

GNB Uropathogens and their Antibiotic Resistance Trends: A Report in Eastern India

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Abstract:

Background: Urinary tract infections are one of the commonest infections in India and worldwide leading to increased mortality and morbidity. Prevalence of bacterial agents causing UTI and its antibiogram varies in different geographical location. Culture and sensitivity testing remains gold-standard for diagnosis of UTI. It is essential to know the local prevalence rate and antibiogram of Uropathogens, before starting an empirical therapy, until arrival of reports. This study was undertaken to study the etiological factors and their antibiogram in a tertiary care hospital in Eastern India.

Methods: In about 5512 admitted and out-patients of KPC Medical College and hospital, Kolkata, freshly passed mid-stream urine from non-catheterized patients and from aseptic catheter port of catheterised patients was collected in a sterile container and was sent to Microbiology Department for a period of one year. The samples were plated in Mac Conkey and UTI agar and incubated at 37°C overnight, following which next day it was processed in Vitek Automated machine for identification and Antibiotic susceptibility testing. Results were analysed using appropriate software.

Results: Of the 5512 urine samples, prevalence of UTI was 8.85% (488). The females (60.86%) were more affected than the males (39.14%). Most susceptible age group susceptible to UTI was the elderly individuals above 60yrs (61.06%) followed by 46-60yr (13.93%). Out of the 488 positive urine growth samples, 447 (91.60%) samples had Gram Negative etiology, 20 (4.09%) samples yielded growth of Gram Positive cocci and 21 (4.31%) samples showed growth of Candida species. Most common pathogen isolated was Escherichia coli (45.90%) followed by Klebsiella sp (34.50%). They are sensitive to Fosfomycin (88.98%), Nitrofurantoin (79.86%), Tetracycline (77.85%), Cotrimoxazole (75.61%), Aminoglycosides (74.94%) and Carbapenems (70.25%).

Conclusion: The study was helpful for giving an idea on Empirical therapy to be used when suspecting UTI. Fosfomycin, Nitrofurantoin, Cotrimoxazole, Aminoglycosides are better to treat UTI empirically. Hygiene, Health education and good Infection control practices can help in decreasing Urinary infection rates.

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Introduction

Urinary Tract infections (UTI) are among the most common bacterial infections in both out-patient and hospitalized patients in India and worldwide[1]. They are the second most common bacterial infections following Lower Respiratory tract infections globally. An estimated 150 million people were infected with UTI per annum worldwide which costed a global economy more than 6 billion US dollars[2]. Urinary tract infection is categorised clinically as urethritis, asymptomatic bacteriuria, cystitis, acute urethral syndrome and pyelonephritis[3]. UTIs can also be grouped into uncomplicated or complicated UTIs. Typically the uncomplicated UTIs affect healthy patients in the absence of structural or neurological anomalies of the urinary tract. However, complicated UTIs are associated with urinary tract

abnormalities that increase susceptibility to infection, such as catheterization or functional disorders or anatomical malformations (e.g., obstructive uropathy, neurogenic bladder, renal failure, pregnancy, and calculi)[1,4]. Patients with UTI mostly present with fever, dysuria, frequency of micturation and lower abdominal pain. It poses significant morbidity and mortality to patients if left untreated [5]. It has been noted that in patients at extremes of age, female sex, patients with immunocompromised diseases like HIV, Cancer, Diabetes, those receiving steroids or prior antimicrobial therapy, prolonged indwelling urinary catheterization, recent urological interventions, and extended duration of hospital stays are at a potential risk for developing UTI[6].

Most UTIs originate in the community and are attributed by common uropathogens like uropathogenic *E. coli* (UPEC) and *Klebsiella* spp., amounting to approximately 75–95 % of the total cases[7]. The other less prevalent organisms are *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Enterococcus faecalis*, and *Staphylococcus aureus*[8]. Delay in appropriate antimicrobial therapy may lead to adverse medical outcomes, increased duration of hospital stay, hamper familial and social relationships and increased economic burden for patients [9]A significant proportion of the Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* species exhibit ESBL and carbapenemase production[10].

Antimicrobial resistance profile of the uropathogens is heterogenous across different strains. A regular monitoring of the antibiotic susceptibility of these pathogens in a particular geographical area is the need of the hour because of increased and varied drug resistance over time [11].

Data from regional microbiology laboratories on local resistance profiles play a crucial role in guiding empirical antimicrobial therapy. Care should be taken to avoid inappropriate and excessive use of antimicrobials [10]. This study is being done to find out the prevalence of Gram Negative Uropathogens and their antibacterial susceptibility pattern in KPC Medical College and Hospital, Kolkata.

Type of study and Study Design: This was a retrospective descriptive cross-sectional study conducted in the department of Microbiology, KPC Medical College and Hospital (KPCMCH), which is a 800 bedded multidisciplinary tertiary care hospital in Kolkata, India, over a total duration of 1 year in the period July 2024 to June 2025.

Study Population: Urine samples of all clinically suspected outpatients with UTI as well as hospitalized patients admitted in KPC Medical College and Hospital, Kolkata was collected after obtaining informed written consent from the patients over the period of 1 year.

Inclusion Criteria

1. Clinically suspected patients with urinary tract infections not on Antibiotics attending the outpatient department of KPC Medical College and Hospital, Kolkata of all ages and gender.
2. Clinically suspected hospitalised patients with urinary tract infections not on antibiotics admitted in all wards and ICUs of KPC Medical College and Hospital, Kolkata of all ages and gender.
3. Urine Samples from both Catheterised and non-catheterised patients.

Exclusion Criteria

1. Clinically suspected patients with urinary tract infections but on Antibiotics within 72hrs prior to sample collection.

2. Patients with neurological dysfunction of urinary bladder (eg: spinal cord injury, multiple sclerosis)
3. Patients with anatomical deformities of the urinary tract (eg: urethral stricture)
4. Patients with vesicourethral reflex.
5. Patients with chronic renal failure.
6. Patients with renal TB, chlamydial infection, viral and parasitic urinary tract infection as it is beyond the scope of our study.

Materials and Methods

History taking: A detailed history (which includes age, gender, history of fever, dysuria, frequency of 83arnataka83n, antibiotic or diuretic usage, co-morbidities etc.) was taken and complete clinical examination was carried out for each case of clinically suspected UTI.

Sample collection and transport to lab: Samples were collected from clean catch midstream; in neonatal cases the samples were collected through suprapubic approach; in catheterised patients, the samples were collected under aseptic conditions using a syringe from the catheter port in a labelled universal sample container. Urine Catheter samples was not collected from the urobag. Discontinuation of all antibiotics 72 hr prior to urine collection for culture and sensitivity is a mandate. Urine samples from patients on antibiotics were excluded from the study. Urine samples were sent to the laboratory within 1-2hr and processed. If immediate transport was not possible, specimens were stored at 4 °C and processed within a maximum of 4 h to prevent bacterial overgrowth or degradation.

Microscopic examination: Microscopic examination of every urine sample to look for the presence of pus cells, RBCs, epithelial cells, casts, and crystals was done.

Culture and sensitivity testing: For isolation and identification, a standard loop of size 4mm was used to place 0.01 mL of urine for inoculation on MacConkey agar and UTI agar (Himedia) and the plates were incubated at 37°C for 24hr – 48 hr for growth of pathogens.

Contaminated samples were ruled out. If there were any multiple growths, the culture was repeated with fresh sample before acceptance of outcomes. The number of colonies were counted to quantify the organisms. Diagnosis of urinary tract infection for a single pathogen is defined based on significant colony count $\geq 10^5$ CFU/ mL for Gram negative and $\geq 10^4$ CFU/mL for Gram positive bacteria. In case of suprapubic puncture urine sample $\geq 10^3$ CFU/ml was considered significant.

Bacterial identification and Antimicrobial susceptibility testing (AST) was performed by automated methods. Gram staining and motility testing was

performed on the culture isolates. Automated methods like VITEK 2 instrument (BioMerieux, Durham, NC, USA) was used for bacterial identification and antibiotic susceptibility testing as per manufacturer’s guidelines Sensitivity against the following antibiotics;3rd Generation Cephalosporins (Ceftriaxone, Ceftazidime, Cefotaxime), Cephamycins (Cefepime, Cefoxitin), Beta-lactamase-Beta lactamase inhibitor combinations (Piperacillin/Tazobactam, Cefuroxime-sulbactam, Amoxicillin-Clavulanic Acid), Carbpenems (Meropenem, Imipenem), Aminoglycosides (Amikacin, Gentamicin), Fluoroquinolones (Ciprofloxacin), Tetracyclin, Trimethoprim/Sulfamethoxazole, Nitrofurantoin and Fosfomycin. The results were interpreted in accordance with CLSI 2024 guidelines. Standard Vittek strains of E. Coli (ATCC 25922), Staphylococcus aureus (ATCC 25912), and Pseudomonas aeruginosa (ATCC 27853) were used in this study as quality control.[12,13]

Statistical Analysis: Data collection and statistical analysis was performed using Microsoft excel version 2021 and SPSS software version 30.

Results

Out of the 5512 Urine samples of clinically suspected patients (both out-patient and admitted in the hospital premises) were received in the Department of Microbiology, KPC Medical College and Hospital, Kolkata, about 488 (8.85%) samples showed positive microbial growth with a significant colony count. Among the 488 patients with significant bacteriuria, females (60.86%) were more affected than the males(39.14%).Most susceptible age group susceptible to UTI was the elderly individuals above 60yrs (61.06%) followed by 46-60yr (13.93%) age bracket. It was observed that least number of laboratory confirmed UTI belonged in 0-15yr (0.82%) age category.

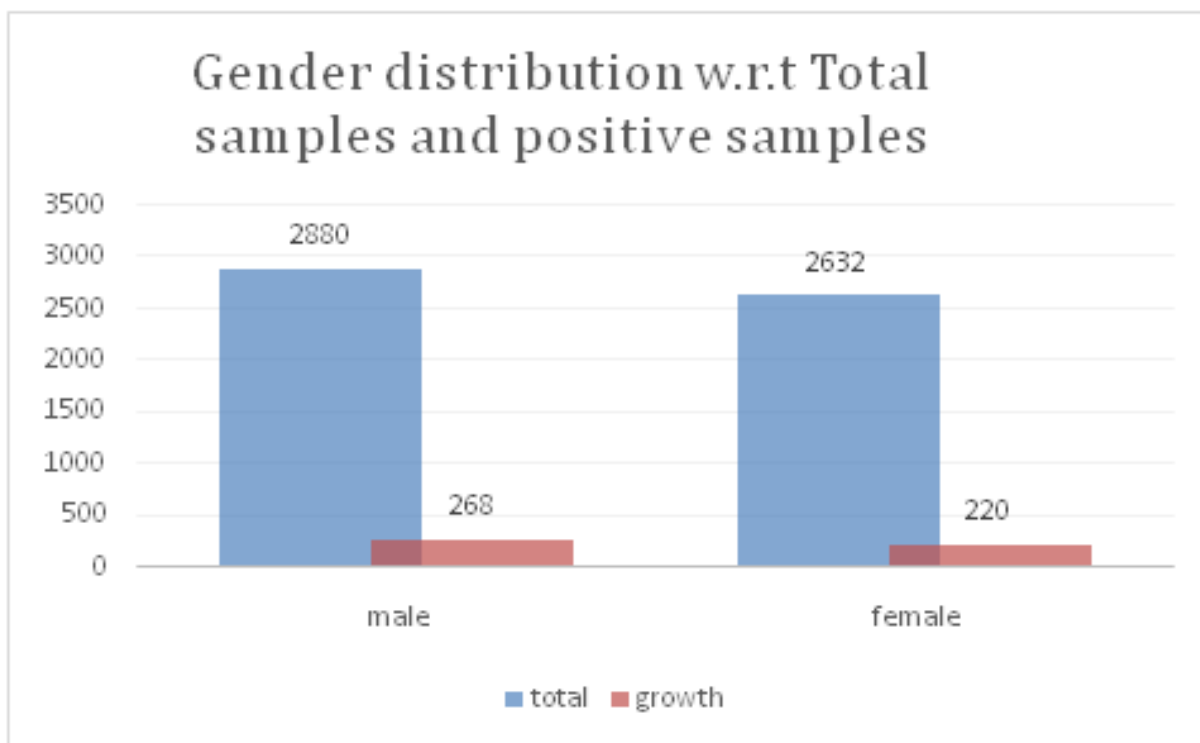


Figure 1: Gender distribution w.r.t Total and positive culture of urine samples

Table 1: Age distribution w.r.t. Total and urine culture positive samples

Age	1-15yrs	16-30yrs	31-45yrs	46-60yrs	>60yrs	Total
Growth	4 (0.82%)	56 (11.48%)	62 (12.71%)	68 (13.93%)	298 (61.06%)	488 (100%)
No Growth	144 (2.86%)	478 (9.52%)	689 (13.72%)	1224 (24.36%)	2489 (49.54%)	5024 (100%)
Total	148 (2.68%)	534 (9.68%)	751 (13.64%)	1292 (23.44%)	2787 (50.56%)	5512 (100%)

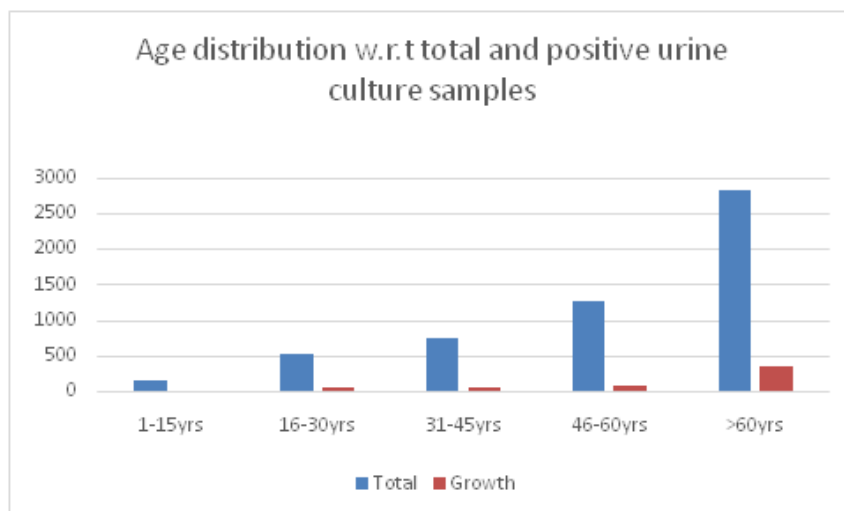


Figure 2: Age distribution of culture positive urine samples

Out of the 488 positive urine growth samples, 447 (91.60%) samples had Gram Negative etiology, 20 (4.09%) samples yielded growth of Gram Positive cocci and 21 (4.31%) samples showed growth of Candida species in the one year period analysed. Most common pathogen isolated was Escherichia coli (n=224, 45.90%) followed by Klebsiella sp (n=168, 34.50%) followed by Pseudomonas aeruginosa (n=24, 4.90%). Other Gram negative bacilli

isolated are Protea group (n=19, 3.90%), Acinetobacter species (n=8, 1.60%) and Enterobacter species (n=4, 0.080%).

In Protea group, Proteus mirabilis (n=9), Proteus vulgaris(n=6) and Morganellamorganni(n=4) were isolated. Staphylococcus aureus(n=8, 1.60%) and Enterococcus sp (n=12, 2.50%) were isolated among Gram positive cocci.

Table 2: Prevalence of Uropathogens isolated

Isolated Pathogens	No. of Pathogens	Percentage (n=488)
Escherichia coli	224	45.90%
Klebsiella sp	168	34.50%
Pseudomonas sp	24	4.90%
Protea group	19	3.90%
Acinetobacter sp	8	1.60%
Enterobacter sp	4	0.80%
Enterococcus sp	12	2.50%
Staphylococcus aureus	8	1.60%
Candida sp	21	4.30%
Total	488	100%

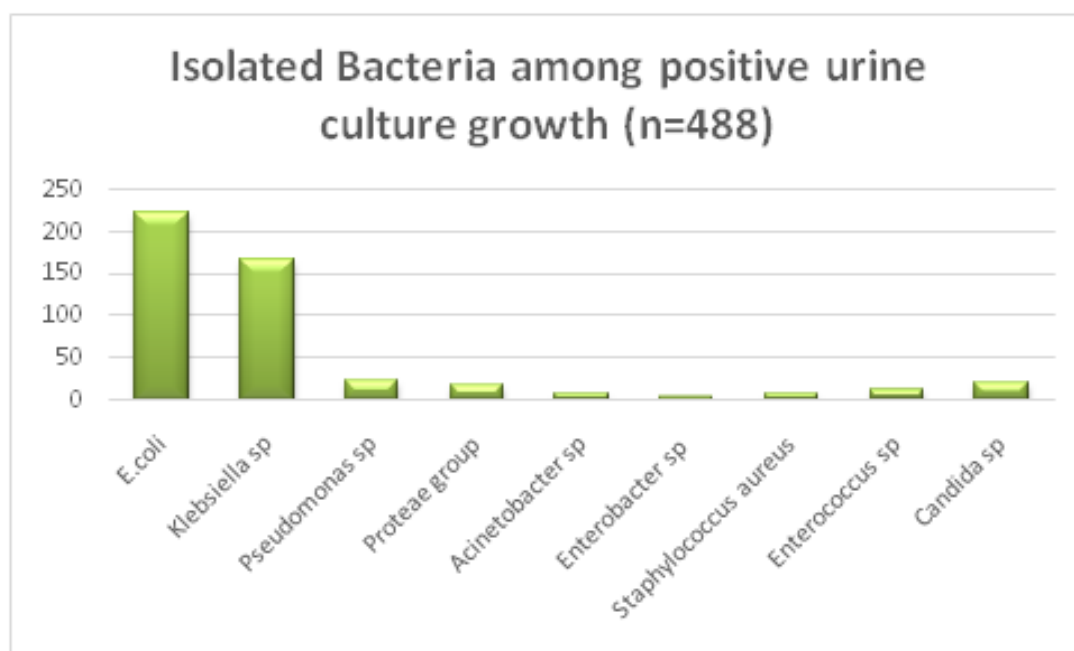


Figure 3: Graphical presentation of prevalence of Uropathogens

Table 3: Antibiotic sensitivity pattern of Gram Negative bacteria in percentages

	E.coli (n=224)	Klebsiella sp (n=168)	Pseudomonas sp (n=24)	Protea group (n=12)	Acinetobacter sp (n=8)	Enterobacter sp (n=4)	Total (n=447)
3 rd Gen Cephalosporins (CTX/CTR/CAZ)	16.07%	34.53%	29.17%	63.2%	25%	50%	26.17%
4 th Gen Cephalosporins (CPM/CX)	73.21%	66.67%	29.17%	31.57%	37.5%	25%	65.54%
BL-BLI (AMC/PTZ/CFS)	69.64%	35.72%	33.34%	52.63%	37.5%	25%	53.25%
Carbapenem (IMP/MRP)	63.31%	72.62%	91.67%	57.89%	25%	100%	70.25%
Aminoglycosides (AMK/GEN)	80.81%	72.62%	62.50%	63.16%	25%	75%	74.94%
Fluroquinolones (CIP/LE)	75%	61.91%	62.50%	57.89%	37.5%	50%	67.78%
Tetracycline	90.41%	78.57%	NT	NT	75%	75%	77.85%
Nitrofurantoin	84.82%	85.72%	70.83%	NT	62.5%	25%	79.86%
Fosfomycin	87.50%	90.47%	NT	NT	NT	NT	87.02%
Cotrimoxazole	85.71%	78.57%	NT	36.84%	62.5%	50%	75.61%
Tigecycline	90.62%	85.72%	87.50%	NT	62.5%	25%	83.67%

*NT- not tested. [CTX – Cefotaxime, CTR – Ceftriaxone, CAZ – Ceftazidime, CPM – Cefepime, CX – Cefoxitin, AMC – Amoxicillin–Clavulanic acid, PTZ – Piperacillin–Tazobactam, CFS – Cefoperazone–Sulbactam, IMP – Imipenem, MRP – Meropenem, AMK – Amikacin, GEN – Gentamicin, CIP – Ciprofloxacin, LE – Levofloxacin]

Antibiotic sensitivity pattern of all Gram Negative bacteria isolated from urine is shown in Table 3. Clustered graphical representation of Antibiotic sensitivity pattern of all Gram negative bacteria (n=447), most common Lactose fermenter isolated (*Escherichia coli*, n=224) and most common non-lactose fermenter isolated (*Pseudomonas aeruginosa*, n=24) is shown in Fig 4. Most GNB are sensitive to Nitrofurantoin (79.86%), Tetracy-

cline(77.85%), Cotrimoxazole (75.61%), Aminoglycosides (74.94%) and Carbapenems (70.25%). *E.coli* and *Klebsiella* sp were highly susceptible to Fosfomycin by 87.50% and 90.47% respectively. We observed that in our study 83.93% of *E.coli* and 65.47% of *Klebsiella* species, 70.83% of *Pseudomonas aeruginosa* were ESBL producers. Carbapenemase production was however lower in the above organisms. 31.69% of *E.coli*, 24.38% of

Klebsiella sp and 8.33% of *Pseudomonas aeruginosa* were Carbapenem resistant.

Discussion

UTI is a very common bacterial infection affecting in a large number of patients, with females being more susceptible than males. The increased risk in females is due to anatomical attributes (such as a short urethra, close proximity to anus etc) and physiological attributes like altered pH, pregnancy, sexual practices, changes in urine osmolarity etc. Several studies have reported a frequency in females compared to males [14,15,16]. Consistent with other researches, our study also found a higher prevalence of UTI in females (60.86%) than in males (39.14%). Among females, those in the reproductive age group of 16-45yrs showed a higher incidence of UTIs (34.35%, 102 out of 297) which aligns with findings from other studies like Maheswary et al (21-35 yrs, 31.05%)[17] and Dash et al(18-37yrs, 29.2%)[18]. However, in the elderly age group males were more commonly affected. Our study revealed that elderly males (>60 y) had a higher incidence of UTI (72.78%, 139 out of 191). These findings were supported by studies [11, 15]. The higher UTI incidence in elderly males may be attributed to the greater prevalence of benign prostate enlargement and neurogenic bladder [18].

The prevalence of bacterial isolates causing UTI and their antibiogram varies in different geographical areas. In our study, the prevalence of UTI was 8.85% (488 culture positive out of 5512 urine samples) which is in concordance with other studies like (Mohapatra et al and Jelly P et al)[10, 20]. Few other studies in India noted a prevalence 37.3% (Sharma et al) and 79.9% (Bhargava et al) due to regional disparities[16, 21].

Gram Negative bacteriuria (91.6%) was more common than UTI caused by Gram Positive bacteria (4.10%) and Yeast (4.30%) which is mirrors studies of Mohapatra et al and Bhargava et al [10,16]. *Candida* growth was 4.30% which was similar to studies of Manjunath et al (4.5%) and Venkatesh et al (6%)[22,23]. *Escherichia coli* was the most common isolate (45.90%) which matches findings of Bhargava et al (54.95%), Huang et al,2022, (43.5%), Ali et al 2022 (49.3%), Jagdeeshan et al (2022, 41.9%) [16,24,25,26]. Second most common isolate was *Klebsiella pneumoniae* (34.50%) which aligns studies of Jalil et al (31.7%) and Guiliano et

al(22.8%)[27,28]. Several studies have reported lesser prevalence of *Klebsiella* Mukherjee et al (10%), Bhargawa et al (6.6%) [16,29].

Malini A et al reported *Pseudomonas aeruginosa* was the most common Non fermentor isolate (53.8%), followed by *Acinetobacter baumannii* (22.2%)[30]. Other studies done by Shobha KL et al., (80.84%) and Somily A et al.,(73.6%) also reported *Pseudomonas* spp.to be the most common non-fermenter to cause UTI[31,32].

Antibiotic Susceptibility testing of GNB showed highest sensitivity with Tigecyclin (83.67%), followed by Nitrofurantoin (79.86%), Tetracyclin (77.85%), Cotrimoxazole (75.61%), Aminoglycosides (74.94%) and Carbapenems (70.25%).

Bouchillon et al., reported >90% sensitivity against MDR GNB including ESBL-producing *E. coli* and *Klebsiella*[33]. Fosfomycin was highly sensitive for *E.coli* (87.50%), *Klebsiella* sp (90.47%) and *Pseudomonas aeruginosa* (87.50%). Similar findings were documented in studies of Karlowsky et al and Sharma et al [34,35]

Increased resistance was noted by all Gram negative bacilli to lower antibiotics like 3rd Generation antibiotics (Cefotaxime, Ceftriaxone and Ceftazidime; 73.34%). In this study we observe that resistance in other groups of Antibiotics like 4th Generation Cephalosporins (34.46%), Beta-lactam- Beta-lactam inhibitor combinations (46.74%) and Fluoroquinolones (32.22%) are on a rise[35,36,37]. Inappropriate usage of Antibiotics like Overuse, Misuse, over the counter supply and using antibiotics in agriculture, fishing and poultry industries have contributed to rise in resistance to Antibiotics. Studies in India have identified key gaps in antibiotic stewardship and proposed improvements through narrative reviews[38]. Research also shows high rates of self-medication and irrational antibiotic use across various low and middle income countries, including Europe, Asia, Africa, and the Americas. For instance, in Saudi Arabia, 78.7% of patients reported antibiotic use without prescription[39]. Antibiotic resistance continues to pose serious challenges globally and highlights the urgent need for better control measures and public health strategies[40].

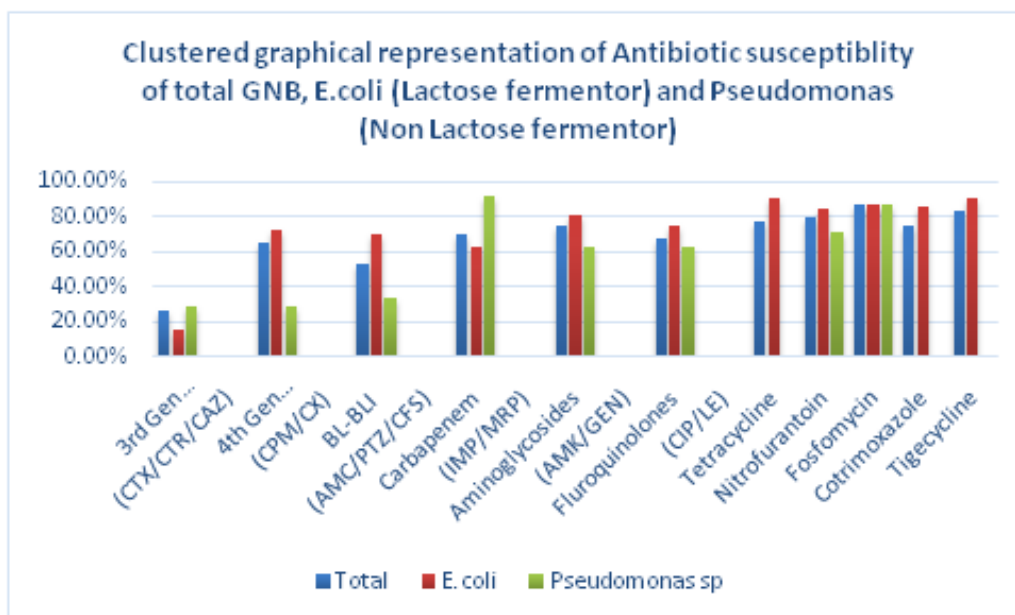


Figure 4: Clustered graphical representation of Antibiotic sensitivity of all GNB (n=447), Lactose fermentor Echerichia coli (n=224) and Non -Lactosefermentor Pseudomonas aeruginosa (n=24)

Conclusion

UTIs have become increasingly problematic and are a major public health concern owing to antibiotic-resistant pathogens.

As per Infectious Diseases Society of America, empirical antibiotic therapy for UTIs should be guided by regional susceptibility patterns, drug availability, and patient clinical background[41]. The study was helpful for giving an idea on Gram Negative pathogens implicated in UTIs and empirical therapy to be used when suspecting UTI.

Fosfomycin, Nitrofurantoin, Cotrimoxazole, Aminoglycosides are better to treat UTI empirically. Individuals should be made aware of the importance of hygiene practices. Health education and good Infection control practices can help in decreasing Urinary infection rates.

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