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Minimum inhibitory concentration creep in *Enterobacterales* – A rising concern

Soumyya Mondal[1], Anwesha Mandal[2], Sipra Saha[3], Partha Sarathi Chakrabarty[4], Debalina Das[5]

[1,2]3rd Year Postgraduate Trainee, [3,5]Assistant Professor, [4]Associate Professor, Department of Microbiology, College of Medicine and Sagore Dutta Hospital, Kamarhati, West Bengal, India

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ABSTRACT

Background: The irrational use of antibiotics has led to the emergence of multidrug-resistant pathogens. The phenomenon of minimum inhibitory concentration (MIC) creeps occurs when organisms start showing raised MIC but within susceptible range giving an indication of the prevalence of rise in resistant pathogens in an area. **Aims and Objectives:** The aims and objectives of the study are to observe the susceptibility pattern among uropathogens and the possibility of MIC creeps among the *Escherichia coli* isolates sensitive to nitrofurantoin. **Materials and Methods:** This retrospective study was done in a tertiary care hospital in Kolkata to observe the susceptibility pattern among uropathogens and the possibility of MIC creeps. We took the data from all inpatient department and outpatient department patients sent for urine culture and sensitivity from October 1st, 2022, to September 30th, 2023. The antimicrobial susceptibility testing and MIC were conducted by VITEK Compact 2. The MIC of nitrofurantoin, one of the most widely used antibiotics for lower urinary tract infections, was calculated to investigate the phenomenon of MIC creep. All the statistical analyses were carried out using the Excel spreadsheet and OpenEpi version 3.01 platform. **Results:** In our study, a total of 631 urine samples were analyzed: 368 were positive with the most common isolate being *E. coli* (53%) followed by *Klebsiella* spp. (12%). Two percent of resistance was observed for fosfomycin and 8% for nitrofurantoin. Extended spectrum beta-lactamase (ESBL) producers *E. coli* was 127 (35%). Overall, 23/368 samples had a MIC ≥128 for nitrofurantoin. Among the ESBL producers, 13/127 had MIC ≥128 for nitrofurantoin. **Conclusion:** In the present study, it was observed that *E. coli* showed a reduced susceptibility for nitrofurantoin indicated by a creeping increase in MIC within normal range. Trends in rising MIC can be an alarming sign for using drugs such as nitrofurantoin judiciously. Antimicrobial stewardship practice should be strongly implemented in hospitals to curb rising resistance and for better prognosis of patients having infectious diseases.

Key words: Minimum inhibitory concentration creep; Nitrofurantoin; Enterobacterales, Extended spectrum beta lactamase

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INTRODUCTION

Urinary tract infection (UTI) is a very common disease encountered in clinical practice, affecting around 150 million people every year.[1] Overuse of antibiotics in many developing countries coupled with poor infection prevention practices is the main cause of multidrug resistance (MDR) among pathogens.[2] Rising antibiotic resistance among microorganisms needs a continuous monitoring system of the susceptibility patterns[1] Among the known bacterial pathogens causing UTI, uropathogenic *Escherichia coli* is the most common organism[3,4] followed by *Klebsiella* spp., *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus* species, and *Pseudomonas aeruginosa*. Despite increasing antimicrobial resistance, nitrofurantoin is considered one of the first-line agents for the treatment of uncomplicated lower UTIs.[4]

In this cross-sectional study, we investigate the rising resistance in *E. coli* to predict future resistance to nitrofurantoin. The susceptibility patterns of all the

Address for Correspondence:
Dr. Debalina Das, Assistant Professor, Department of Microbiology, College of Medicine and Sagore Dutta Hospital, Kamarhati, West Bengal, India. **Mobile:** +91-9433563885. **Email:** drdebalinadas63885@gmail.com

uropathogens isolated were observed along with minimum inhibitory concentrations (MIC) for *E. coli*.

Aims and objectives

The aims and objectives of the study are to observe the susceptibility pattern among uropathogens and the possibility of MIC creeps among the Escherichia coli isolates sensitive to nitrofurantoin.

MATERIAL AND METHODS

This retrospective study was conducted for a period of 1 month from January 1st, 2024, to January 31st, 2024 after obtaining permission from the Institutional Ethics Committee. We took the data from all inpatient department and outpatient department patients sent for urine culture and sensitivity from October 1st, 2022 to September 30th, 2023 in a tertiary care hospital in Kolkata, West Bengal. A total number of 6100 urine samples were processed in the laboratory for the identification of urinary tract pathogens from October 1st, 2022, to September 30th, 2023. Clean catch mid-stream urine samples were collected from suspected UTI patients. Then, routine microscopy and culture of those samples were done in 5% sheep blood agar and MacConkey agar.

The identification of bacterial pathogens with significant bacteriuria and their antimicrobial susceptibility testing (AST) with MIC readings were conducted by VITEK 2 Compact.[5] The identification of extended-spectrum beta-lactamase (ESBL) producers among *E. coli* was identified with VITEK Compact 2 AST cards, additionally by a double disk diffusion test. Retrospective data were extracted as an Excel sheet from the VITEK automated ID AST system. The statistical analyses were carried out using the Excel spreadsheet and OpenEpi version 3.01 platform. The descriptive data were expressed as mean and categorical data were expressed as ratio, proportion, and percentage. $P<0.05$ is considered significant.

Inclusion criteria

Data from all bacterial pathogens with significant bacteriuria isolated from urine culture from October 1st, 2022, to September 30th, 2023, were included in this study.

Exclusion criteria

Samples with mixed growth and pathogens other than bacteria isolated from urine culture were excluded from this study.

RESULTS

In our study, a total of 6100 urine samples of clinically suspected UTI cases were analyzed over a period of 1 year. Out of 6100 urine samples, only 631 (1%) urine samples showed significant bacteriuria and the remaining 5469 (99%) samples were either insignificant growth, contamination, or were sterile. Among these 631 growth that were processed, 542 (86%) were from females and 89 (14%) from male patients.

Out of the total 631 uropathogens, the most common isolate was *E. coli* (n=368, 58%) followed by *Klebsiella* spp. (n=81, 12%). Among the Gram-positive organisms, *Enterococcus* spp. (n=58, 9%) was the most common. Other organisms such as *Staphylococcus*, *P. aeruginosa*, *Enterobactor* spp, *Proteus* spp., and *Staphylococcus saprophyticus* were also isolated. Some occasionally found organisms were *Morganella* spp, *Acinetobacter* spp, and *Citrobacter* spp. The pattern of isolation of these organisms is shown in Figure 1.

The resistance pattern of the isolated *E. coli* in the urine samples is shown in Figure 2. The highest percentage of resistance was observed for ampicillin (85%) followed by amoxicillin-clavulanic acid (50%). Two percent of resistance was observed for fosfomycin and 8% for nitrofurantoin.

Out of 368 *E. coli* isolated in these, 127 (35%) were ESBL producers and 97 (26%) were carbapenemase-producing *E. coli* (Figure 3).

We found a significant correlation between ESBL production and nitrofurantoin resistance in *E. coli*. Among 241 ESBL non-producer *E. coli,* only 10 isolates were found to have nitrofurantoin MIC >128. Out of 127 ESBL *E. coli,* 13 samples were found to have nitrofurantoin MIC >128 (uncorrected Chi-square value 4.563, P=0.01634) (Table 1).

There is an increase in the mean MIC of nitrofurantoin for *E. coli* from December 2022 to April 2023, and there is again a rise in the mean MIC of nitrofurantoin from July 2023 to October 2023 (Figure 4).[6]

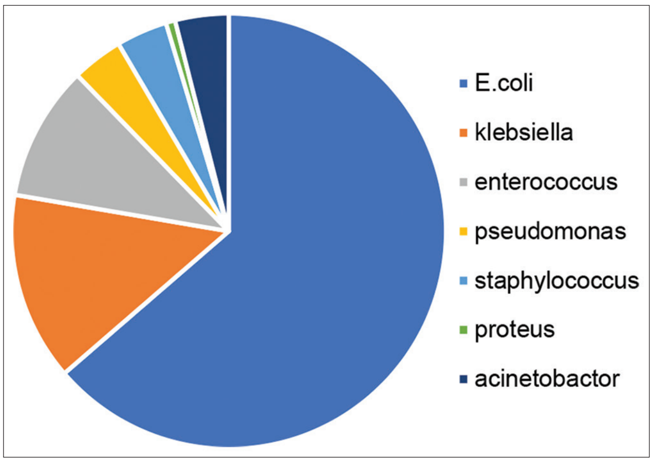

Figure 1: Distribution of uropathogens in the urine samples

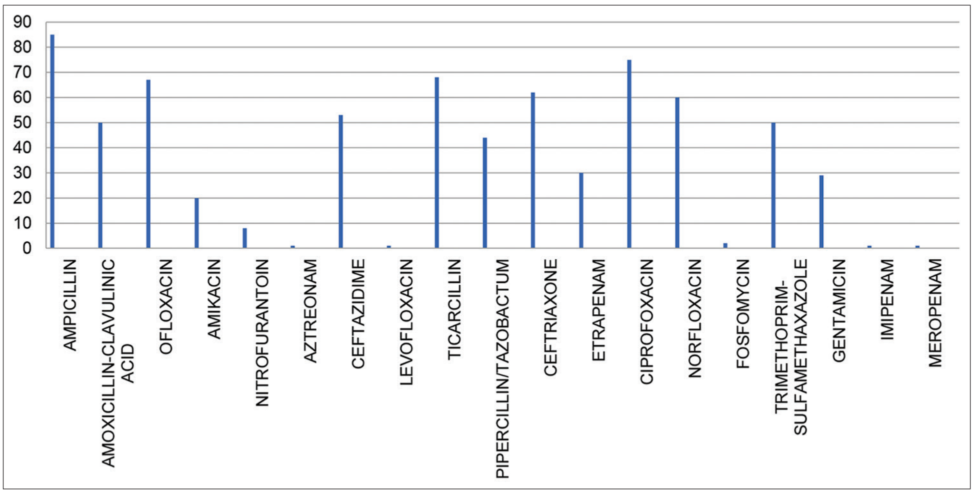

Figure 2: Resistance pattern of *Escherichia coli* isolated from urine samples

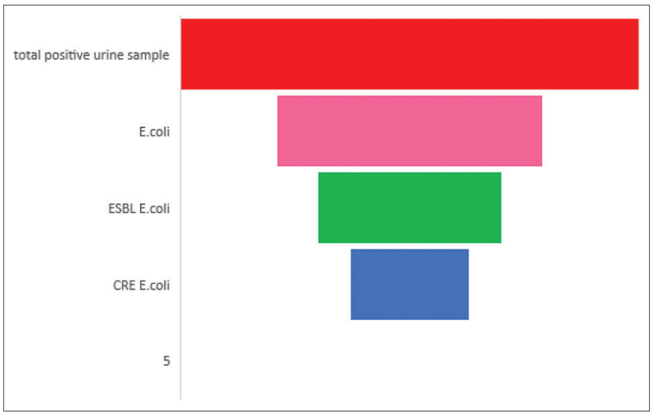

Figure 3: Proportion of extended-spectrum beta-lactamase and carbapenem-resistant *Enterobacteriaceae* to all isolated *Escherichia coli*

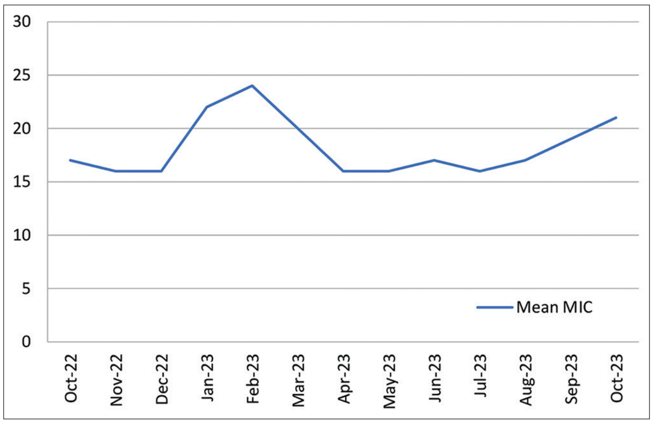

Figure 4: Increase in mean minimum inhibitory concentration of nitrofurantoin within the sensitive minimum inhibitory concentration range of *Escherichia coli*

DISCUSSION

In the present study, we analyzed the prevalence of uropathogens for 1-year period. The most common uropathogens found in the present study are *E. coli* (58%) followed by *Klebsiella* spp. (12%). This was similar to some previous studies.[1-4]

Table 1: Nitrofurantoin MIC distribution in ESBL non-producing *E. coli* and ESBL *E. coli*

Nitrofurantoin MIC	ESBL non-producer *E. coli* (n=241) (%)	ESBL *E. coli* (n=127) (%)
<16	159 (66)	60 (47)
32	28 (12)	26 (20)
64	33 (14)	13 (11)
128	11 (5)	15 (12)
>128	10 (4)	13 (10)
Total	241	127

MIC: Minimum inhibitory concentration, ESBL: Extended-spectrum beta-lactamase

UTI occurred more commonly in females, 86% were from females and 14% from male patients, which is similar to earlier studies by Gautam et al.,[5] where the occurrence in males and females was 45.2% and 77.4%, respectively. The validation for increased frequency of UTI among females could be the presence of a short urethra and proximity to the rectal flora.[7]

In the present study, the most common isolate, *E. coli*, showed a high level of resistance to some of the commonly used antibiotics such as ampicillin (85%) and amoxicillin-clavulanic acid (50%).[8,9] Gram-negative bacteria such as *E. coli* and *Klebsiella* spp. belonging to the *Enterobacteriaceae* have several factors responsible for their attachment to uroepithelium. They colonize the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptors.[7]

The increasing trend toward MDR among uropathogens has become a serious global public health problem. Fortunately, 8% of resistance was observed for nitrofurantoin and 2% for fosfomycin. These findings are similar to previous studies such as those by Ravishankar et al., Belete et al., and Daoud et al.[10-12]

Out of the total 631 samples, *E. coli* was isolated from (51%) of samples, among them 35% were ESBL producers. This was similar to a previous study.[13,14] There is a significant correlation between nitrofurantoin resistance in *E. coli* and ESBL production of *E. coli* (uncorrected Chi-square value 4.563, P=0.01634), hence indicating that nitrofurantoin resistance may be related to beta-lactamase production. A similar increasing nature of resistance was also observed in some previous studies.[15,16]

Nitrofurantoin and fosfomycin are the most common first-line drugs in the treatment of UTIs since date.[17] Sorlozano-Puerto et al. found that resistance to nitrofurantoin was associated with modifications of NfsA, NfsB, and RibEproteins.[18] In our study, we noted the emergence of *E. coli* isolates showing reduced susceptibility for nitrofurantoin as shown by increasing MICs (Figure 4). The phenomenon of MIC creep has been well established in vancomycin against *S. aureus*, where a rising MIC value is noted within a normal range.[19] This phenomenon may have developed in uropathogens as well as is observed in our study with *E. coli* against nitrofurantoin. This rising trend indicates a probable shift in the nitrofurantoin MICs.

Limitations of the study

1. Duration is very short only one year
2. ESBL and carbapenemase detection was not done by molecular test.

CONCLUSION

Nitrofurantoin is a widely used drug for treating lower UTIs in clinical practice in India. It has been known to be an effective antibiotic with low reported resistance, but our study shows a slowly increasing resistance pattern in MIC for nitrofurantoin. We have used the term "MIC creep" in nitrofurantoin for *E. coli* here since it is comparable to other MIC creeps such as in vancomycin for *S. aureus*.[19]

This rising trend indicates a probable shift in the nitrofurantoin MICs, which is an alarming sign to stop using antibiotics unnecessarily. Vigilant use of antibiotics and prevention of their misuse can be conducted by forming strong antimicrobial stewardship teams and implementation antimicrobial guidelines in hospital setups and following them constantly.

REFERENCES

1. Flores-Mireles AL, Walker JN, Caparon M and Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269-284.
   https://doi.org/10.1038/nrmicro3432
2. Foxman B and Brown P. Epidemiology of urinary tract infections: Transmission and risk factors, incidence, and costs. Infect Dis Clin North Am. 2003;17(2):227-241.
   https://doi.org/10.1016/s0891-5520(03)00005-9
3. Patel HB, Soni ST, Bhagyalaxmi A and Patel NM. Causative agents of urinary tract infections and their antimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians prevent antibiotic misuse. J Family Med Prim Care. 2019;8(1):154-159.
   https://doi.org/10.4103/jfmpc.jfmpc_203_18
4. Bono MJ, Leslie SW and Reygaert WC. Uncomplicated urinary tract infections. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470195 [Last accessed on 2023 Nov 13].
5. Gautam G, Gogoi S, Saxena S, Kaur R and Dhakad MS. Nitrofurantoin susceptibility pattern in gram-negative urinary isolates: In need of increased vigilance. J Lab Physicians. 2021;13(3):252-256.
   https://doi.org/10.1055/s-0041-1731106
6. CLSI. Performance Standards for Antimicrobial Susceptibility Testing Guideline. 33rd ed. CLSI Document M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023. p. 58-71.
7. Sharma D, Preston SE and Hage R. Emerging antibiotic resistance to bacterial isolates from human urinary tract infections in Grenada. Cureus. 2019;11(9):e5752.
   https://doi.org/10.7759/cureus.5752
8. Sanjee SA, Karim ME, Akter T, Parvez MA, Hossain M, Jannat B, et al. Prevalence and antibiogram of bacterial uropathogens of urinary tract infections from a tertiary care hospital of Bangladesh. J Sci Res. 2017;9(3):317-328.
   https://doi.org/10.3329/jsr.v9i3.31677
9. Lagunas-Rangel FA. Antimicrobial susceptibility profiles of bacteria causing urinary tract infections in Mexico: Single-centre experience with 10 years of results. J Glob Antimicrob Resist. 2018;14:90-94.
   https://doi.org/10.1016/j.jgar.2018.03.004
10. Ravishankar U, Sathyamurthy P and Thayanidhi P. Antimicrobial resistance among uropathogens: Surveillance report from South India. Cureus. 2021;13(1):e12913.
    https://doi.org/10.7759/cureus.12913
11. Belete MA and Saravanan M. A systematic review on drug resistant urinary tract infection among pregnant women in developing countries in Africa and Asia; 2005-2016. Infect Drug Resist. 2020;13:1465-1477.
    https://doi.org/10.2147/IDR.S250654
12. Daoud N, Hamdoun M, Hannachi H, Gharsallah C, Mallekh W and Bahri O. Antimicrobial susceptibility patterns of *Escherichia coli* among tunisian outpatients with community-acquired urinary tract infection (2012-2018) [published correction appears in Curr Urol. 2021;15(4):241]. Curr Urol. 2020;14(4):200-205.
    https://doi.org/10.1159/00049923
13. Azuma T, Uchiyama T, Zhang D, Usui M and Hayashi T. Distribution and characteristics of carbapenem-resistant and extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* in hospital effluents, sewage treatment plants, and river water in an urban area of Japan. Sci Total Environ. 2022;839:156232.
    https://doi.org/10.1016/j.scitotenv.2022.156232
14. Devi LS, Broor S, Rautela RS, Grover SS, Chakravarti A and Chattopadhya D. Increasing prevalence of *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M-type extended-spectrum beta-lactamase, carbapenemase, and NDM-1 in patients from a rural community with community acquired infections: A 3-year study. Int J Appl Basic Med Res. 2020;10(3):156-163.

https://doi.org/10.4103/ijabmr.IJABMR_360_19

15. Tulara NK. Nitrofurantoin and fosfomycin for extended spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumo*niae. J Glob Infect Dis. 2018;10(1):19-21.

    https://doi.org/10.4103/jgid.jgid_72_17

16. Resman-Targoff BH and Salvatore DJ. Treatment options for urinary tract infections caused by extended-spectrum B-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. J Acad Hosp Med. 2015;7(1):1-5.

17. Shakti L and Veeraraghavan B. Advantage, and limitations of nitrofurantoin in multi-drug resistant Indian scenario. Indian J Med Microbiol. 2015;33(4):477-481.

    https://doi.org/10.4103/0255-0857.167350

18. Sorlozano-Puerto A, Lopez-Machado I, Albertuz-Crespo M, Martinez-Gonzalez LJ and Gutierrez-Fernandez J. Characterization of fosfomycin and nitrofurantoin resistance mechanisms in *Escherichia coli* isolated in clinical urine samples. Antibiotics (Basel). 2020;9(9):534.

    https://doi.org/10.3390

19. Diaz R, Ramalheira E, Afreixo V and Gago B. Evaluation of vancomycin MIC creep in *Staphylococcus aureus*. J Glob Antimicrob Resist. 2017;10:281-284.

    https://doi.org/10.1016/j.jgar.2017.04.007

Authors Contribution:
SM- Definition of intellectual content, literature survey, prepared the first draft of the manuscript, implementation of the study protocol, data collection, data analysis, and manuscript preparation**; AM-** Literature survey and preparation of figures; **SS**- Review manuscript; **PSC-** Coordination and manuscript revision, **DD-** Concept, design, clinical protocol, manuscript preparation, design of the study, statistical analysis and interpretation and submission of the article.

Work attributed to:
College of Medicine and Sagore Dutta Hospital, Kamarhati, Kolkata, India.

Orcid ID:
Dr. Soumyya Mondal - https://orcid.org/0009-0000-0008-5823
Anwesha Mandal - https://orcid.org/0009-0004-9658-2072
Sipra Saha - https://orcid.org/0009-0001-5812-6605
Partha Sarathi Chakrabarty - https://orcid.org/0009-0004-0866-5493
Debalina Das - https://orcid.org/0000-0002-2116-6662

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