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TWO-YEAR ANALYSIS OF ANTIBIOTIC SUSCEPTIBILITY CHANGES IN URINE ISOLATES FROM SUB-HIMALAYAN MEDICAL COLLEGE, NORTH INDIA

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ABSTRACT

Urinary tract infections (UTIs) represent a prevalent community-onset bacterial ailment, with Uropathogenic Escherichia coli (UPEC) as the primary causative agent. However, the escalating emergence of antibiotic resistance poses a significant challenge in managing these infections, potentially leading to therapeutic impasses. Global estimates attribute approximately 700,000 annual deaths to antimicrobial resistance (AMR), projecting a staggering 10 million deaths annually by 2050 if current trends persist. This retrospective analysis, conducted at the Microbiology Department of DRPGMC Kangra, Tanda, Himachal Pradesh, from June 2021 to July 2023, scrutinized urine culture results following CLSI guidelines. A descriptive overview of urine sample culture distributions between July 2021 to June 2022 and July 2022 to June 2023 revealed a substantial influx of samples: 7341 (36.8%) and 12,607 (63.2%) respectively. Predominantly, E. coli, Klebsiella pneumoniae, and Pseudomonas species comprised the major isolates. Notably, all isolates exhibited a discernible decline in antimicrobial sensitivity, underscoring a worrying trend of resistance proliferation. The mounting rates of antibiotic resistance pose a daunting challenge to clinicians, limiting viable treatment options for UTIs caused by these multi-drug resistant organisms. Contributory factors including inappropriate broad-spectrum antibiotic use, inadequate hygiene practices, immunosuppression, and prolonged hospital stay further exacerbate the risk of Multi-Drug Resistant (MDR) infections.

Keywords: Urinary tract infections(UTI), Uropathogenic Escherichia coli, Antimicrobial resistance, Multi-Drug Resistant organisms(MDRO), CLSI.

INTRODUCTION

Urinary tract infections (UTIs) represent a pervasive concern in community health, affecting approximately 150 million individuals annually worldwide. Alarmingly, up to 75% of females experience UTIs by the age of 24, with a relapse rate of 15–25% within this demographic [1,2].
Clinically, UTIs manifest as bacteriuria accompanied by urinary symptoms and can afflict both the lower and upper urinary tracts. Lower UTIs, termed cystitis, exhibit symptoms such as

dysuria, frequency, urgency, and suprapubic tenderness. However, the presence of lower UTI symptoms doesn't preclude the concurrent existence of upper UTIs [3,4].

The treatment of UTI can be classified into uncomplicated and complicated based on the choice of treatment [5] Predominantly caused by Gram-negative bacteria like Escherichia coli, Klebsiella spp., Proteus mirabilis, and Pseudomonas aeruginosa, alongside Gram-positive Staphylococcus aureus, UTIs present a substantial healthcare burden [6].

Uropathogenic Escherichia coli (UPEC) emerges as the primary etiological agent and a leading cause of UTIs worldwide. However, the rising challenge of treatment inefficacy and therapeutic impasses due to increasing antibiotic resistance looms large [7].

The rampant misuse of antimicrobials in UTI management significantly fuels the emergence of antimicrobial resistance (AMR), posing a grave global threat. Estimates suggest AMR accounts for about 700,000 annual deaths, with a projected toll exceeding 10 million deaths yearly by 2050 if current trends persist [8,9]. Notably, comprehensive antimicrobial treatment often commences before laboratory results, leading to frequent antibiotic misuse. In India, the distribution and resistance patterns of community-acquired uropathogens remain relatively understudied [10,11,12].

This study aims to compare the antibiotic susceptibility of uropathogens following CLSI guidelines, facilitating the formulation of empirical therapy guidelines for UTIs while awaiting culture sensitivity reports. Furthermore, it sheds light on shifting susceptibility patterns among common uropathogens in North India, advocating for periodic monitoring to mitigate therapeutic failures and curb the escalating antibiotic resistance crisis.

MATERIAL AND METHODS

Study Design and Methodology

A retrospective analysis of urine sample cultures was conducted at the Microbiology Department of DRPGMC Kangra, Tanda, Himachal Pradesh, spanning from June 2021 to July 2023.

Sampling and Processing

Urine specimens were collected in sterile wide-mouth universal containers and promptly processed on the same day. A semi-quantitative approach, employing a calibrated 4mm diameter loop for primary organism isolation, facilitated the transfer of 10µl of urine per loop. Following this, samples were inoculated onto Mac Conkey agar plates and aerobically incubated at 37°C for 24-48 hours. Cultures exhibiting colony counts $\geq 10^5$cfu/ml were identified as positive for UTI, while the absence of growth indicated sterile samples. Identification of uropathogens from positive cultures relied on standard biochemical reactions, with a significant bacterium defined by a urine culture yielding $\geq 10^5$ CFU/ml, considering $<10^5$ CFU/ml as insignificant.[15]

Antimicrobial Susceptibility Testing

The assessment of antimicrobial susceptibility was conducted on Mueller-Hinton agar plates utilizing the Kirby-Bauer disc diffusion method, following established operational procedures. Test organism suspensions in sterile saline were standardized to 0.5 McFarland units. A uniform seeding process on Mueller Hinton agar plates preceded a 10-minute drying period before

applying antibiotic discs. Subsequent incubation of plates at 37°C for 16-18 hours allowed the measurement and recording, in millimeters, of clear zones surrounding the antibiotic discs. The susceptibility and resistance data evaluation adhered strictly to the guidelines outlined by the Clinical Laboratory Standards Institute.[16]

RESULTS

In our investigation, 19,948 urine samples were collected from different departments within Dr. Rajendra Prasad Government Medical College from July 2021 to June 2023. This period was subdivided into two yearly segments: July 2021 to June 2022 and July 2022 to June 2023. During the initial year of the study, we received 7341 urine samples, accounting for 36.8% of the total samples, while in the subsequent year, from July 2022 to June 2023, there was a notable increase with 12,607 urine samples received, constituting 63.2% of the overall samples collected during the study period. (Table & Fig. 1)

Table 1: Yearly Distribution of Urine samples

Samples	**Numbers (%)**
1. First Year(July 2021 to June 2022)	7341(36.8%)
2. Second Year(June 2022 and July 2023)	12,607(63.2%)
TOTAL	**19,948**

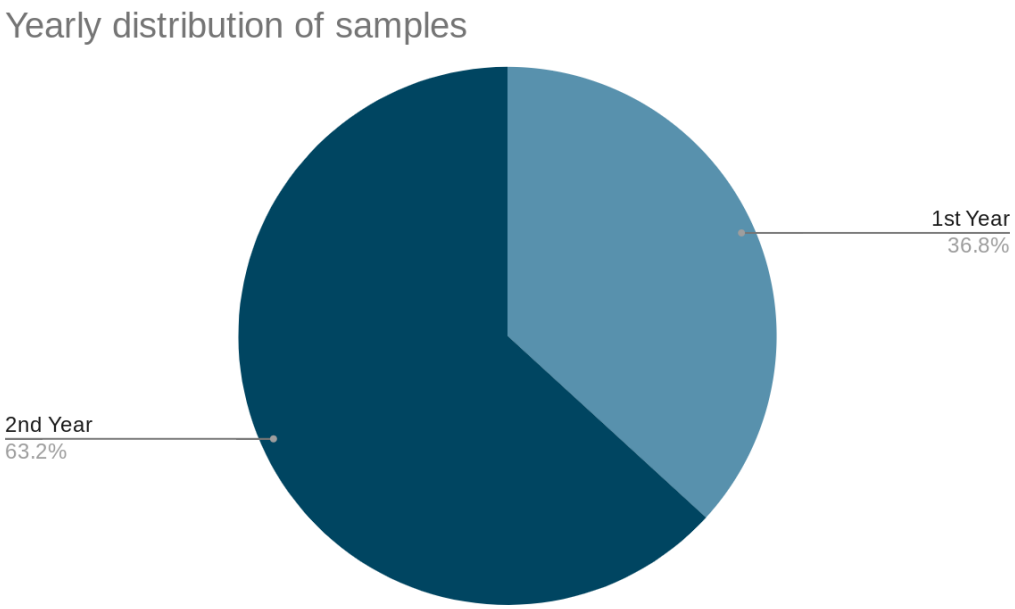

Fig. 1: Yearly Distribution of Urine samples

In the initial year of our study, among the 7341 urine samples received, a majority of 5350 samples (72.9%) were determined to be sterile, while 1457 samples (19.8%) exhibited significant

pathogenic growth, and 504 samples (6.8%) indicated insignificant contaminants. In the subsequent year, out of the 12,607 urine samples received, 9048 samples (71.7%) were identified as sterile, 2846 samples (22.6%) displayed significant pathogenic growth, and 713 samples (5.7%) showed insignificant or contaminant growth. This comparison indicates a consistent pattern of sterile samples along with fluctuations in significant pathogenic growth and insignificant or contaminant findings between the two study years.(Fig.2)

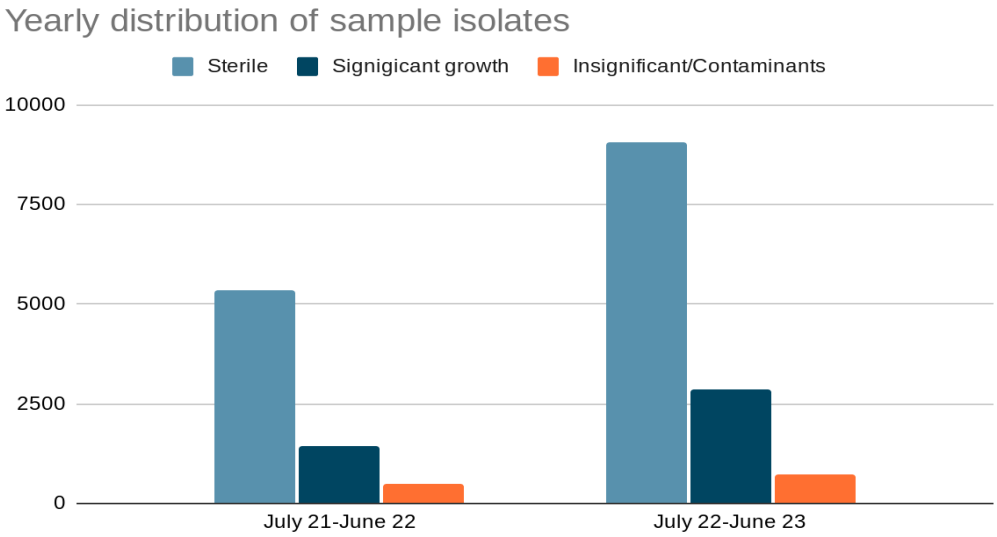

Fig. 2: Yearly distribution of sample isolates

During the first year of our study, among the 1457 urine samples exhibiting significant pathogenic growth, the majority were attributed to specific pathogens. Escherichia coli accounted for the highest proportion with 856 cases (58.9%), followed by Klebsiella pneumoniae with 131 cases (8.9%), and Pseudomonas aeruginosa with 68 cases (4.6%). Other identified pathogenic isolates constituted 402 cases, amounting to 27.6% of the total samples displaying significant pathogenic growth.

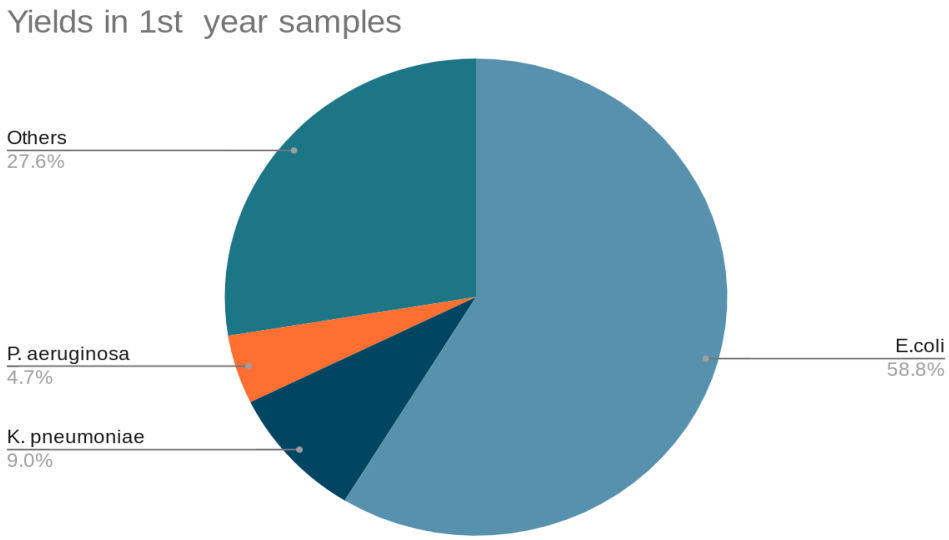

Fig. 3: Significant Bacterial isolates from samples of 1st Year

In the subsequent year of our study, among the 2846 urine samples displaying significant pathogenic growth, a comparable trend to the previous year was observed in the distribution of pathogens. Escherichia coli continued to be predominant, accounting for 1440 cases (50.6%), followed by Klebsiella pneumoniae with 166 cases (5.8%), and Pseudomonas aeruginosa with 106 cases (3.7%). The remaining pathogenic isolates totaled 1134 cases, representing 39.9% of the samples demonstrating significant pathogenic growth

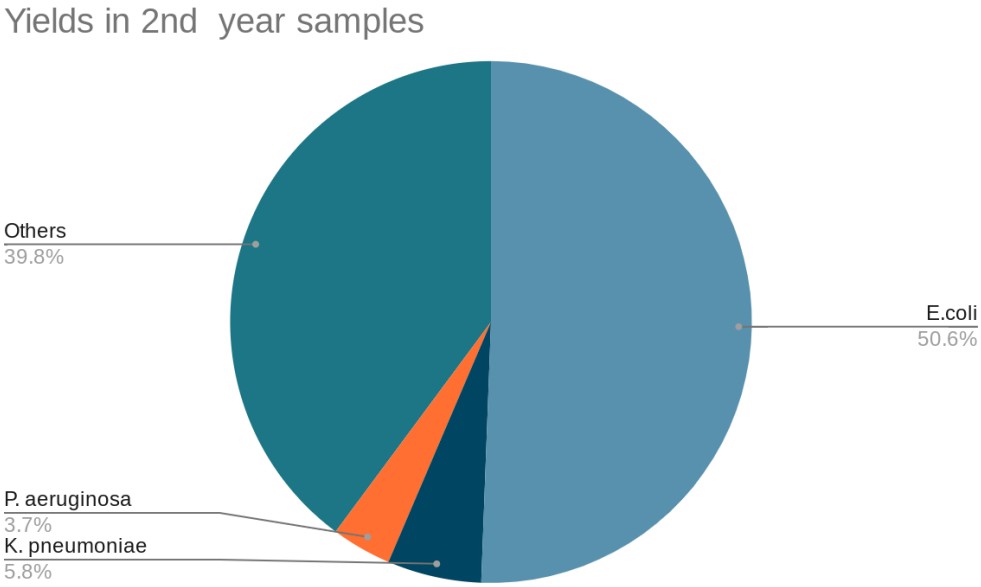

Fig. 4: Significant Bacterial isolates from samples of 2nd Year

Antimicrobial susceptibility testing(AST) was done on these major bacterial isolates from urine samples and their AST pattern was compared yearly changing trend of the susceptibility was done for the antibiotics as per the CLSI, 2023 guidelines.

The antimicrobial susceptibility testing (AST) conducted on major Escherichia coli isolates from urine samples revealed a shifting pattern in susceptibility over two years. Notably, there was a substantial decrease in susceptibility to Cefazolin, dropping from 36% in the first year to 32% in the second year. Ampicillin Sulbactam displayed a marginal decrease in susceptibility by 3.5% between the two years. Contrarily, Fosfomycin exhibited a noteworthy increase in susceptibility, rising from 93% in the first year to 97% in the second year (Table 2)

Table 2: Major changes in the susceptibility pattern in *E. coli* isolates from urine samples over 2 years.

Name of the antibiotic	July 2021-June 2022	July 2022-June 2023
	susceptibility (n/n) %	
1. Ampicillin sulbactam	80.9%	77.4%
2. Norfloxacin	51.1%	47.3%
3. Nitrofurantoin	91%	89%
4. Cefazolin	36%	32%
5. Fosfomycin	93%	97%

The susceptibility analysis conducted on Klebsiella pneumoniae isolates revealed distinctive changes in antibiotic susceptibility over the evaluated periods. There was a noteworthy decline in susceptibility to Ampicillin Sulbactam, decreasing from 57.1% to 45.3%. Similarly, Nitrofurantoin exhibited reduced susceptibility, dropping from 76% to 64%. Contrarily, an encouraging trend was observed in the susceptibility to Cefazolin, which increased from 29% to 31.5%.(Table 3)

Table 3: Major changes in the susceptibility pattern in *K. pneumoniae* isolates from urine samples over 2 years.

Name of the antibiotic	July 2021-June 2022	July 2022-June 2023
	susceptibility (n/n) %	
1. Ampicillin-sulbactam	57.1%	45.3%
2. Norfloxacin	61.9%	59%
3. Nitrofurantoin	76%	64%
4. Cefazolin	29%	31.5%
5. Ceftriaxone	58.8%	53.6%

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The analysis of Pseudomonas aeruginosa isolates revealed notable shifts in antibiotic susceptibility within the evaluated period. There was a considerable decrease in susceptibility to Piperacillin and Tazobactam, declining notably from 93.3% to 60.3%. Conversely, an encouraging trend was observed in susceptibility to Amikacin, which increased from 85.7% to 89.4%. (Table 4)

Table 4: Major changes in the susceptibility pattern in *P. aeruginosa* isolates from urine samples over 2 years.

Name of the antibiotic	July 2021-June 2022	July 2022-June 2023
	susceptibility (n/n) %	
1. Pip-tazpbactam	93.3%	60.3%
2. Ciprofloxacin	87.9%	85%
3. Amikacin	85.7%	89.4%
4. Cefepime	84%	82%

DISCUSSION

This study offers crucial insights into the landscape of antimicrobial resistance among uropathogens, providing a foundation for enhanced empirical treatment strategies. Globally, a concerning escalation in antimicrobial resistance has been well-documented [17–23]. Consistent with earlier reports from various regions of India and beyond, our findings corroborate the prevalence of E. coli as the primary UTI pathogen, closely followed by Klebsiella spp [24,25].

In our investigation, E. coli exhibited declining susceptibility to cefazolin and Ampicillin sulbactam, aligning with Fasugba et al.'s longitudinal study on Five-Year Antimicrobial Resistance Patterns of Urinary Escherichia coli [26]. Similarly, Klebsiella pneumoniae samples showcased maximal resistance to cefazolin and penicillin combinations, yet a relatively lower

resistance to Nitrofurantoin. This trend of reduced susceptibility across antibiotics echoes findings akin to Rocca et al.'s study [27]. Intriguingly, our study noted an augmented sensitivity trend for cefazolin, possibly indicating restrained usage of oral cephalosporins in Klebsiella pneumoniae treatment.

Beyond E. coli and Klebsiella pneumoniae, Pseudomonas aeruginosa, for its biofilm-forming capability, displayed an alarming escalation in antimicrobial resistance across all antibiotics—a trend mirroring Reghal's findings in a four-year analysis of urinary tract infections [28]. Interestingly, there was an observed enhancement in aminoglycoside sensitivity over consecutive years, potentially indicative of reserved use of Amikacin aligning with CLSI 2023 guidelines.

The observed shifts in antimicrobial resistance underscore the pressing need for vigilance and dynamic therapeutic strategies. These findings illuminate the evolving resistance among uropathogens, emphasizing the importance of tailored treatment approaches guided by ongoing surveillance of susceptibility profiles.

CONCLUSION

Urinary tract infections stand as one of the most prevalent conditions across all age groups in medical practice. However, the surge in antibiotic resistance poses a significant challenge, limiting clinicians' choices for effective antibiotics against multi-drug resistant organisms. Contributing factors such as unwarranted use of broad-spectrum antibiotics, inadequate hygiene practices, immune system suppression, and prolonged hospital stays amplify the risk of multi-drug resistant (MDR) infections. To address this escalating concern, hospitals require enhanced record-keeping systems and a forward-looking surveillance approach. Regular and systematic monitoring is crucial, enabling the tracking of evolving levels and patterns of antibiotic resistance, thereby facilitating informed and timely interventions to counter the changing landscape of infections and their resistance profiles.

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