In Vitro Activity of Nitrofurantoin against Multi Drug Resistant Enterobacteriaceae Isolated from Urine

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Abstract

Background: Amongst the Enterobacteriaceae, *Escherichia coli* account for 75% to 95% of cases of Urinary Tract Infection (UTI), the remaining being *Klebsiella* and *Proteus mirabilis* . The first-line treatment for community-acquired uncomplicated cystitis is nitrofurantoin with advantages over new drugs being high urinary concentration and minimal toxicity.

Objectives: To study the susceptibility pattern of urinary isolates of Enterobacteriaceae and *in-vitro* activity of Nitrofurantoin against MDR Enterobacteriaceae.

Methods: The urinary samples received in the laboratory were cultured and sensitivity was performed by disc diffusion method. Sensitivity profile of all Enterobacteriaceae was noted and MIC of 130 MDR isolates to Nitrofurantoin was determined using Agar Dilution Method and VITEK 2 Compact system.

Results: Most common Enterobacteriaceae isolated from urine was *Escherichia coli* (77.22%) followed by *Klebsiella pneumoniae* (20.87%) and *Proteus spp* (1.92%). In patients of MDR UTI, *Escherichia coli* was most common (73.1%) followed by *Klebsiella pneumoniae* in 26.9%. Majority of MDR Enterobacteriaceae were sensitive to Nitrofurantoin by all the three methods, 61.5% by Disc Diffusion and Agar Dilution, while as 66.2% by VITEK 2.

Conclusion: MDR Enterobacteriaceae isolated from urine have a high level of susceptibility to Nitrofurantoin which can be used as a dependable treatment option for UTIs in Kashmir. Besides, low-cost disc diffusion method can be safely used for antibiotic susceptibility testing of urinary isolates to nitrofurantoin as the results are quite comparable to the gold standard test. Nitrofurantoin can be used as first line drug for treatment of UTI in absence of antibiotic sensitivity results.

Keywords: Urinary tract infection • *Escherichia coli* • Cystitis • Nitrofurantoin • Antibiotic resistance • Pyelonephritis

Introduction

UTIs are among the most frequent bacterial infections. It is estimated that there are about 150 million cases worldwide per annum; they account for over 8 million physician visits per year in the USA and for 1%-6% of all consultations in general practice in the UK each year [1-3]. The prevalence of UTIs depends on demographics, medical interventions and co-morbidities.

An inflammation of the urinary tract can occur in urethra (urethritis), bladder (cystitis), kidneys and renal pelvis (pyelonephritis), epididymis (epididymitis) or prostate gland (prostatitis) in males. In extreme cases infection overspills to the bloodstream and manifests as sepsis, severe sepsis or septic shock. A "significant" bacteriuria is conventionally defined as ≥ 10^8 cfu/L or ≥ 10^5 cfu/ml of one bacterial species in a clean-catch mid-stream urine as first proposed by Kass, in 1956 [4]. Kass found that lower numbers of bacteria generally indicated contamination during sample collection, although bacterial loads of 10^4-10^5 cfu/mL were difficult to interpret. Nevertheless, approximately one-fourth of patients presenting with symptoms suggesting acute uncomplicated UTIs yield no bacterial growth on urine culture or counts between 10^2 cfu/ ml-10^4 cfu/ ml [5].

In general, antimicrobial treatment in UTI should be reserved only for patients with clinical symptoms and signs, except for asymptomatic bacteriuria (>10^5 cfu/mL) in pregnancy and in transurethral resection of prostate where mucosal bleeding is likely to occur [6]. Local epidemiology, resistance profiles, and patient factors (age, gender, medical interventions, and comorbidities such as diabetes, immunosuppression, catheterization, neuromuscular disorders, renal transplantation) should be taken into consideration before deciding on antimicrobial therapy.

Clinical laboratories should be also aware of natural (inherent) resistance profiles of common uropathogens. For example *Proteus mirabilis* is naturally resistant to nitrofurantoin and colistin, Enterobacter spp., *Citrobacter freundii* are naturally resistant to ampicillin, amoxicillin, co-amoxiclav, first generation cephalosporins and cefoxitin [7]. Monitoring antimicrobial resistances, combined with reasonable antibiotic therapy should help to reduce the rate at which resistance emerges and spreads. It should also seek to minimise the super-infection rate with Clostridium difficile. Guidelines for empirical treatment of UTIs need to be regularly reviewed and updated.

The upsurge of drug resistant uropathogens in recent years has perplexed the treatment of Urinary Tract Infections (UTIs), causing an escalation in morbidity and mortality. Many multi drug resistance organisms including *Escherichia coli* retain susceptibility to two old antibiotics, nitrofurantoin and fosfomycin. Advantages over new drugs are their higher urinary concentrations and minimal toxicity [8].

Methods

Study design

A Cross-sectional study was carried out in the Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences (SKIMS) Soura, Srinagar Kashmir.

Study period

The study was carried out from January 2021 to December 2021 for a period of one year.

Study population

Patients who presented with symptoms suggestive of urinary tract infection at OPD and inpatient care at Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Kashmir and were advised urinary culture and sensitivity during the study period.

Source of sample

All routine urinary samples received in the Department of Microbiology from inpatient as well as outpatient department during the study period were considered eligible for the study.

Sample size

After considering the inclusion and exclusion criteria a total of 130 samples were recruited during the study period.

Processing of sample

The urinary samples received in the laboratory were cultured and identified as per standard guidelines and sensitivity was performed by disc diffusion method as per CLSI guidelines. Sensitivity profile of all Enterobacteriaceae was noted and those which were found multidrug resistant were preserved in 20% glycerol at -20ºC. All the work was done in biosafety cabinet IIB. Equipment, media, reagents and consumables used in the study are mentioned in Appendix-I.

On the day of testing, the urinary MDR strains of Enterobacteriaceae were revived in batches by sub-culturing them on Hi-Chrome agar prepared as per manufacturer's instructions (Appendix-II).

Then the MIC of Nitrofurantoin of the isolates was determined using:

- Agar Dilution Method (Figure 1).
- VITEK 2 Compact system (Figure 2).

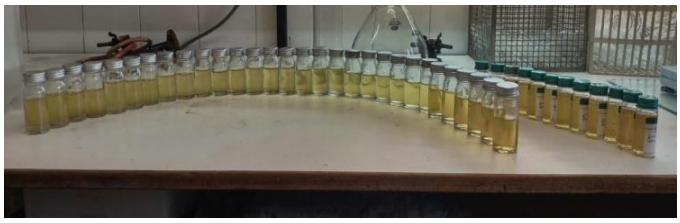

Figure 1. Agar dilution method.

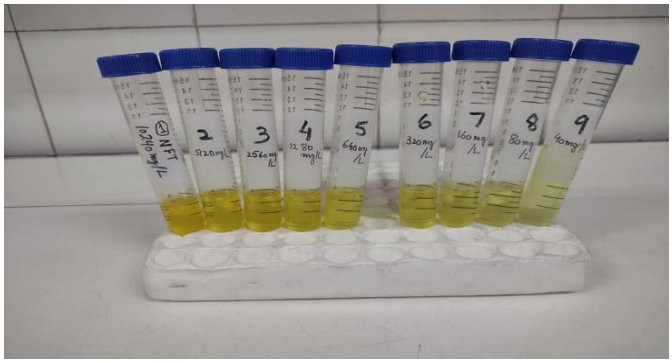

Figure 2. VITEK 2 compact system.

Agar dilution method

Mueller Hinton Agar was used as the medium for performing agar dilution. Agar Dilution Method involved the incorporation of different concentrations of Nitrofurantoin into Mueller-Hinton Agar followed by the application of standardized number of cells to the surface of the agar plate. Growth was assessed after incubation at 37°C for 16 h-20 h.

Reading and interpreting the results:
Results for a particular isolate were considered valid only when the control plate showed growth. The lowest concentration of Nitrofurantoin at which no visible bacterial growth on the agar plate was observed by the naked eye, was considered the MIC for that particular isolate. A classification based on an *in vitro* response of an organism to an antimicrobial agent was adopted which divides the result into one of the three responses which include:

- Susceptible (S)- a category that implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.
- Intermediate (I)- a category that includes isolates with antimicrobial agent minimal inhibitory concentrations that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. and
- Resistant (R) – a category that implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate minimal inhibitory concentrations that fall in the range in which specific microbial resistance mechanisms (e.g., β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

VITEK 2 compact system

(BioMérieux) was used for confirmation of microbial identification and antibiotic susceptibility of MDR Enterobacteriaceae.

Results

The mean age of participants was 37.13 years with SD of 21.68. Majority of patients were aged >10 years (83.1%). Majority (61.5%) of patients were females. The patients were recruited both from OPD as well as IPD, with 59.2% from OPD & 40.8% from IPD (Table 1).

Table 1. Demographic profile of the study population declaration of patient consent.

Variables	Frequency	%
Sex M/F	51/79	60.76/39.23
OPD/IPD	59.2	59.2/40.8
Age (years) 37.13 ± 21.68, IQR of 22 years to 54 years		

Majority of samples (69.59%) were sterile, while as 17.66% had clinically significant Bacteraemia (Table 2).

Table 2. Distribution of urinary samples.

Urinary Samples	Number	Percentage
Sterile Samples	5547	69.59
Contaminated Samples	855	10.73
Yeast	161	2.02
Clinically Significant Bacteriuria	1408	17.66
Total	7971	100

Overall, the most common Enterobacteriaceae isolated from urine was *Escherichia coli* (77.22%) followed by *Klebsiella pneumonia* (20.87%) and *Proteus* spp (1.92%).In patients of MDR UTI, *Escherichia coli* (73.1%) was most commonly isolated followed by *Klebsiella pneumoniae* in 26.9% (Table 3).

Table 3. Distribution of urinary isolates of Enterobacteriuria

Enterobacteriuria isolated from urine	Total		MDR	
	Number	Percentage	Number	Percentage
E. coli	766	77.22	95	73.1
Klebsiella spp.	207	20.87	35	26.9
Proteus spp.	19	1.92	0	0
Total	992	100	130	100

Among samples from IPD the maximum resistance was observed with Ceftazidime (99%) followed by Ciprofloxacin (97.2%), Ceftriaxone (95.4%), Cefepime (93.5%) and Piperacillin+Tazobactam (90.8%) with least resistance for Nitrofurantoin (36.9%) and Amikacin (42%).

Among samples from OPD the maximum resistance was observed with Ceftazidime (96%) followed by Cefepime (95.2%), Piperacillin + Tazobactam (93.5%), Ciprofloxacin (93.4%) (Table 4).

Table 4. Resistance pattern of urinary isolates of *E. coli* to various antibiotics using disc diffusion method.

Antibiotic	Resistance Pattern of *E. coli* (n/%)		
	IPD (n=222)	OPD (n=544)	Total (766)
	(n/%)	(n/%)	(n/%)
Amikacin	93/42	213/39.2	306/40
Gentamicin	135/61	281/51.7	417/54.4
Cefoperazone + Sulbactam	167/75.3	364/67	532/69.4

Piperacillin + Tazobactam	202/90.83	509/93.5	710/92.72
Cefepime	207.5/93.49	517.9/95.2	725.4/94.7
Ceftazidime	220/99	522/96	742/96.9
Ceftriaxone	212/95.36	484/89	696/90.84
Ciprofloxacin	216/97.24	508/93.4	724/94.51
Imipenem	179/80.6	460/84.5	639/83.4
Meropenem	141/63.3	319/58.7	46060
Nitrofurantoin	82/36.9	233/42.9	315/41.2
Cotrimoxazole	197/88.6	469/86.3	666/87

On Disc Diffusion maximum resistance was observed to AMP (100% cases) followed by CTX (98.5%), CEF (97.7%), CIP (93.1%) and CPM (80.8%) (Figure 3).

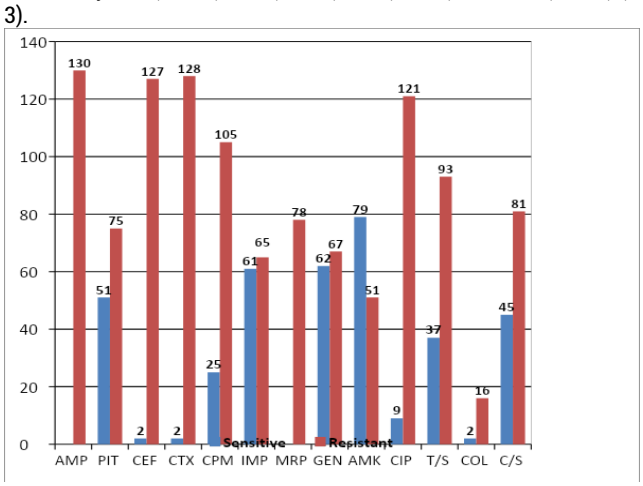

Figure 3. Diffusion maximum resistance.

The percentage of sensitive samples was similar on Disc Diffusion and Agar Dilution (61.5% each) and slightly higher on VITEK (66.2%) (Figure 4).

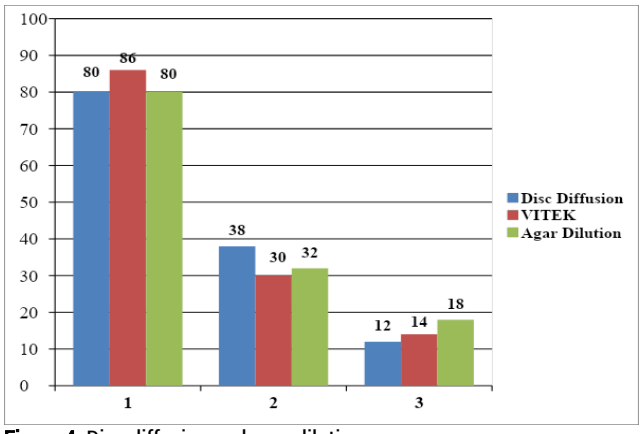

Figure 4. Disc diffusion and agar dilution.

Majority of *Escherichia coli* samples were sensitive to Nitrofurantoin on all the three methods (disc diffusion: 74.7%, VITEK: 78.9%, agar dilution: 76.8%) as compared to *Klebsiella pneumoniae* (Disc Diffusion: 25.7%, VITEK: 31.4%, agar dilution: 20%) (Figure 5).

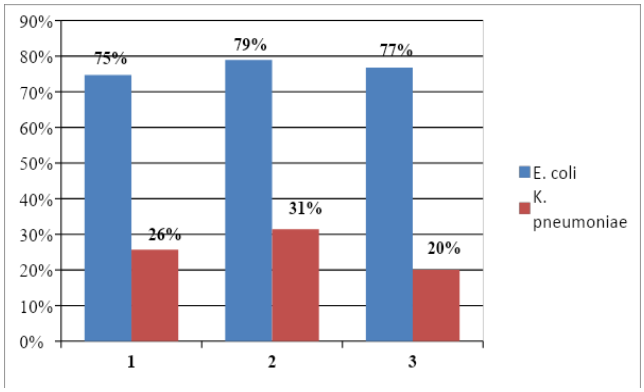

Figure 5. Agar dilution and disc diffusion.

A Kappa of 0.67 represents a substantial amount of agreement between the agar dilution & disc diffusion methods in assessing the sensitivity of Enterobacteriaceae isolated from urine to Nitrofurantoin (Figure 6).

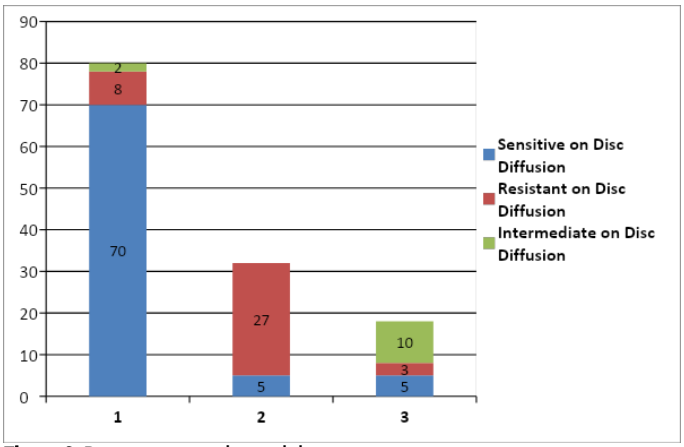

Figure 6. Represents a substantial amount.

Discussion

Urinary tract infections are one of the commonest primary infections seen in health care settings. The patients often present with vague complaints and asymptomatic bacteriuria requiring laboratory confirmation for successful treatment. At present, the emergence of resistance to regularly used antibiotics has left limited options for treatment.

All the patients who presented with complaints of symptomatic urinary tract infection at OPD and IPD during the study period were considered eligible for the study and those urinary isolates found to be multidrug resistant Enterobacteriaceae were included. A total of 7971 urinary samples were received in Bacteriology laboratory at SKIMS, Soura from 1st January 2021 up to 31st December 2021, out of which majority of samples (69.59%) were sterile, while as 17.66% had clinically significant Bacteriuria. Overall, the most common Enterobacteriaceae isolated from urine was *E. coli* (77.22%) followed by *K. pneumoniae* (20.87%) and *Proteus* spp. (1.92%).

In a study conducted by Sonali Bhattar et al.,in 2020 (New Delhi) to review the susceptibility pattern of the uropathogens isolated from the patient population referred to a super speciality hospital antimicrobial susceptibility test of the isolates was done by VITEK-2 compact system, they also found that *E. coli* was the most frequent uropathogen [9]. Fajfr et al., in 2017 (Czech Republic) also conducted a study to review the susceptibility pattern of the uropathogens isolated from the patient population referred to their respective tertiary care hospital wherein also *E. coli* was the most frequent uropathogen seen [10]. Safar Farjaniaet al, in 2009 published their research work on causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran in which they found that *E. coli* was the most common etiological agent of UTI (74.6%), followed by *Klebsiella* spp (11.7%) [11].

A total of 130 isolates were found to be multidrug resistant Enterobacteriaceae in our study. Out of these 130 MDR Enterobacteriaceae the most common isolated organism was *E. coli* (73.1%), while as in rest (26.9%) it was *K. pneumoniae*. Similar findings were found in a study conducted by Sonali Bhattar et al.,in 2020 [9]. Safar Farjaniaet al., in 2009 also concluded that the antimicrobial resistance patterns of the causes of UTI are highly variable and continuous surveillance of trends in resistance patterns of uropathogens is important [11].

In our study majority of the study participants (61.5%) were females. This predominance of females over males in our study was due to the fact that more females had reported to the OPDs and IPDs as they are more susceptible to the UTIs due to the anatomical structure of the female urinary genital tract [12]. In this study we also found that in majority of female patients (76.3%), *E. coli* was isolated from their urine samples, while as in rest (23.8%) it was *K. pneumoniae*. Similarly, in male study subjects *E. coli* (68%) was isolated more frequently from their urine samples, as compared to *K. pneumoniae* isolated in 32%. In our study we found that the proportion of isolates sensitive to Nitrofurantoin was more among females than males on Disc Diffusion, VITEK as well as on agar dilution; though the difference was not statistically significant. Garima Gautam et al., in 2021 found that about 28.82% of the isolates were of the paediatrics age group and most of the isolates belonged to females (64.69%) [13]. We also found that majority

of samples were from patients aged>10 years (83.1%) and less from paediatric age group.

In our study, study participants were recruited both from OPD as well as IPD, with 59.2% from OPD and 40.8% from IPD by convenient sampling as no pre-decided sample size was estimated for the study. The proportion of *E. coli* was more among OPD (79.2%) as compared to IPD (64.2%) with a significant p Value. In the present study we found that the proportion of samples sensitive to Nitrofurantoin was more from OPD patients as compared to samples from IPD patients on Disc Diffusion, VITEK as well as on agar dilution; though on disc diffusion and VITEK the difference was not statistically significant but on agar dilution the difference (70% against 49%) was statistically significant with a p Value of 0.03 (Figure 7).

Majority of MDR *Enterobacteriaceae* were sensitive to Nitrofurantoin by all the three methods, 61.5% by disc diffusion and agar dilution, while as 66.2% by VITEK. This sensitivity was more in case of *E. coli* and lesser in cases of *K. pneumoniae*. Majority of *E. coli* isolates were sensitive to Nitrofurantoin by all the three methods (Disc diffusion: 74.7%, VITEK: 78.9%, Agar dilution: 76.8%), while as in case of *K. pneumoniae* the sensitivity pattern was quite different and very low (Disc diffusion: 25.7%, VITEK: 31.4%, Agar dilution: 20%). These differences in the sensitivity patterns of *E coli* and *K pneumoniae* were statistically significant with a p Value of <0.05.

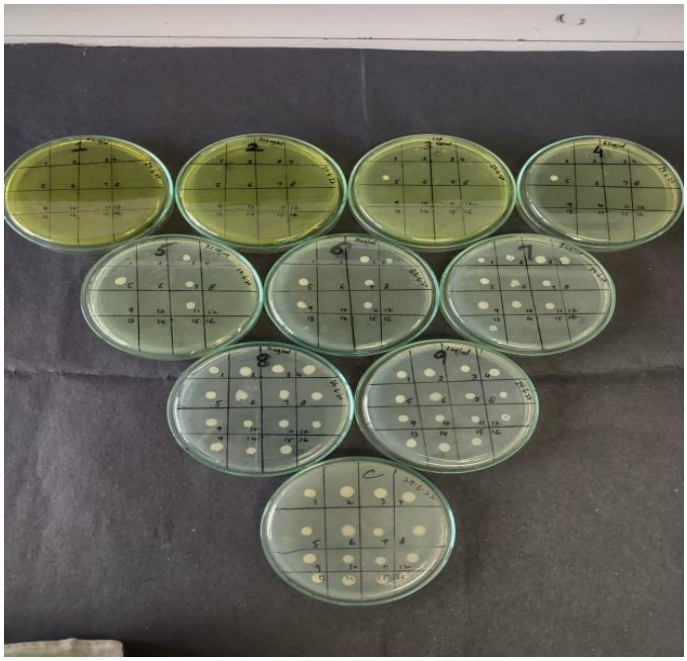

Figure 7. Culture plates.

James Kashanian et al., in 2008 (NY USA) conducted a study to re-evaluate the first and second line therapies for treating uncomplicated urinary tract infection. The overall susceptibilities of *E. coli* to various drugs varied significantly. Among all drugs, Nitrofurantoin had the most pronounced and consistent activity against *E. coli* [14]. Mujde Eryilmaz et al., in 2009 (Turkey) studied the antimicrobial resistance rate of 110 *E. coli* strains isolated from UTIs in Etlik Lokman Hekim Hospital, Ankara, Turkey [15]. They found that isolates were susceptible to Fosfomycin and Nitrofurantoin.

In our study the percentage of sensitive samples was similar on Disc Diffusion and Agar Dilution (61.5% each) and slightly higher on VITEK (66.2%). We found a moderate amount of agreement (Kappa: 0.5) between the VITEK and Agar Dilution methods in calculating MIC of Nitrofurantoin. We also found a substantial amount of agreement (Kappa: 0.73) between the VITEK and Agar Dilution methods in assessing the sensitivity of Enterobacteriaceae isolated from urine to Nitrofurantoin. Similar findings were observed by Suneeta Meena et al study wherein they went to detect susceptibility of nitrofurantoin against Enterobacteriaceae isolates from Urinary Tract Infection (UTI) of patients from outdoor and indoor departments (108). In their study they found there was a moderate amount of agreement (Kappa: 0.6) between the VITEK and Agar Dilution methods in calculating MIC of Nitrofurantoin. It we also found in their study that there was a substantial amount of agreement (Kappa: 0.77) between the VITEK and Agar Dilution methods in assessing the sensitivity of Enterobacteriaceae isolated from urine to Nitrofurantoin.

In our study we found that there was a substantial amount of agreement (Kappa: 0.67) between the Disc Diffusion and Agar Dilution methods in assessing the sensitivity of Enterobacteriaceae isolated from urineto Nitrofurantoin. Similar findings were found by Suneeta Meena et al [16]. They found in their moderate amount of agreement (Kappa: 0.60) between the Disc Diffusion and Agar Dilution methods in assessing the sensitivity of *Enterobacteriaceae* isolated from urineto Nitrofurantoin.

In our study, on Disc Diffusion maximum resistance was observed with ampicillin (100% cases) followed by ceftriaxone (98.5%), Ceftazidime (97.7%), Ciprofloxacin (93.1%) and Cefipime (80.8%). Guillermo V Sanchezin 2012 conducted a study to examine the *in vitro* antimicrobial resistance data of *Escherichia coli* isolates obtained from urine samples of U.S. outpatients between 2000 and 2010 using the Surveillance Network [16]. They found that from 2000 to 2010, the antimicrobial resistance of urinary *E. coli* isolates to ciprofloxacin and TMP-SMX among outpatients increased substantially.

Zi-Xing Zhong et al in 2020 in China, found that MICs of 11 antibiotics were determined against 12 clinical isolates. The MICs for nitrofurantoin ranged from 8 μg/ml to 32 μg/ml and all 12 clinical UPEC strains were susceptible.These12 clinical UPEC strains were classified as MDR *E. coli*. *In vitro* testing of Nitrofurantoin combinations indicated a synergistic action against all UPEC strains.

In our study we found that majority of *E. coli* isolates were sensitive to Nitrofurantoin on Disc Diffusion (74.7%), on VITEK (78.9%) as well as on Agar Dilution (76.8%), while as lesser proportion of *K. pneumoniae* isolates were sensitive to Nitrofurantoin on Disc Diffusion (25.7%), VITEK (31.4%) and Agar Dilution (20%). All these differences were statistically significant. Similar results of higher resistance of *K. pneumoniae* have also been found by many researchers. Garima Gautam et al, in 2021 in their study reported that out of total of 500 isolates, 20.17% isolates were resistant to nitrofurantoin while as much higher resistance was seen in *Klebsiella* sp. (44.61%) [15].

Conclusion

It can be concluded from our study that the MDR Enterobacteriaceae in urine (*Escherichia coli* and *Klebsiella pneumoniae*) have a high level of susceptibility to Nitrofurantoin which can be used as a dependable treatment option for UTIs in Kashmir.

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