

**Nitrofurantoin susceptibility profile versus other antibiotics tested in uropathogens- a retrospective study from India.**

**RUNNING TITLE- Nitrofurantoin susceptibility amongst inpatient uropathogens.**

**KEYWORDS: Nitrofurantoin, Antimicrobial drug resistance, Urinary Tract Infections.**

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## **ABSTRACT**

### **Introduction**

Urinary Tract Infection (UTI) is one of the most common bacterial infections encountered by clinicians worldwide. The emergence of multidrug-resistant uropathogens necessitates a review of their susceptibility profiles. This study aims to assess the susceptibility trends of uropathogens to a panel of drugs, with special emphasis on Nitrofurantoin (NFT).

### **Methods**

A retrospective analysis was conducted on 2,099 mid-stream clean catch urine samples processed by standard microbiological methods. Clinical and Laboratory Standards Institute (CLSI) guidelines (2019) were followed. Statistical analysis was performed.

### **Results**

Out of all samples, 212 were culture positive. *Escherichia coli* (34.9%) and *Enterococcus* spp. (15.1%) were the most common Gram-negative and Gram-positive organisms, respectively. Gram-negative isolates were most susceptible to Colistin (97.38%), followed by NFT (69.35%). Gram-positive uropathogens were most sensitive to Linezolid (100%), followed by Vancomycin and NFT, each with 92.45% susceptibility.

## **Conclusion**

The increase in antibiotic resistance among various uropathogens underscores the need for surveillance data to inform the appropriate selection of antibiotics. Our study highlights that, among the panel of antibiotics tested, NFT appears to be a viable alternative for treating multidrug-resistant uropathogens.

## **INTRODUCTION**

Urinary Tract Infection (UTI) is one of the most common bacterial infections encountered by clinicians worldwide, significantly contributing to the workload in clinical microbiology laboratories (1,2).

UTI is a general term referring to infection or inflammation of any part of the urinary tract (1). It is divided into two types based on the site of involvement: upper UTI and lower UTI . The prevalence of UTI varies depending on age, sex, catheterization, hospitalization, and previous exposure to antimicrobials (4 The overall prevalence of UTI ranges from 8.7% to 90.1% (5). UTI is more common in females than in males due to factors such as sexual activity, hormonal changes, a shorter urethra, and the proximity of the urethral orifice to the anus (3,6).

UTIs are most often caused by bacteria but can also involve fungal, viral, and parasitic pathogens. Gram-negative bacteria cause 90% of UTI cases, while Gram-positive bacteria

account for only 10%. *Escherichia coli* (70-95%) is noted as the predominant uropathogen (7).

The increase in multidrug-resistant (MDR) organisms has become an alarming situation globally, including in India. A study from South India found an overall prevalence of MDR organisms at 54% (8). Another study from India highlighted that 23% of uropathogenic *E. coli* were ESBL producers, and around 12% were MDR uropathogenic *E. coli* (9). A study from eastern North India reported that 96% of the isolated uropathogens were MDR organisms, with 40.4% of *E. coli* isolates being ESBL producers (10). Commonly used oral antibiotics like cotrimoxazole and fluoroquinolones are no longer effective, especially in inpatient settings (11). Moreover, higher-end antibiotics such as third-generation cephalosporins and carbapenems are losing effectiveness due to the rapid emergence of Extended Spectrum Beta-Lactamases (ESBLs) and carbapenemases, respectively (11,12). Furthermore, the development of novel antibiotics is lagging. This situation has necessitated revisiting age-old antimicrobials such as nitrofurantoin for their activity against these multidrug-resistant uropathogens (3,13).

Nitrofurantoin is an example of an oral antibiotic that was approved by the Food and Drug Administration (FDA) in 1953 for the treatment of lower UTIs. It was widely used for uncomplicated UTIs until the 1970s when trimethoprim-sulfamethoxazole and newer beta-lactam antibiotics became available (14). Due to the paucity of data regarding effective drugs for MDR uropathogens, this study was undertaken to assess the susceptibility trends of all uropathogens isolated to a panel of drugs, with special reference to nitrofurantoin.

## **MATERIALS AND METHODS**

This retrospective study was conducted in the Department of Microbiology at a tertiary care hospital in New Delhi, India. A convenient sampling method was used, including 2,099 urine

samples received over six months from December 2019 to May 2020. Culture sensitivity testing was performed as part of routine diagnostics during patients' inpatient stays, for which patient consent was not required. All data from test requisition forms were recorded in laboratory registers and maintained in desktop records, from which it was analyzed.

### **Ethical approval and consent**

Since this was a retrospective analysis, departmental-level approval was obtained from the bacteriology section. According to institutional policy, departmental ethical clearance suffices for retrospective studies; hence, both departmental and sectional clearances were obtained. Every effort was made to ensure patient confidentiality during data collection. All available measures were taken to maintain confidentiality related to patient details while compiling data for the study.

The study included urine samples from adult inpatients (aged >18 years) of both sexes. Urine samples from outpatient attendees and individuals under 18 years were excluded from the study.

### **Specimen collection and processing**

Midstream urine samples were collected in wide-mouth, leak-proof, screw-capped sterile universal containers with properly filled test requisition forms, duly signed by the referring doctor (15). Samples were processed within 2-4 hours of collection for aerobic culture and sensitivity testing. Uropathogens were isolated on Blood agar and MacConkey agar using a 0.01 mm calibrated loop for the semi-quantitative method. The plates were incubated overnight at 37°C and observed for discrete growth for characterization and identification of the pathogen. Colony counts  $>10^5$  colony-forming units (CFU)/ml in patients with no risk factors were considered significant, while in symptomatic patients (where history was provided in test requisition forms), colony counts of  $>10^3$  CFU/ml were considered

significant (15). Bacterial identification was performed using colony characteristics, Gram staining, catalase, oxidase, coagulase production, and various biochemical tests, including Triple Sugar Iron, indole, citrate utilization, urea hydrolysis, sulfide-indole motility (SIM) medium, mannitol salt agar, DNase, and bile esculin hydrolysis (16).

### **Antibiotic susceptibility testing**

Antibiotic susceptibility testing was performed on each isolated bacterium using the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines. Bacterial suspensions were prepared by emulsifying 3–5 pure colonies in nutrient broth and adjusted to 0.5 McFarland standards. A sterile cotton swab was then dipped into the suspension and swabbed onto the surface of Mueller-Hinton agar plates. Standard antibiotic discs were placed aseptically, and the inoculated Mueller-Hinton agar plates were incubated at 37 °C for 16–18 hours. The diameters of the zones of complete inhibition were measured using a ruler and reported as sensitive, intermediate, or resistant according to CLSI 2019.

All antibiotic discs were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra: Nitrofurantoin (300 µg), Amikacin (30 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Ampicillin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Cefoxitin (30 µg), Piperacillin/Tazobactam (100/10 µg), Ceftazidime (30 µg), Amoxiclav (20/10 µg), Cefuroxime (30 µg), Colistin (10 µg), Nalidixic acid (30 µg), Vancomycin (30 µg), Linezolid (30 µg), Clindamycin (2 µg), Erythromycin (15 µg), and Penicillin (10 U).

### **Quality control and Quality assurance**

Standard Operating Procedures (SOPs) were strictly followed, ensuring that culture media and antibiotic disks met expiration date and quality control parameters. Quality control was

performed according to SOPs. Each new lot was checked with the reference strains: *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *P. aeruginosa* (ATCC 27853) (17).

### **Statistical analysis**

SPSS software version 21.0 was used for data analysis. A two-tailed Chi-square test was used to compare categorical variables. A p-value <0.05 was considered significant.

## **RESULTS**

Of the total 2,099 samples, 2,049 (97.6%) were received from females and 50 (2.4%) from males. Out of all samples, 212 (10.1%) were culture positive. Among the 212 culture-positive samples, 16 (0.8%) were from males and 196 (9.3%) were from females (p-value < .00001, significant).

Gram-negative organisms accounted for 159 (75%) of the isolates, outnumbering Gram-positive organisms, which accounted for 53 (25%). The most commonly isolated uropathogen was *Escherichia coli* with 74 isolates (34.9%), followed by *Klebsiella pneumoniae* with 41 isolates (19.4%), *Acinetobacter* spp. with 14 isolates (6.6%), *Pseudomonas aeruginosa* with 10 isolates (4.7%), *Enterobacter* spp. and *Proteus mirabilis* with 6 isolates each (2.8%), *Klebsiella oxytoca* with 5 isolates (2.4%), and *Citrobacter* spp. with 3 isolates (1.4%) as depicted in Table 1.

**Table 1.** Distribution of uropathogens isolated (n=212)

Organism	Number of isolates	Percentage (%)
<b>Gramnegative organisms - 159 (75%)</b>		
<i>Escherichia coli</i>	74	34.9
<i>Klebsiellapneumoniae</i>	41	19.4
<i>Acinetobacter spp.</i>	14	6.6

<i>Pseudomonas aeruginosa</i>	10	4.7
<i>Enterobacterspp.</i>	6	2.8
<i>Proteus mirabilis</i>	6	2.8
<i>Klebsiellaoxytoca</i>	5	2.4
<i>Citrobacterspp.</i>	3	1.4
<b>Grampositive organisms- 53 (25%)</b>		
<i>Enterococcus spp.</i>	32	15.1
<i>Staphylococcus aureus</i>	16	7.5
Coagulase negative <i>Staphylococcus</i> (CoNS)	5	2.4
Total	212	100

Gram-negative isolates were most susceptible to Colistin (97.38%), followed by Nitrofurantoin (69.35%), Netilmicin (55.17%), Imipenem (52.83%), and Amikacin (50.94%). Gram-positive uropathogens were most sensitive to Linezolid (100%), followed by Vancomycin and Nitrofurantoin, each with 92.45% sensitivity.

**Table-2** Antimicrobial susceptibility profile of uropathogens.

Name of the drug	Sensitivity n (%)	Resistance n (%)	Total isolates tested
<b>Gram negative organisms</b>			
Amoxi-clav	30 (22.22)	105 (77.78)	135

<b>Nitrofurantoin</b>	<b>86 (69.35)</b>	<b>38 (30.65)</b>	<b>124</b>
Piperacillin-Tazobactam	40 (25.15)	119 (74.85)	159
Ceftriaxone/cefotaxime	12 (8.88)	123 (91.12)	135
Cefuroxime	9 (6.66)	126 (93.34)	135
Imipenem	84 (52.83)	75 (47.17)	159
Ertapenem	33 (25.58)	96 (74.42)	129
Amikacin	81 (50.94)	78 (49.06)	159
Netilmicin	80 (55.17)	65 (44.83)	145
Ciprofloxacin	27 (18.62)	118 (81.38)	145
Nalidixic Acid	24 (17.77)	111 (82.23)	135
Cotrimoxazole	33 (22.15)	116 (77.85)	149
Colistin	149 (97.38)	4 (2.62)	153
<b>Gram positive organisms</b>			
<b>Nitrofurantoin</b>	<b>49 (92.45)</b>	<b>4 (7.55)</b>	<b>53</b>
Ciprofloxacin	19 (35.85)	34 (64.15)	53
Vancomycin	49 (92.45)	4 (7.55)	53
Linezolid	53 (100)	0	53
Penicillin	0	21 (100)	21
Gentamicin Low dose <sup>a</sup>	14 (66.67)	7 (33.33)	21
High dose gentamicin <sup>b</sup>	17 (53.12)	15 (46.88)	32
Cotrimoxazole <sup>a</sup>	8 (38.09)	13 (61.91)	21
Ampicillin	23 (43.39)	30 (56.61)	53
Cefoxitin <sup>a</sup>	6 (28.57)	15 (71.43)	21

<sup>a</sup>only for *Staphylococcus* spp.



<sup>b</sup> only for *Enterococcus* spp.

*Citrobacter* spp. was 100% sensitive to Nitrofurantoin, followed by *E. coli*, *K. pneumoniae*, and *K. oxytoca* with 90.5%, 36.5%, and 20% sensitivity, respectively. *Staphylococcus aureus* and CoNS were 100% sensitive to Nitrofurantoin, while *Enterococcus* spp. showed 87.5% sensitivity.

<b>Antibiotics</b>	<b><i>E.coli</i> (n=74)</b>	<b><i>K. pneumoniae</i> (n=41)</b>	<b><i>Acinetobacter</i> spp. (n=14)</b>	<b><i>P. aeruginosa</i> (n=10)</b>	<b><i>Enterobacter</i> spp. (n=6)</b>	<b><i>Proteus mirabilis</i> (n=6)</b>	<b><i>K. oxytoca</i> (n=5)</b>	<b><i>Citrobac ter</i> spp. (n=3)</b>	<b><i>Enterococ cus</i> spp. (n=32)</b>	<b><i>Staphyloco ccus aureus</i> (n=16)</b>	<b>Coagulase negative <i>Staphylococcus</i> spp. (CoNS) (n=5)</b>
Nitrofurantoin	90.5%	36.5%	NA <sup>b</sup>	NA	16.6%	IR <sup>a</sup>	20%	100%	87.5%	100%	100%

**Table 3.** Susceptibility profile of uropathogens to nitrofurantoin.

<sup>a</sup>-IR-intrinsic resistance (according to CLSI 2019) (17)

<sup>b</sup>-NA-Not Applicable (according to CLSI 2019)(17)



## DISCUSSION

This study provides important information regarding antimicrobial susceptibility trends in both Gram-negative and Gram-positive uropathogens. Our findings may serve as a foundation for exploring the administration of Nitrofurantoin (NFT) among multidrug-resistant uropathogens.

A predominance of female patients was observed, similar to studies conducted by Akhter et al. and Naik et al. (19, 20). Since the majority of samples were from females, the culture-positive cases were also higher in females, at 9.3%, consistent with the findings of Akhter et al. (19). The high prevalence of UTI in females of reproductive age is attributed to the proximity of the urethral meatus to the anus, a shorter urethra, sexual intercourse, incontinence, and poor toilet hygiene (19, 20).

Out of 2,099 samples received, 212 (10.1%) were culture-positive, which aligns with other studies by Kasew et al., Nahar et al., and Tesfa et al. (21, 22, 23). Differences in prevalence could be due to varied geographical distribution, length of study, sample size, and seasonal variation (23).

The study reinforces the well-known fact that *E. coli* is the leading uropathogen, comparable to other studies by Derby et al. and Kasew et al. (21, 24). We observed high resistance among Gram-negative uropathogens towards ciprofloxacin, ceftriaxone, and cotrimoxazole, which are commonly used antibiotics for UTI treatment. These results were comparable to those of Goyal et al. (3). Gram-negative uropathogens remained predominantly sensitive to Netilmicin, Imipenem, and Amikacin, which are parenterally administered and reserved for inpatient use. Among Gram-negative uropathogens, NFT, an age-old oral drug, showed good sensitivity, similar to the results seen by Goyal et al. (3).

All Gram-positive isolates were sensitive to Linezolid, whereas Vancomycin and Nitrofurantoin each showed 92.45% sensitivity. Goyal et al. (3) found contrasting results with maximum sensitivity to Nitrofurantoin (76%), followed by Linezolid (69%) and Vancomycin (58%). Additionally, we highlighted that *Staphylococcus aureus* and CoNS were 100% sensitive to Nitrofurantoin, while *Enterococcus* spp. showed 87.5% sensitivity.

The majority of *E. coli* isolates were sensitive to Nitrofurantoin at 90.5%, which is consistent with findings by Goyal et al., Gautam et al., and Neelima A et al. (3, 11, 12). We found *K. pneumoniae* isolates to be 36.5% sensitive to Nitrofurantoin, similar to results obtained by Goyal et al. and Gautam et al. (3, 11).

The most commonly isolated Gram-positive uropathogen was *Enterococcus* spp. at 15.1%, comparable to results seen by Akhtar et al. (19). We reported that out of the total VRE isolates, 40% were sensitive to Nitrofurantoin (2 out of 5 isolates). The incidence of VRE in the current study is 2.34%, comparable to that obtained by Meena et al. (2.7%) (13).

Due to the retrospective nature of the study, crucial data and result findings cannot be correlated with the clinical diagnosis, associated signs, symptoms, and comorbidities. However, clinicians have been informed about the summary findings of the drugs, which may be beneficial in future probable cases of UTI.

## CONCLUSION

The escalating challenge of antibiotic resistance among uropathogens necessitates vigilant surveillance to guide effective treatment strategies. This study highlighted that Gram-negative uropathogens, led by *Escherichia coli*, predominated among isolates, particularly affecting female patients. Our findings underscored the efficacy of nitrofurantoin as a valuable option against multidrug-resistant Gram-negative bacteria, positioning it favorably compared to traditional therapies like carbapenems and aminoglycosides. Notably, Gram-positive uropathogens, including *Staphylococcus aureus* and Coagulase-negative *Staphylococci*, exhibited high susceptibility to nitrofurantoin, suggesting its potential for broader clinical use.

Furthermore, our identification of 40% sensitivity among vancomycin-resistant enterococci (VRE) to nitrofurantoin prompts reconsideration of its role in treating challenging infections. However, prudent stewardship of nitrofurantoin is crucial to mitigate resistance emergence and preserve its efficacy. Future research should explore expanded use of nitrofurantoin in the treatment of multidrug-resistant urinary tract infections, emphasizing species-specific identification and susceptibility testing to optimize therapeutic outcomes.

### Authors contribution statement

**Study conception and design:** Dr. Bhawna sharma, Dr. Anuja Gupta

**Data collection:** Dr. Anuja Gupta

**Analysis and interpretation of results:** Dr. Anuja Gupta, Dr. Bhawna Sharma

**Draft manuscript preparation:** Dr. Anuja Gupta, Dr. Bhawna Sharma

All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

**Ethics statement:** This retrospective analysis received departmental-level approval from the bacteriology section of the institution. In accordance with institutional policy, departmental ethical clearance suffices for retrospective studies; therefore, both departmental and sectional clearances were obtained. Throughout the study, rigorous efforts were undertaken to safeguard patient confidentiality. All necessary measures were implemented to uphold the confidentiality of patient information during data collection and compilation.

**Conflicts of interest:** There are no conflicts of interest and no details of population has been disclosed to anywhere in the study.

**Funding:** No external funding was required as urine sample processing is a part of routine investigations in inpatient admissions.

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