Original Article

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Assessment of Drug Sensitivity of Different Urinary Isolates to Nitrofurantoin and its Comparison with Other Drugs- A Retrospective Study

Microbiology Section

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ABSTRACT

Introduction: Uncomplicated Urinary Tract Infection (UTI) is one of the most common indications for antibiotic use in the community. However, gram negative organisms that are predominant cause of UTI are becoming increasingly resistant to commonly used antibiotics. Consequently, empiric therapy is likely to fail resulting in increasing number of patients with uncomplicated UTI requiring hospitalisation for intravenous antibiotics as there are no oral treatment options left.

Aim: To assess the susceptibility trends of urinary isolates to panel of antibiotics with particular reference to nitrofurantoin.

Materials and Methods: The present retrospective study was conducted at Adesh Hospital Bathinda, Punjab, India, for a period of one year from April 2020 to April 2021. Kass criteria was followed for interpretation of significant bacteriuria according to which significant growth is considered if number of colony is more than 10^5 Colony Forming Unit (CFU)/mL. Culture positive were analysed by gram staining and on the basis of colony characteristics, gram staining, final identification was done using Vitek 2 compact system. Statistical analysis was done using Chi-square test.

Results: A total of 392 urinary isolates were identified during the study period. Out of the 392 isolates, 316 (80.6%) were gram negative isolates and 76 (19.4%) were gram positive isolates. Maximum sensitivity was shown to nitrofurantoin, 88% in case of gram negative isolates and 76% in case of gram positive isolates.

Conclusion: Nitrofurantoin is an effective therapeutic agent in the treatment of UTI. It has been used for a long time, but the emergence of antibiotic resistance and the decline in newly developed antibiotics has increased interest in the treatment of bacterial UTI with this antibiotic.

Keywords: Antibiotic resistance, Gram negative bacteria, Urinary tract infection

INTRODUCTION

The UTIs are amongst the most common infection seen in outpatient settings. It is defined as multiplication of organisms in the urinary tract. It is usually associated with the presence of polymorphs and 10^5 organisms in the Midstream Urine (MSU) sample. It is divided into two types depending upon site of involvement: Lower UTI and Upper UTI. Symptoms include dysuria, frequency, urgency and suprapubic tenderness. It is much more common in females than males due to anatomical and physiological reasons. Frequent use of antibiotics has led to increased antimicrobial resistance in urinary pathogens [1]. Uncomplicated UTI is one of the most common indications for antibiotic use in the community. They pose significant challenges for empiric treatment in outpatient settings. However, gram negative organisms that are predominant cause of UTI are becoming increasingly resistant to commonly used antibiotics. There are a few newer antibiotics on the horizon and those have been recently approved are mostly for intravenous use. This resulted in the need for re-evaluating the efficacy of old antimicrobials such as nitrofurantoin for their activity against these uropathogens.

Nitrofurantoin is an example of such old drug which was approved by Food and Drug Administration (FDA) in 1953. Many multidrug resistant organisms are becoming increasingly susceptible to nitrofurantoin. Advantage of this drug over newer one is its high urinary concentration, low serum concentration, minimal toxicity and almost nil impact on bowel flora [2]. Nitrofurantoin is an ideal prophylactic agent which is absorbed by oral route, mainly excreted in urine, active against putative pathogens. It is well tolerated, lacks side-effects and toxicity and has low cost. It has given consistently good results, and has often been used as a comparator in many clinical trials in prevention of recurrent UTI in adult women in older times. Keeping in mind the increasing resistance of uropathogens and to aid in developing the antibiogram of the hospital, the study was conducted to assess the susceptibility trends of urinary isolates to panel of antibiotics with particular reference to nitrofurantoin.

MATERIALS AND METHODS

This retrospective study was conducted in Bacteriology section of Microbiology Laboratory, Adesh Hospital Bathinda, Punjab, India. Data of last one year was collected (April 2020 to April 2021). Further, the data was analysed from May to July 2021. All the urine samples received in the laboratory for urine culture and sensitivity were included for the study. Ethical permission was taken from the Research Committee of the Institute with reference to letter number IRC 27.10.2020. Out of the 1,190 total urine samples received 392 organisms were isolated and these were included for further study of antibiotic susceptibility testing using the Vitek 2 compact system.

Inclusion criteria: All the urine samples received in the laboratory for urine culture and sensitivity were included in the study. Since, it was a retrospective study, the whole of the data was collected from hospital records.

Exclusion criteria: The samples which were not properly packed or labelled or with incomplete clinical data were excluded.

Study Procedure

The samples were received in universal sterile containers appropriately labelled and analysed within two hours after collection. Routine

microscopic examination of urine samples was done to look for pus cells, red blood cells and epithelial cells. Urine culture was done by semiquantitative method. A loopful 0.001 mL of well mixed uncentrifuged urine was inoculated on blood agar and MacConkey agar. The samples were streaked with the help of sterile inoculating loop on MacConkey agar and blood agar and were incubated for 24 hours at 37°C aerobically to check the bacterial growth. The plates were then examined macroscopically for bacterial growth. Kass criteria was followed for interpretation of significant bacteriuria according to which significant growth was considered if number of colony count is more than 10^5 CFU/mL.

Culture positive were analysed by gram stain and on the basis of colony characteristics, gram staining, final identification was done using Vitek 2 compact system. For gram positive organisms GP card was used and for gram negative GN card was used. For antimicrobial sensitivity testing in case of gram positive P628 card was used which included following panel of antibiotics gentamicin, ciprofloxacin, erythromycin, clindamycin, nitrofurantoin, vancomycin, teicoplanin, linezolid. For Antimicrobial Susceptibility Testing (AST) of gram negative organisms N280 card was used in case of lactose fermenting organisms and N281 was used in case of non lactose fermenting organisms. This card included following panel of antibiotics amikacin, gentamicin, ceftriaxone, cefepime, ciprofloxacin, nitrofurantoin, cotrimoxazole and piperacillin tazobactam.

STATISTICAL ANALYSIS

Data was analysed and effectiveness of nitrofurantoin over other newer drugs was calculated in frequencies and percentages. Statistical analysis was done using Chi-square test. A p-value <0.05 was considered to be statistically significant.

RESULTS

Out of the 1,190 total urine samples received 392 (32.94%) were culture positive and out of them 316 (80.6%) were gram negative isolates and 76 (19.4%) were gram positive isolates. A total of 251 (64%) were female patients and 141 (36%) were male. Mean age of the patients in this study was 42.3 years, mostly young sexually active females. Distribution of patients in hospital is shown in [Table/Fig-1].

Departments	Number	Percentage
Outpatient department	188	48
Inpatient department	118	30
Emergency	86	22

[Table/Fig-1]: Distribution of patients in hospital (n=392).

The urinary isolates majorly comprised 198 (50.50%) *Escherichia coli*, followed by 88 (22.50%) *Klebsiella pneumoniae*, 56 (14.28%) *Enterococcus faecalis* [Table/Fig-2].

Name of the organism	N (%)
Escherichia coli	198 (50.50)
Klebsiella pneumoniae	88 (22.50)
Enterococcus faecalis	56 (14.28)
Staphylococcus aureus	20 (5.10)
Pseudomonas aeruginosa	19 (4.80)
Acinectobacter baumannii	7 (1.80)
Citrobacter koseri	4 (1.02)

[Table/Fig-2]: Incidence of bacterial isolates from urine samples of patients.

Total gram negative isolates were 316. Gram negative isolates showed maximum sensitivity to nitrofurantoin (88%) followed by respectively by amikacin (73%) and piperacllin/tazobactum (54%) [Table/Fig-3]. Total gram positive isolates were 76. Gram positive isolates showed maximum sensitivity to nitrofurantoin (76%) followed by linezolid (69%) and teicoplanin (69%) each [Table/Fig-4].

Name of drug	Sensitivity n (%)	Resistance n (%)	p-value
Amikacin	230 (73)	86 (27)	<0.001 highly significant
Gentamicin	151 (48)	165 (52)	
Ceftriaxone	61 (19)	255 (81)	
Cefepime	129 (41)	187 (59)	
Ciprofloxacin	48 (15)	268 (85)	
Nitrofurantoin	**278 (88)**	**38 (12)**	
Cotrimoxazole	114 (36)	202 (64)	
Piperacillin/tazobactam	170 (54)	146 (46)	

[Table/Fig-3]: Antimicrobial resistance pattern of gram negative microorganisms.

Name of drug	Sensitivity n (%)	Resistance n (%)	p-value
Gentamicin	36 (47)	40 (53)	<0.001 highly significant
Ciprofloxacin	28 (37)	48 (63)	
Erythromycin	24 (32)	52 (68)	
Clindamycin	16 (21)	60 (79)	
Nitrofurantoin	**58 (76)**	**18 (24)**	
Vancomycin	44 (58)	32 (42)	
Teicoplanin	52 (69)	24 (31)	
Linezolid	52 (69)	24 (31)	

[Table/Fig-4]: Antimicrobial resistance pattern of gram positive microorganisms.

DISCUSSION

Different rates of antimicrobial sensitivity have been reported in urinary isolates by various authors. Apart from the progressive erosion nitrofurantoin appears to have good clinical and microbiological efficacy for UTI caused by common uropathogens. This is the preferred drug used for uncomplicated UTI caused by *Escherichia coli* [3]. Also, prescribed for recurrent UTI and in older women with low glomerular filtration rate [4,5].

In this retrospective study, a total of 392 (32.94%) samples out of 1,190 total samples received in laboratory had significant bacteriuria and confluent growth and were included. Maximum cases of UTI were from Outpatient Department (OPD) (48%) followed by Inpatient Department (IPD) (30%) and emergency (22%). It is not an emergency condition, so footfall of patients is more in OPD. Gender distribution shows that, 251 (64%) were female patients and 141 (36%) were males. Females are at high risk of UTI compared to males due to short urethra, close proximity to anus, use of diaphragmatic condom and spermicidal jellies. Similar results were found in a study by Neelima A and Kiranmai regarding male female ratio [6].

Out of the 1,190 total urine samples received 392 (32.94%) were culture positive and AST testing was done. Out of the 392 isolates 316 (80.6%) were gram negative isolates and 76 (19.4%) were gram positive isolates. The results were concordant with another study by Brumfitt W et al., where 80.3% of infections were by gram negative bacteria [7]. In another study, enterococci which is gram positive has been recognised as the second pathogenic agent of UTI [8]. Gram negative organisms are more common cause of lower UTI due to spread from perianal region whereas gram positive are associated with upper UTI due to haematogenous spread.

In current study the 392 (32.94%) urinary isolates comprised 198 (50.50%) *Escherichia coli*, followed by 88 (22.50%) *Klebsiella pneumoniae*, 56 (14.28%) *Enterococcus faecalis*, 20 (5.10%) *Staphylococcus aureus*, 19 (4.80%) *Pseudomonas aeruginosa*, 7 (1.80%) *Acinetobacter baumannii* and 4 (1.02%) *Citrobacter koseri.* In a similar study by Al Zarouni M et al., results were concordant [9]. Similar results were found in another study by Brumfitt W et al., [7]. So, in most of the studies the most common organism responsible for causing UTI was *Escherichia coli*. This may be attributed to Secreted Autotransporter Toxin (SAT), it has toxic effect against epithelial cell lining of urinary tract [10]. Current

results in relation to the most common uropathogen are concordant with the above two studies [7,9].

These isolates were tested against various antibiotics to determine their sensitivity and resistance patterns. The pathogens associated with UTI are multidrug resistant and similar resistance was noted in present study. In this study the gram negative urinary isolates were highly resistant to ciprofloxacin (85%), ceftriaxone (81%) and cotrimoxazole (64%), the three most commonly used antibiotics in the outpatient setting for treatment of UTI. Although, they remained predominantly sensitive to amikacin (73%), piperacillin and tazobactam (54%), these are parenterally administered and reserved for inpatient use. Nitrofurantoin is an oral drug to which they showed good sensitivity of 88%. The results were concordant with a study conducted by Khoshbakht R et al., where majority of the isolates were sensitive to nitrofurantoin (87.12%) [11]. They are similar to results documented by Shalini et al., Kibret M and Abera B, and Rijal A et al., [12-14]. Present results were similar to study by Raja NS where maximum isolates were from female patients (77%), 90% were *Escherichia coli* followed by *Klebsiella* spp showing maximum sensitivity to nitrofurantoin of 93% and 42%, respectively [15].

In case of gram positive isolates maximum sensitivity was shown to nitrofurantoin (76%) followed by linezolid (69%), teicoplanin (69%) and vancomycin (58%). In this study, the rate of resistance to nitrofurantoin was comparatively higher as compared to other studies. This may be attributed to increased usage of nitrofurantoin in recent times as compared to studies in historical era.

It is therefore recommended that routine microbiological analysis of urine samples to be carried out before administration of drugs for the treatment of UTIs. In doing so, development of unusual resistance among such strains could easily be detected and thus helped in better treatment and management of those infected by these pathogens. A Europe based surveillance study under antimicrobial resistance epidemiology survey on cystitis project suggested fosfomycin, nitrofurantoin and pivmecillinam as an effective agent for the treatment of uncomplicated UTIs [16]. Multiple novel agents approved are in the pipeline and nitrofurantoin is one such agent that would be suitable in our country for treatment of UTI.

Limitation(s)

Molecular analysis could not be performed for knowing genetic mechanism of drug resistance.

CONCLUSION(S)

Nitrofurantoin is an effective therapeutic agent in the treatment of UTI. It has been used in older times for a considerable long period but the upcoming resistance has increased interest in its use again. Time to time analysis of AST pattern of UTI isolates and to include molecular analysis for resistant strains is important.

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