ORIGINAL ARTICLE

RISING RESISTANCE IN UROPATHOGENS WITH AN INDICATION OF NITROFURANTOIN MIC CREEP

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Background: The irrational use of antibiotics has led to the emergence of multi drug resistant pathogens. The phenomenon of 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 **Methods:** A cross sectional study in a large tertiary care hospital in North India to observe the susceptibility pattern among uropathogens and the possibility of MIC creeps. The Antimicrobial Susceptibility Testing (AST) and Minimum Inhibitory Concentration (MIC) were conducted by Vitek Compact 2. The identification of Extended Spectrum Beta Lactamase (ESBL) producers and Carbapenem Resistant Enterobacteriaceae (CRE) among Escherichia coli were noted. The MIC 50 and MIC 90 for Nitrofurantoin, the most widely used antibiotic for lower UTI, was calculated to investigate the phenomenon of MIC creep. **Results:** In our study, a total of 2522 urine samples were analyzed: 1538 (61%) were positive with the commonest isolate being *E. coli* (n=736, 47.8%) followed by *Klebsiella spp.* (n=178, 11%). Less than 10% of resistance was observed for Fosfomycin, Amikacin, Nitrofurantoin, Imipenem, Meropenem and Colistin. ESBL producers and CRE *E. coli* were 528 (72% of 736) and 79 (11% of 736) respectively. Overall, 119/736 samples had an MIC ≥128. Amongst the ESBL producers, 96/528 had MIC ≥128 and amongst the CRE, 13/79 had MIC ≥128. **Discussion:** *E. coli* can be used to reflect the trends in development of resistance. In the current study, it was observed that *E. coli* showed a reduced susceptibility for Nitrofurantoin indicated by a creeping increase in MIC albeit within normal range. **Conclusion:** Trends in rising MIC should alert prescribers to use drugs such as Nitrofurantoin judiciously. Antimicrobial stewardship practices should be strongly implemented in hospitals to curb rising resistance and obtain better treatment outcomes for patients with infectious diseases.

Keywords: Urinary tract Infection; Hospital acquired infection; Extended Spectrum Beta-lactamase; Carbapenem-resistant Enterobacteriaceae; MIC (Minimum inhibitory concentration) creep; Antimicrobial Stewardship

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INTRODUCTION

Urinary tract infection (UTI) is very commonly encountered in clinical practice, affecting around 150 million people every year.[1] Irrational use of antibiotics due to over-the-counter availability in many developing countries coupled with poor infection prevention practices lead to emergence of multi drug resistance among pathogens.[2] Rising antibiotic resistance among uropathogens, makes it important to conduct periodic reviews of the susceptibility patterns. This information is relevant due to organisms mutating to resistant forms as well as indicating injudicious use of certain antimicrobial agents in that locality.[3] Among all the known bacterial pathogens causing UTI, uropathogenic *E. coli* is the most implicated organism[4] followed by *Klebsiella spp.*, *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus* species and *Pseudomonas aeruginosa*[5–7]. With increasing antimicrobial resistance, standard guidelines (Infectious Diseases Society of America and European Society for Microbiology and Infectious Diseases) recommend Nitrofurantoin as one of the first-line agents for the treatment of uncomplicated lower UTI.[8,9]

In this cross-sectional study conducted for a period of one year, we investigate rising resistance in *E. coli* to predict future resistance to the frequently used antibiotic, Nitrofurantoin. The susceptibility patterns of all the uropathogens isolated were observed along with Minimum

Inhibitory Concentrations, MIC50 and MIC90 for *E. coli*.

MATERIAL AND METHODS

A retrospective study was conducted for a period of one year, including patients from IPD and OPD in a tertiary care hospital of New Delhi, India. A total number of 2522 samples were processed in the laboratory for the identification of urinary tract pathogens from January 2019 to January 2020. Clean catch midstream urine samples were subjected to routine microscopy, Gram staining, and culture in 5% sheep blood agar and MacConkey agar was done. Samples with mixed growth and pathogens other than bacteria were excluded.

The identification of bacterial pathogens responsible for significant bacteriuria was observed with culture and biochemical characteristics. The antimicrobial susceptibility testing (AST) with MIC readings, was conducted by Vitek 2 Compact with N235, N280 and GP 628 AST cards on all samples with significant bacteriuria.[10] The identification of Extended Spectrum Beta Lactamase (ESBL) producers and Carbapenem Resistant Enterobacteriaceae among Escherichia coli (CRE) were identified with Vitex Compact 2 AST cards.[11,12] Retrospective data was extracted as an excel sheet from VITEK automated ID AST system and *E. coli* isolates were arranged in increasing order of MIC for Nitrofurantoin to calculate MIC 50 and MIC 90 for Nitrofurantoin.[12,13]

RESULTS

In our study a total of 2522 urine samples of clinically suspected UTI cases were analyzed over a period of one year. Of these, 1538 (61%) were positive urine samples showing significant bacteriuria and the remaining 984 (39%) samples were either non-significant growth, polymicrobial flora or were sterile. Out of the 1538 samples that were processed, 926 (60.2%) were from adult females and 612 (39.8) from adult male patients.

Out of the total 1538 uropathogens, the commonest isolate was *E. coli* (n=736, 47.8%) followed by *Klebsiella spp.* (n=178, 11%). Among the Gram-positive organisms, *Staphylococcus spp.* (n=130, 8.45%) was the most common. Other organisms like *Pseudomonas aeruginosa*, *Enterococcus spp*, Proteus *spp*., *S. 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 Tetracyclines (96%) followed by Nalidixic acid (86%) and Ampicillin (81%). Less than 10% of resistance was observed for Fosfomycin, Amikacin, Nitrofurantoin, Imipenem, Meropenem and Colistin. Out of the total 1538 samples, *E. coli* was isolated from 736 (47.8%) samples. Out of these, 528 (72% of 736) were Extended Spectrum Beta Lactamase (ESBL) producers and 79 *E. coli* (11% of 736) were Carbapenem resistant (CRE) Figure-3.

MICs for Nitrofurantoin for the *E. coli* isolates were noted from Vitek 2 compact. Amongst all *E. coli* isolates MIC 90 for Nitrofurantoin was 128 (662nd isolate) and MIC 50 was 32 (368th isolate). Amongst the ESBL producers, MIC 90 was 128 (476th isolate) and MIC 50 was 32 (264th isolate). Similarly, for the CRE, MIC 90 was 128 (71st isolate) and MIC 50 was 32 (40th isolate). Overall, 119/736 samples had an MIC ≥128. Amongst the ESBL producers, 96/528 had MIC ≥ 128 and amongst the CRE, 13/79 had MIC ≥ 128. This is shown in Figure-4 and Table-1.

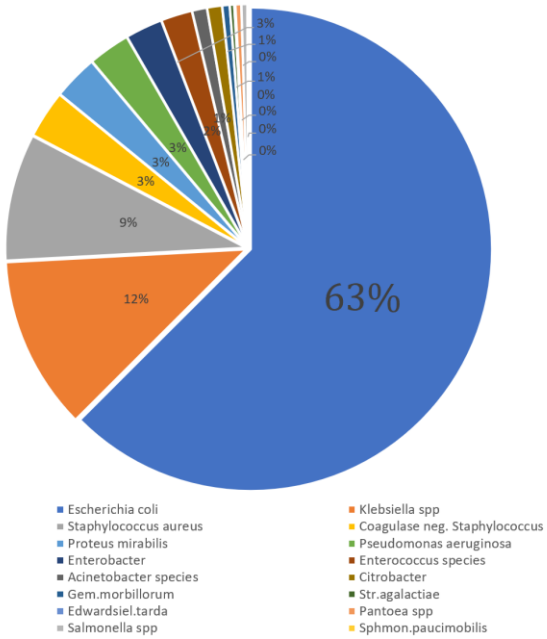

Figure-1: The pattern of isolation of Uropathogens

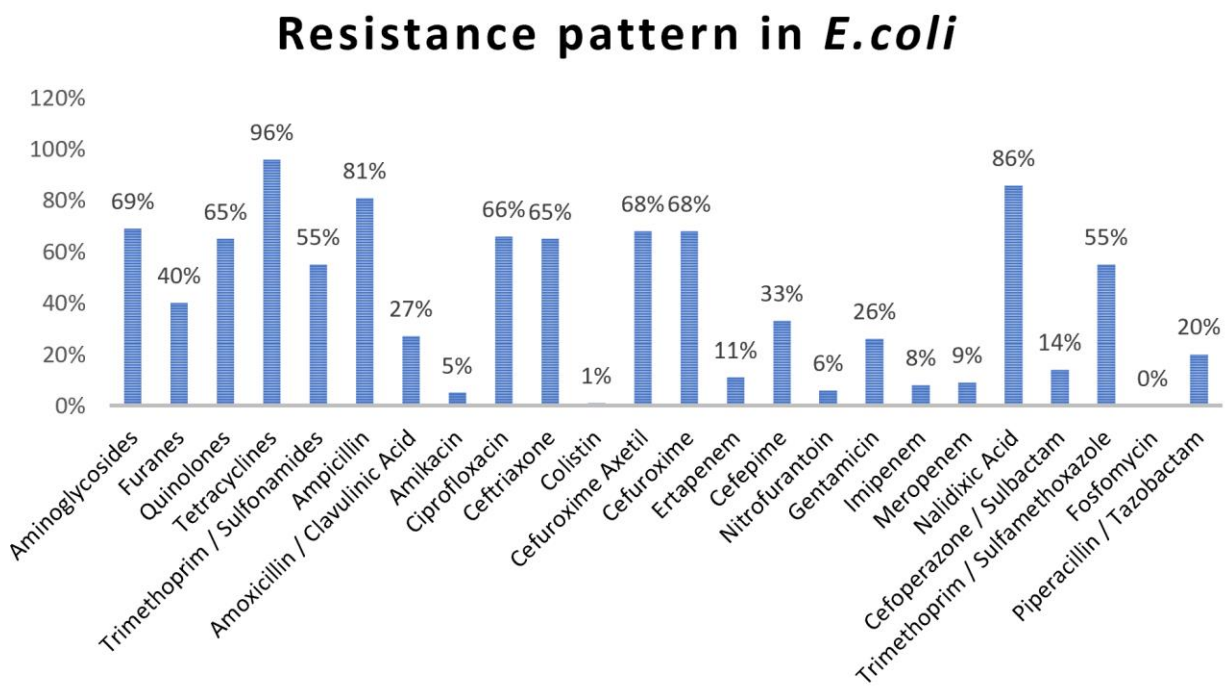

Figure-2: Resistance pattern of the isolated *E. coli*

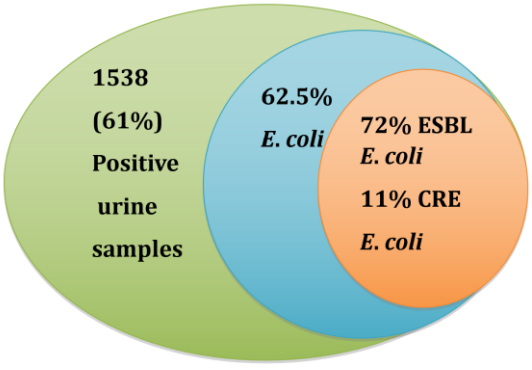

Figure-3: Ratio of ESBL and CRE *E. coli* in urine samples.

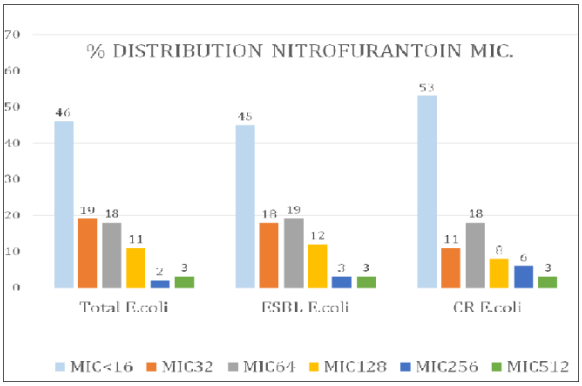

Figure-4: Percent distribution of Nitrofurantoin MIC amongst all *E. coli,* ESBL and CR *E. coli*

TOTAL *E.coli*(736)		ESBL *E.coli*(528)		CRE *E.coli*(79)	
MIC90 (662nd Isolate) = 128		MIC90 (476thIsolate) = 128		MIC90 (71stIsolate) = 128	
MIC50 (368th Isolate) = 32		MIC50 (264th Isolate) = 32		MIC50 (40th Isolate) = 32	
MIC DISTRIBUTION OF NITROFURANTOIN (%) IN *E.coli*					
119/736 (16%) had MIC ≥128		96/528(18%) had MIC ≥128		13/79(17 %) had MIC ≥128	
<16	336 (47%)	<16	236 (45%)	<16	42 (53%)
32	137 (19%)	32	96 (18%)	32	9 (11%)
64	134 (18%)	64	100 (19%)	64	15 (19%)
128	84 (11%)	128	64 (12%)	128	6 (8%)
256	16 (2%)	256	17(3%)	256	5 (6%)
>512	19 (3%)	>512	15 (3%)	>512	2 (3%)

Table-1: MIC distribution of Nitrofurantoin (%) in *E. coli*

DISCUSSION

In the present cross-sectional study, we analyzed the prevalence of uropathogens for a one-year period. UTI occurred more commonly in females (n=926, 60.2%) as compared to males (n=612, 39.8 %) which is similar to earlier studies by Dash *et al*[14] and Shah *et al*[15] where the occurrence in 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.

The most common uropathogens found in the present study are *Escherichia coli* (n=736, 47.8%) followed by *Klebsiella spp* (n=178, 11%). This was similar to previous studies carried out by Meena *et*

al[16] and Shah *et al*[15] from India; Solórzano *et al*.[17] from Spain; Eure *et al*.[18] and Sharma *et al*.[19] from Grenada; and Lagunas-Rangel[20] from Mexico. 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 receptor.[21] Many other studies have stated the preponderance of gram-negative organisms responsible for UTI with *E. coli* and *Klebsiella* occurring most frequently, similar to our study.[22]

Diseases caused by drug resistant pathogens causes a mortality of around 700,000 people each year. Studies estimate this could swell to 10 million a year by 2050 if no action is taken[19] The increasing trend towards multi drug resistance (MDR) among uropathogens has become a serious global public health problem.[24]

In the present study, the most common isolate, *E. coli*, showed a high level of resistance to some of the commonly used antibiotics like Tetracyclines (96%), Nalidixic acid (86%) and Ampicillin (81%). Fortunately, less than 10% of resistance was observed for Nitrofurantoin, Fosfomycin, Amikacin, Imipenem, Meropenem and Colistin. These findings are similar to previous studies such as those by Uma Ravishankar *et al*[22], MA Belete *et al*[25] and Daoud N *et al*[26].

In developing countries, increase in antibiotic resistance can be attributed to antibiotic abuse caused by "over the counter" availability of antibiotics. A major part of resistance is also attributed to misuse by the veterinary and farming industries. In many instances in hospitals or private clinics, antimicrobial treatment is initiated before the laboratory results are reported which leads to incorrect and often incomplete use of antibiotics by patients. An important attributing factor is also that research and development of newer antibiotics is rare since it may not be as profitable for pharmaceutical companies as compared with lifestyle illness medications due to rapidly developing resistance.

Out of the total 1538 samples, *E. coli* was isolated from 736 (47.8%) samples, 528 (72% of 736) were Extended Spectrum Beta-lactamase producers and 79 *E. coli* (11% of 736) were Carbapenem resistant. This was similar to study conducted by Devi LS *et al*.[27] where 60.6% ESBL and 8% CRE (*E. coli*) were isolated.

With the advent of multidrug resistance among uropathogens, use of Nitrofurantoin and Fosfomycin as first line drugs in treatment of UTI has been increasing. Solórzano-Puerto A *et al* found that resistance to Nitrofurantoin was associated with modifications of NfsA, NfsB, and RibE proteins.[28] In our study. we noted the emergence of *E. coli* isolates inclining to a reduced susceptibility for Nitrofurantoin as shown by increasing MICs. The phenomenon of MIC creep has been well established in Vancomycin against *Staphylococcus aureus*, where a rising MIC value is noted albeit within a normal range.[29] This phenomenon may have developed in uropathogens as well as is observed in our study with *E. coli* against Nitrofurantoin.

The MIC_{50} represents the MIC value at which ≥50% of the isolates in a test population of the bacteria are inhibited by the antibiotic; it is equivalent to the median MIC value. The MIC_{90} represents the MIC value at which ≥90% of the strains within a test population of the bacteria are inhibited by the antibiotic; the 90th percentile. The MIC 50 and MIC 90 are used for studying large test populations with multiple isolates as important parameters for reporting susceptibility test results. In our study, the MIC_{50} is still in the susceptible breakpoint range as shown in Table-1 and Figure-4. Percentage of isolates with MIC_{90} ≥128 was similar without any significant difference (*p*-value >.05) among the three categories: Total *E. coli*, ESBL *E. coli* and CRE *E. coli*, hence indicating that it may be unrelated to Beta-lactamase production. Similar increasing nature of resistance was also observed by Solórzano-Puerto *et al*[28] and Shakthi *et al*[23]. This rising trend, unrelated to Beta-lactamase production indicates a probable shift in the Nitrofurantoin MICs. Such rising trends are a warning that resistance is beginning to develop, and inadvertent use of antibiotics must stop. The emergence and spread of these resistance mechanisms, including transferable resistance could compromise the future use of Nitrofurantoin for treating UTIs.

CONCLUSION

In our study we have encouraging susceptibility results of uropathogens to Nitrofurantoin, Fosfomycin, Carbapenems and Amikacin. Nitrofurantoin is a widely and frequently used drug for treating lower UTI in clinical practice in North India. It has been known to be an effective antibiotic with low reported resistance; however 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 Staphylococcus aureus.[29]

The indiscriminate use of Nitrofurantoin is commonly observed as an empirical treatment even before laboratory results are available. At other times, laboratory reports are used in the absence of consistent initial history leading to inappropriate antibiotic therapy for asymptomatic bacteriuria.

A limited number of oral options of antibiotics are available to treat ESBL producing Enterobacteriaceae infections. Hence it is only prudent to reserve less toxic oral antibiotics such as Nitrofurantoin for management of culture-confirmed lower UTI infections. It is highly recommended for all prescribers and treating physicians to consult with infectious diseases specialists and liaise with Microbiologists to interpret susceptibility results with disease.

This study shows the importance of surveillance, creation of local antibiograms with analysis of resistance trends and MICs regularly. Vigilant use of antibiotics and prevention of their misuse can be conducted by forming strong antimicrobial stewardship teams and robust implementation of antimicrobial guidelines in hospital set ups.

AUTHOR'S CONTRIBUTION

AH, RG: Literature search. AH, SR: Conceptualization of study design. RG, MRA: Data collection. AD, SD, and RG: Data Analysis. AH, SR, and SN: Data interpretation. AH, KK, RG, and SD: Write up. SN, MRA, and AD: Proof reading.

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