patients, particularly those in the reproductive age group, showed higher infection rates due to recognized anatomical and physiological factors, while elderly individuals and male patients exhibited higher frequencies of multidrug-resistant infections.

A major outcome of the study was the alarming prevalence of antimicrobial resistance. Commonly prescribed antibiotics such as amoxicillin, fluoroquinolones, and trimethoprim-sulfamethoxazole

showed resistance rates exceeding fifty percent, indicating that traditional empirical therapies may no longer be reliable. A substantial proportion of isolates were identified as extended-spectrum beta-lactamase (ESBL) producers, reflecting widespread beta-lactam resistance. Although carbapenems remained effective against most isolates, the emergence of carbapenem-resistant strains signaled an early warning for future therapeutic challenges.

More than half of the culture-positive isolates were multidrug resistant, highlighting intense antibiotic selection pressure in the community. Elevated multiple antibiotic resistance (MAR) index values suggested extensive antibiotic exposure due to over-the-counter availability, incomplete treatment courses, empirical prescribing, and self-medication practices. The association between ESBL production and multidrug resistance indicated the possible transfer of resistance genes through mobile genetic elements, increasing the risk of rapid dissemination within healthcare and community settings.

The study emphasized the importance of routine culture and sensitivity testing, updated local antibiograms, and evidence-based prescribing practices. It highlighted the urgent need for antimicrobial stewardship programs, stricter regulation of antibiotic distribution, infection prevention measures, and public awareness campaigns promoting responsible antibiotic use.

The research also discussed broader contributing factors, including limited diagnostic infrastructure in rural and semi-urban areas, delayed laboratory reporting, healthcare access challenges, socioeconomic disparities, patient behavior, and environmental reservoirs of resistance. The findings underscored the importance of adopting a One Health approach that integrates human, environmental, and veterinary perspectives in resistance control.

Furthermore, the study stressed the value of strengthening laboratory capacity, implementing surveillance networks, integrating molecular diagnostics, and promoting interdisciplinary collaboration among clinicians, microbiologists, policymakers, and public health authorities. Future research involving longitudinal monitoring, genomic analysis, and environmental studies was recommended to better understand resistance evolution and transmission dynamics.

In conclusion, the study established that UTIs in Kangra district are characterized by high infection burden, predominance of Gram-negative pathogens, widespread multidrug resistance, significant ESBL production, and emerging carbapenem resistance. These findings highlight antimicrobial resistance as a serious and evolving public health challenge requiring immediate, coordinated, and sustained interventions through stewardship, surveillance, education, policy enforcement, and improved diagnostic infrastructure.

Summary of Key Findings and Discussion

The present study investigated the prevalence, antimicrobial resistance patterns, multidrug resistance (MDR), and molecular characterization of uropathogens among patients in Kangra district, Himachal Pradesh. Out of 400 urine samples analyzed, 248 (62%) were culture positive, indicating a significant burden of urinary tract infections (UTIs) in the region. Urinary Tract Infection was most common among females and individuals aged 26–35 years, while elderly patients showed higher rates of multidrug-resistant infections.

Major Uropathogens Identified

The predominant pathogen isolated was Escherichia coli (58.9%), followed by Klebsiella pneumoniae and other Gram-negative bacteria. Gram-negative organisms accounted for 87.9% of all isolates, confirming their dominant role in UTIs globally and nationally. The high prevalence of E. coli reflects its strong virulence mechanisms such as fimbriae-mediated adhesion, biofilm formation, and iron acquisition systems.

Antimicrobial Resistance Patterns

The study revealed alarming resistance levels to commonly prescribed antibiotics:

- Amoxicillin – 70%
- Ciprofloxacin – 59%
- Trimethoprim-sulfamethoxazole – 63%

These findings indicate extensive misuse and overuse of antibiotics in the community. However, Nitrofurantoin retained high effectiveness (84% sensitivity), supporting its continued use as a first-line treatment for uncomplicated UTIs.

Multidrug Resistance and ESBL Production

A high MDR prevalence of 54.8% was observed, showing that more than half of the isolates were resistant to multiple antibiotic classes. Extended-spectrum beta-lactamase (ESBL) production was detected in 43.1% of Gram-negative isolates, significantly limiting treatment options. Carbapenem resistance, although lower (7.8%), is concerning because carbapenems are considered last-resort antibiotics.

The study also demonstrated a strong relationship between ESBL production and MDR patterns, emphasizing the growing threat of resistant uropathogens in both hospital and community settings.

MAR Index Findings

The Multiple Antibiotic Resistance (MAR) index analysis showed that 76.6% of isolates originated from high-risk antibiotic exposure environments. High MAR values indicate:

- Excessive antibiotic use
- Increased resistance pressure
- Greater risk of treatment failure
- Circulation of resistant strains within the community

The study confirmed a significant association between higher MAR index values and MDR prevalence.

Molecular Characterization

The integration of 16S rRNA sequencing improved pathogen identification accuracy and strengthened the scientific reliability of the findings. Molecular confirmation reduced the risk of misidentification associated with conventional biochemical techniques and provided insight into regional strain distribution.

Demographic Trends

- Females showed higher overall UTI prevalence due to anatomical and hormonal factors.
- Males exhibited a higher proportion of complicated and MDR-associated infections.
- Elderly patients demonstrated increased resistance patterns because of repeated antibiotic exposure and comorbidities.

Public Health Implications

The findings highlight a serious antimicrobial resistance burden in Kangra district and emphasize the need for:

- Revision of empirical antibiotic guidelines
- Regular regional antibiogram updates
- Antimicrobial stewardship programs
- Restriction of unnecessary cephalosporin and fluoroquinolone use
- Improved infection control measures
- Regulation of over-the-counter antibiotic sales

Study Strengths

The study's major strengths include:

- Large sample size (400 samples)
- Molecular confirmation using 16S rRNA sequencing
- Comprehensive MDR and MAR index analysis
- Demographic-based epidemiological evaluation
- First baseline regional resistance data from Kangra district

Limitations

The study was limited by:

- Cross-sectional design
- Lack of whole genome sequencing
- Single-district sampling
- Absence of environmental resistance analysis

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