Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

ISSN NO. 2320-5407

Article DOI:10.21474/IJAR01/17612
DOI URL: http://dx.doi.org/10.21474/IJAR01/17612

## RESEARCH ARTICLE

# BACTERIAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF UROPATHOGENS CAUSING URINARY TRACT INFECTIONS: OBSERVATIONSINA NEW TERTIARY CARE INSTITUTE OF A RURAL AREA OF NORTHERN INDIA

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## Manuscript Info

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***Manuscript History***
Received: 28 July 2023
Final Accepted: 31 August 2023
Published: September 2023

***Key words:-***
Urinary Tract Infections, Bacterial Infections, Antimicrobial Drug Resistance, Antibiogram

## Abstract

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**Introduction:** Urinary tract infections (UTIs) are among the most common and burdensome diseases affecting outpatients and hospitalized patients, *Escherichia coli* being the most commonly implicated bacterium. UTIs have been treated empirically in locations where microbiological facilities are either non-existent or too expensive. This study was conducted to fill in our lack of knowledge regarding the bacterial composition and antimicrobial susceptibility patterns of uropathogens in Raebareli (Uttar Pradesh), India, and to help formulate an antibiotic policy.

**Materials and Methods:** This retrospective study was conducted at a 600-bed tertiary care hospital in rural India, from 11th August 2021 to 10th July 2022. The study excluded patients who were on antibiotic therapy and had been hospitalized a week before their OPD visit. Midstream urine samples collected aseptically were cultured semi-quantitatively. Organisms with significant colony counts were identified using routine biochemical techniques and antimicrobial susceptibility was tested against various antibiotics as per CLSI guidelines. The data was condensed and analyzed using SPSS version 23 software's descriptive statistics feature.

**Results:** A total of 5,982 urine samples were considered eligible during the study period. Most of the patients were 19-39 years old (40.67%), and the majority were females (55.25%). Most of the samples were received from General Medicine (32.77%). 056 (17.65%) samples showed significant bacterial growth. 258 (4.31%) samples were not considered for further analysis due to contamination during collection. *E. coli* was the most common organism isolated (53.5%), followed by *Enterococcus faecalis* (21.4%). *E. coli* isolates exhibited good sensitivity to Fosfomycin (90.97%), Gentamicin (80.71%), and Imipenem (80%). Vancomycin and Linezolid showed >90%

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susceptibility to all the gram-positive isolates. 71.79% of the Staphylococcal isolates were MRSA.

**Discussion**: This study marks the primary investigation into the susceptibility patterns displayed by bacterial strains causing urinary tract infections in Raebareli, Uttar Pradesh (UP) and enables a comparison with other areas of the nation. Age, sex and pyuria were all crucial factors in the incidence of UTI, and must be considered when assessing and managing UTI patients. There was no significant difference in the prevalence of UTI among various departments. *E. coli* is the leading cause of UTI worldwide, as in our study. The bacterial isolates exhibited noteworthy resistance to the first-line therapeutic agents which are commonly employed for treating UTIs. Our results indicate that fosfomycin, gentamicin, and nitrofurantoin are viable options for treating *E. coli* infections, while carbapenems and piperacillin-tazobactam can potentially manage other Gram-negatives. Vancomycin, linezolid and nitrofurantoin were the available options for Gram-positive isolates,

**Conclusion:** Our study has revealed a significant number of Gram-negative bacterial isolates, with *Escherichia coli* being the main cause of UTIs. We have also conducted a comprehensive analysis of antibiogram data. Based on our findings, Nitrofurantoin and Fosfomycin are recommended to initially treat UTIs in our region. These results will apply to our inpatient and outpatient facilities and other departments within our organization.



## Introduction:-

In the realm of infectious diseases, urinary tract infections (UTIs) are among the most common and burdensome diseases affecting outpatients and hospitalized patients.[1] It poses a significant challenge to health care, leading to increased morbidity, escalating healthcare costs, extended hospital stays, and an overall decline in the quality of life for affected individuals.[2] Recognizing the severity of this issue, healthcare providers and policymakers have increasingly turned their attention toward identifying strategies and implementing preventive measures to combat UTIs effectively.*Escherichia coli* is the most commonly implicated bacterium, followed by *Klebsiella pneumoniae*, *Staphylococcus* species, *Proteus*species, *Pseudomonas aeruginosa*, *Enterococcus* species, and *Enterobacter*species. It is worth noting that the sequence of predominance may vary among these bacterial strains.[3]

UTIs account for 150 million cases worldwide, costing $6 billion in healthcare.These are typically treated empirically due to a paucity of resources for urine culture analysis, leading to inappropriate antibiotic use, especially in rural and small-town areas. The utilization of susceptibility data obtained from nearby microbiological facilities, thus, plays a crucial role in guiding the empirical selection of antibiotics for treating these infections. Drug resistance among uropathogens is also rising, warranting regular antibiotic susceptibility (AS) testing.[4,5] Moreover, the antibiotic sensitivity of bacterial uropathogens exhibits temporal and geographical variations.Hence, it is imperative to perform susceptibility screening in every area to ensure the generation of current and comprehensive epidemiological data.[6]

Regrettably, there exists a dearth of comprehensive exploration into the resistance profile of community-acquired uropathogens throughout different geographical areas in India.[7] UTIs have been treated empirically in locations where microbiological facilities are either non-existent or too expensive.We undertook this study to fill in our lack of knowledge regarding the bacterial composition and antimicrobial susceptibility patterns of uropathogens in Raebareli (Uttar Pradesh), India. Knowledge thus obtained is a prerequisite to formulating antibiotic policyfor our institute. [8]

## Materials and Methods:-

**Study setting:**

This retrospective study was conducted in the Department of Microbiology at a 600-bed tertiary care hospital in rural Raebareli, North India, serving a population of more than 40 lakhs. A retrospective dataset spanning a year ($11^{th}$August 2021 to $10^{th}$July 2022) was obtained through manual collection from the microbiology laboratory logbook, a paper-based repository of laboratory findings.

**Study population:**

We receivedurine samples from 6600 individuals, irrespective of age and sex, who sought treatment at our hospital's outpatient departments (OPDs) and were clinically diagnosed with urinary tract infections by their treating physicians.

The study excluded patients who had been hospitalized a week before their OPD visit to rule out hospital-acquired infections.The study participants who were on antibiotic therapy were also excluded from the study. Ethical permission was obtained from AIIMS Raebareli's Institutional Ethics Committee (IEC).

**Sample Collection and Processing:**

Midstream urine samples collected aseptically in an adequately labelled sterile, leak-proof, wide-mouthed container were carefully transported to the Microbiology laboratory and subjected to processing within 4 hours. A semi-quantitative culture was performed on HiCrome UTI agar plates (HiMedia Laboratories, Mumbai, India), and the organisms were identified using routine biochemical techniques.The reports were confidently produced using data from clinical presentation, Gram stain microscopy, and culture growth.A urine specimen was considered positive for UTI if the growth of an organism was detected at a concentration of at least $10^5$ colony-forming units per millilitre (CFU/mL) or if $10^4$ CFU/mL were detected along with the presence of more than 1 pus cells per 7 high-power field during microscopic examination. [9,10]

**Identification of Bacterial isolates and Antimicrobial Susceptibility Testing:**

The identification of bacterial isolates was performed employing standard microbiological techniques, specifically by examining culture and biochemical attributes. Variousstandard biochemical testswere conducted to identify all bacterial isolates present.[11,12]Kirby Bauer's standard disc diffusion method was employed to assess the isolates' susceptibility to antibiotics. Interpretation was done as per CLSI guidelines. [13]

Gram-negative bacteria were tested for susceptibility to different antibiotics including ceftriaxone (CTR), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (PIT), gentamicin (GEN), levofloxacin (LE), cotrimoxazole (COT), ertapenem (ETP), imipenem (IMP), meropenem (MRP), amikacin (AK), ceftazidime (CAZ), minocycline (MIN), nitrofurantoin (NIT) and fosfomycin (FOS).Antibiotics tested for Gram-positive cocci wereampicillin (AMP),penicillin (P), cefoxitin (CX), nitrofurantoin, high-level gentamicin (HLG), levofloxacin, fosfomycin, tetracycline (TET), cotrimoxazole, teicoplanin (TEI), vancomycin (VA), linezolid (LZ).[14]

**Data analysis:**

The data was condensed and analyzed using SPSS version 23 software's descriptive statistics feature. Investigators monitored data quality and completeness throughout the collection, after collection, and after entering data into SPSS for statistical analysis. A p-value of $< 0.05$ was deemed statistically significant for all tests at the 95% confidence level.

## Results:-

A total of 5,982 urine samples were considered eligible during the study period. Most of the patients were 19-39 years old (40.67%), and the majority were females (55.25%). Most of the samples were received from General Medicine (32.77%) followed by the Urology department (21.78%), together accounting for more than half the samples.Table 1shows the demographic distribution of the patients from whom urine samples were received.

**Table 1:-**

| Age (in years) | n (%) |
|---|---|
| 0-18 | 697 (11.65%) |
| 19-39 | 2433 (40.67%) |

| | |
|---|---|
| 40-59 | 1759 (29.41%) |
| >60 | 1093 (18.27%) |
| **Total** | **5982 (100%)** |
| **Sex** | **n (%)** |
| M | 2677 (44.75%) |
| F | 3305 (55.25%) |
| **Total** | **5982 (100%)** |
| **Department** | **n (%)** |
| CTVS | 17 (0.28%) |
| Cardiology | 24 (0.40%) |
| Dental | 10 (0.17%) |
| Dermatology | 39 (0.65%) |
| ENT | 22 (0.37%) |
| General Medicine | 1960 (32.77%) |
| General Surgery | 735 (12.29%) |
| OBGY | 1008 (16.85%) |
| Paediatrics | 394 (6.58%) |
| Neurosurgery | 27 (6.58%) |
| Neurology | 82 (1.37%) |
| Ophthalmology | 43 (0.72%) |
| Orthopaedics | 81 (1.36%) |
| Urology | 1303 (21.78%) |
| Paediatric Surgery | 87 (1.46%) |
| PMR | 30 (0.50%) |
| Psychiatry | 19 (0.32%) |
| Trauma and Emergency | 69 (1.15%) |
| COVID-ICU | 32 (0.53%) |
| **Total** | **5982 (100%)** |

Out of all the samples that were analyzed, 1096 (18.32%) showed a significant presence of pus cells, i.e., 1 per 7 high power field (HPF) and 1056 (17.65%) had noticeable bacterial growth (colony count $\geq 10^5$). However, 258 (4.31%) samples were not considered for further analysis due to contamination during collection. Additionally, 27 samples with a colony count $\geq 10^5$ of *Candida* spp. were excluded.

Table 2 shows that patients who are female, older, and have pus cells in their urine sample are more likely to develop UTI.

**Table 2:-** Significant growth in urine culture in various age groups.

| **Age (in years)** | **No Growth** | **Significant Growth** | **Total** |
|---|---|---|---|
| 0-18 | 543 (82.15%) | 118 (17.85%) | 661 (100%) |
| 19-39 | 2012 (85.25%) | 348 (14.75%) | 2360 (100%) |
| 40-59 | 1372 (81.38%) | 314 (18.62%) | 1686 (100%) |
| >60 | 741 (72.86%) | 276 (27.14%) | 1017 (100%) |
| Total | 4668 (81.55%) | 1056 (18.45%) | 5724 (100%) |
| **p-value is 0.000** | | | |

**Table 3:-** Significant growth in urine culture in different genders.

| **Sex** | **No Growth** | **Significant Growth** | **Total** |
|---|---|---|---|
| M | 2146 (83.34%) | 429 (16.66%) | 2575 (100%) |
| F | 2522 (80.09%) | 627 (19.91%) | 3149 (100%) |
| Total | 4668 (81.55%) | 1056 (18.45%) | 5724 (100%) |
| **p-value is 0.002** | | | |

**Table 2:-** Correlation between significant pus cells in urine and significant growth in culture.

| Pus Cells | No Growth | Significant Growth | Total |
|---|---|---|---|
| Insignificant | 3987 (84.49%) | 732 (15.52%) | 4719 (100%) |
| Significant | 681 (67.76%) | 324 (32.24%) | 1005 (100%) |
| Total | 4668 (81.55%) | 1056 (18.45%) | 5724 (100%) |
| **p-value is 0.000** | | | |

After analysing samples from various units of General Medicine, General Surgery, Obstetrics and Gynaecology, and Paediatrics, it was discovered that only patients in General Medicine Unit III had a statistically significant risk of developing UTI. However, there was no difference observed among the other disciplines, as shown in Tables 3 and 4.

**Table 3:-**

| Department | No Growth | Significant Growth | TotalSamples Received |
|---|---|---|---|
| General Medicine | **1520** | **357** | **1960** |
| General Surgery | **573** | **123** | **735** |
| **OBGY** | **810** | **165** | **1008** |
| Paediatrics | **302** | **65** | **394** |
| Urology | **1010** | **252** | **1303** |
| Total | **4215** | **962** | **5400** |
| p-value is 0.55 | | | |

Note: Only the 5 departments with the most samples were analysed.

**Table 4:-**

| Department and Unit | No Growth | Significant Growth | TotalSamples Received |
|---|---|---|---|
| General Medicine I | 682 | 159 | 864 |
| General Medicine II | 654 | 150 | 850 |
| General Medicine III | 184 | 48 | 246 |
| **Total** | **1520** | **357** | **1960** |
| **p-value is 0.041** | | | |
| General Surgery I | 330 | 76 | 430 |
| General Surgery II | 243 | 47 | 305 |
| **Total** | **573** | **123** | **735** |
| p-value is 0.6405 | | | |
| OBGY I | 662 | 130 | 821 |
| OBGY II | 103 | 22 | 128 |
| OBGY III | 45 | 13 | 59 |
| **Total** | **810** | **165** | **1008** |
| p-value is 0.655 | | | |
| Paediatrics I | 117 | 32 | 158 |
| Paediatrics II | 120 | 16 | 146 |
| Paediatrics III | 63 | 17 | 88 |
| Paediatrics IV | 2 | 0 | 2 |
| **Total** | **302** | **65** | **394** |
| p-value is 0.169 | | | |

Note: The Urology Department do not have Units, hence not included in this table.

Bacteria grew in 97.51% (n=1056) of the samples that showed significant growth, whereas only 2.49% (n=27) grew *Candida* spp. (Fig. 1)

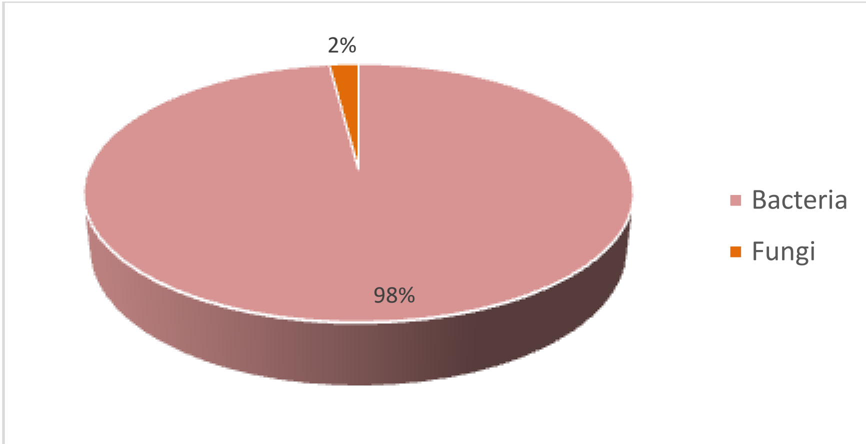



**Figure 1:-** Distribution of Microorganisms.

*E. coli* was the most common organism isolated (53.5%), accounting for more than half of the isolates, followed by *Enterococcus faecalis* (21.4%). Other organisms isolated were *Klebsiella* spp. (6.25%), *P. aeruginosa* (4.07%), *Proteus* spp. (1.79%), *Enterococcus faecium* (4.73%), *Staphylococcus aureus* (3.69%), etc.(Fig. 2)

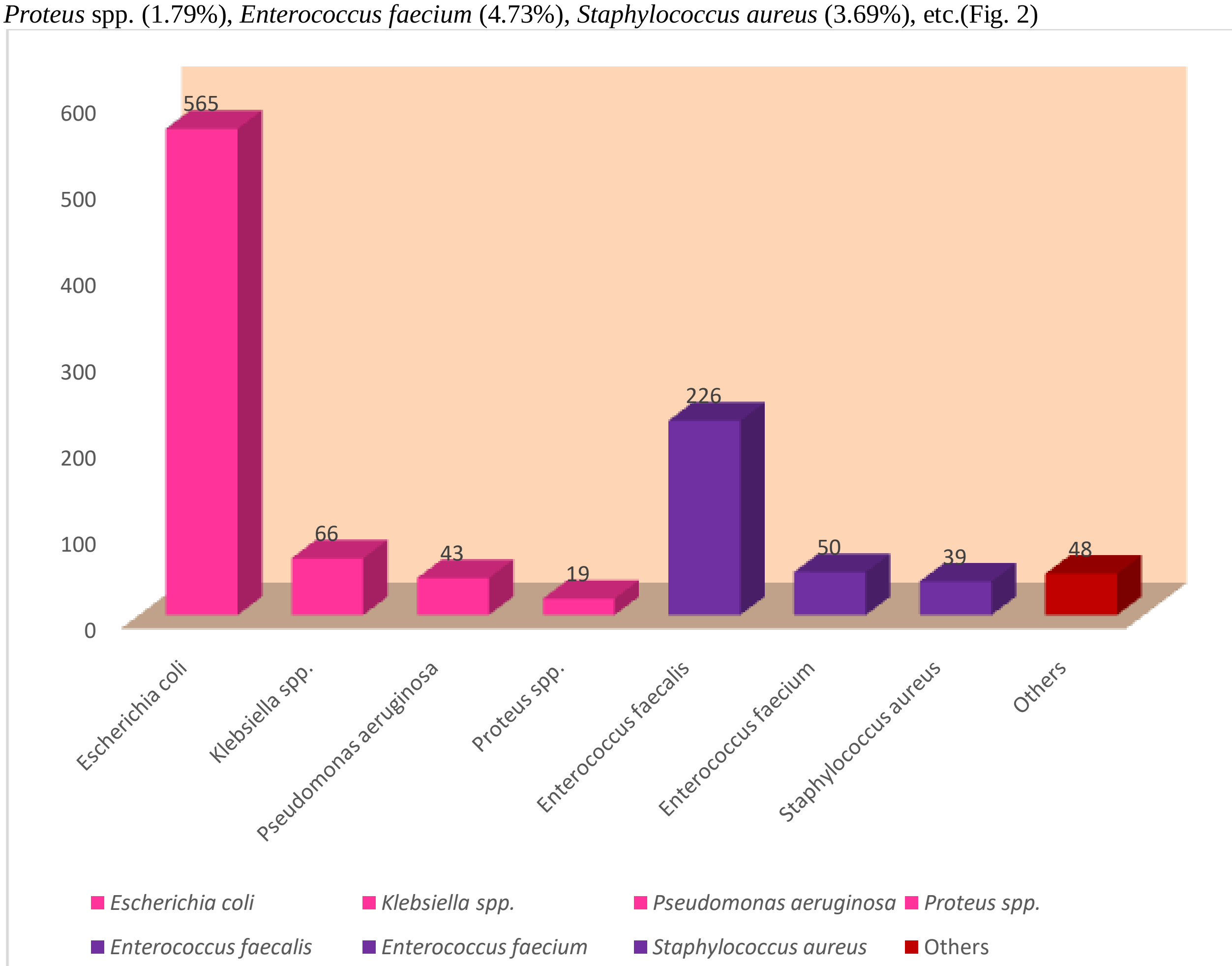
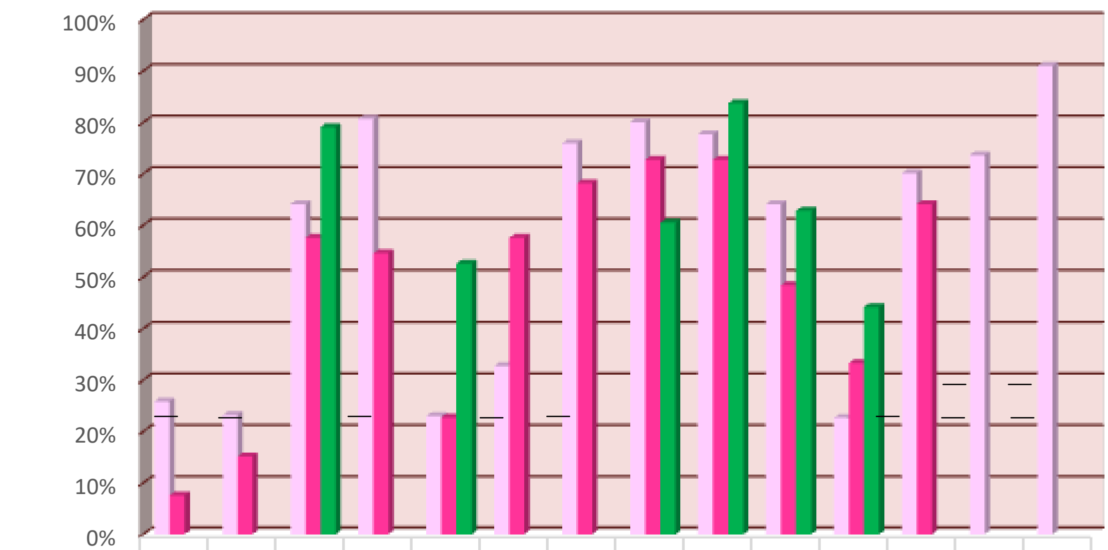



**Figure 2:-** Bacterial uropathogens isolated from urine.

*E. coli* isolates exhibited good sensitivity to Fosfomycin (90.97%), Gentamicin (80.71%), and Imipenem (80%) and were reasonably sensitive to Nitrofurantoin, Minocycline Meropenem, and Ertapenem (>70%). Klebsiella spp. was, however, sensitive to carbapenems (≈70%), followed by Minocycline, Cotrimoxazole, and Piperacillin-tazobactam

(≈60%). For *P. aeruginosa*, the most sensitive drugs were Meropenem and Piperacillin-tazobactam (≈80%). Figure 3 displays the antibiotic sensitivity pattern of the Gram-negative isolates.

**Figure 3:-** Antibiotic Sensitivity Pattern of Gram-negative Isolates.

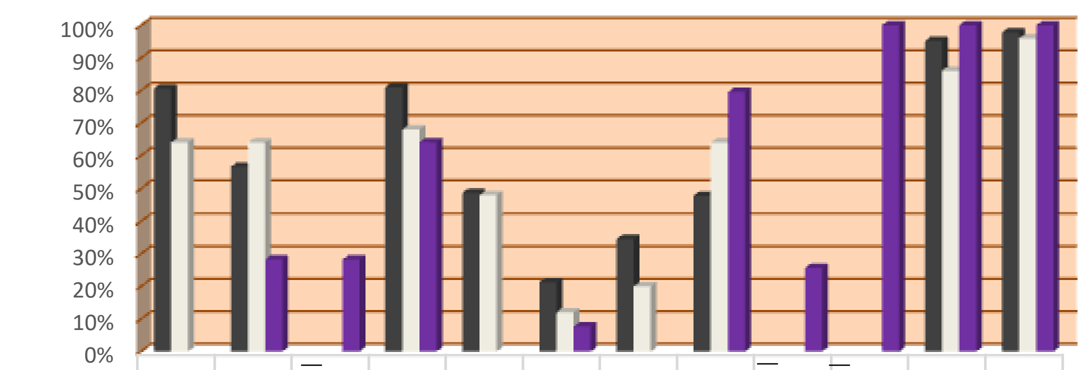



| | CTR | AMC | PIT | GEN | LE | COT | ETP | IMP | MRP | AK | CAZ | MIN | NIT | FOS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | 25.84% | 23.18% | 64.07% | 80.71% | 23.01% | 32.74% | 75.93% | 80% | 77.69% | 64.07% | 22.65% | 70.09% | 73.63% | 90.97% |
| *Klebsiella spp.* | 7.57% | 15.15% | 57.57% | 54.54% | 22.72% | 57.57% | 68.18% | 72.72% | 72.72% | 48.48% | 33.33% | 64.12% | | |
| *P. aeruginosa* | | | 79.07% | | 52.49% | | | 60.46% | 83.72% | 62.79% | 44.19% | | | |

CTR: Ceftriaxone; AMC: Amoxicillin-Clavulanic acid; PIT: Piperacillin-Tazobactam; GEN: Gentamicin; LE: Levofloxacin; COT: cotrimoxazole; ETP: ertapenem; IMP: imipenem; MRP: meropenem; AK: amikacin; CAZ: ceftazidime; MIN: minocycline; NIT: nitrofurantoin; FOS: fosfomycin

Vancomycin and Linezolid showed >90% susceptibility to all the isolates, 100% in the case of *S. aureus*. Nitrofurantoin showed good susceptibility to all, but the isolates were mainly resistant to Levofloxacin and Ampicillin or Penicillin. 71.79% of the Staphylococcal isolates were methicillin-resistant *Staphylococcus aureus* (MRSA).Figure 4 illustrates the antibiotic sensitivity pattern of the Gram-positive isolates.

**Figure 4:-** Antibiotic Sensitivity Pattern of Gram-positive Isolates.




| | AMP | P | CX | NIT | HLG | LE | FOS | TET | COT | TEI | VA | LZ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. faecalis* | 80.53% | 56.60% | | 80.97% | 48.67% | 21.24% | 34.51% | 47.79% | | | 95.13% | 97.78% |
| *E. faecium* | 64% | 64% | | 68% | 48% | 12% | 20% | 64% | | | 86% | 96% |
| *S. aureus* | | 28.21% | 28.21% | 64.10% | | 7.69% | | 79.49% | 25.64% | 100.00 | 100% | 100% |

AMP:Ampicillin; P:Penicillin; CX:Cefoxitin;NIT:Nitrofurantoin;HLG:High-level Gentamicin;LE: Levofloxacin; FOS: Fosfomycin;TET:Tetracycline;COT:Cotrimoxazole;TEI: Teicoplanin; VA: Vancomycin;LZ:linezolid

## Discussion:-

This study marks the primary investigation into the susceptibility patterns displayed by bacterial strains causing urinary tract infections in Raebareli, Uttar Pradesh(UP). The research provides noteworthy laboratory data and enables a comparison of the situation in our region with other areas of the nation. Our research revealed that 17.65% of urine samples taken from patients who sought medical attention at our hospital's outpatient clinics yielded substantial bacterial pathogens. It is essential to take note that the prevalence of UTI in our community is comparatively higher than in other countries such as Tehran, Iran (6.3%), Libya (13.9%), and Ethiopia (15.9%). [15-18] Contrastingly, the prevalence was lower compared to reports from Madhya Pradesh, India (65.5%) and Bareilly, UP, India (45.3%). [19,20]

Based on our study's findings, individuals aged 19-39 and 40-59 are most susceptible to UTI. However, those over 60 had a remarkably higher culture positivity rate of 27.14%. These results indicate that age is a critical factor in the incidence of UTI, and healthcare practitioners must consider it when assessing and managing patients. In their retrospective study conducted in Ethiopia, Kasew et al. presented findings aligning with the research.[21] However, Mohapatra et al. and Kabew et al. reported the highest prevalence of UTI among age groups 19–35 years (56.9%) and 21-30 years (27.16%), respectively.[22,23]

Studies have shown that women are more prone to UTIs because their urethra, which is closer to the rectum, is shorter than men's. [1,15] Our study supports this finding, revealing that the occurrence of UTIs in females (19.91%) was significantly higher than in males (16.66%). Studies by Kasew et al. and Sood et al. found similar results. [21,24] However, the latter reported a higher incidence of UTIs among males over 60 than females in the same age group.

Neupane et al.'s research has revealed that pyuria may not be a definitive indicator of significant bacteriuria in UTI cases. [25] Nevertheless, a greater number of pus cells in the urine sample can increase the chances of a positive culture result. Our study findings corroborate these observations. Only a few studies compared the prevalence of UTI among various departments and there was no significant difference among them, as in this study, suggesting other underlying factors, presumably age and sex, are more crucial.[26]

The diagnosis of UTI relies on the presence of clinical symptoms and a positive urine culture. Bacteria are the most common cause of UTI, followed by fungi. Uropathogenic *E. coli* (UPEC) is the leading cause of UTI, accounting for 80% of cases, according to a review by Klein et al. Our study also revealed that bacterial growth was present in 98% of culture-positive cases, with *E. coli* being the most frequently identified bacteria (n=565, 53.5%), followed by E. faecalis (n=226, 21.4%). [27,28]

The medical community is facing a serious problem with the increase of bacterial uropathogens that are resistant to antibiotics. This has caused a reduction in treatment options for UTIs, which has led to a worrying connection between infections caused by antimicrobial-resistant bacteria and higher rates of treatment failure, hospitalizations, financial burden, and death. The absence of new antibiotics has exacerbated this problem, making it a significant cause for concern. [2,4]

When dealing with UTIs, making informed decisions about antibiotics is crucial to achieve optimal outcomes. The Gram-negative isolates exhibited noteworthy resistance to the primary therapeutic agents cotrimoxazole, ampicillin, Augmentin, ceftriaxone, and levofloxacin, which are commonly employed for treating UTI, as per findings from the antibiotic sensitivity tests. Fosfomycin (90.97%), gentamicin (80.71%), and nitrofurantoin (73.6%) were highly effective in treating *E. coli* isolates. Furthermore, other Gram-negative isolates exhibited moderate sensitivity to carbapenems (approximately 70%) and piperacillin-tazobactam (around 60%). These results indicate that fosfomycin, gentamicin, and nitrofurantoin are viable options for treating *E. coli* infections, while carbapenems and piperacillin-tazobactam can potentially manage other Gram-negatives.Previous Indian studies have demonstrated that nitrofurantoin and fosfomycin are viable oral initial treatment options for community-acquired urinary tract infections. [22,24]Vancomycin (≈90%), linezolid (>90%), and nitrofurantoin (≈70%) were effective against the Gram-positive isolates, with 71.79% of Staphylococcal isolates being MRSA. These results were consistent with studies by Sweih et al. and Chooramani et. al.[29,30]

However, our study has few limitations. Firstly, we did not explore UTI-causing anaerobic bacteria, fungi, and viral agents due to limited laboratory resources. Secondly, due to variations in sensitivity rates between healthcare facilities, the findings may lack generalizability and reproducibility in different healthcare settings of UP state.

## Conclusion:-

Our study has revealed a significant number of Gram-negative bacterial isolates, with *Escherichia coli* being the main cause of UTIs, followed by Klebsiella pneumoniae. We have also conducted a comprehensive analysis of antibiogram data. Based on our findings, Nitrofurantoin and Fosfomycin are recommended to initially treat UTIs in our region. These results will apply to our inpatient and outpatient facilities and other departments within our organization.

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