

Nitrofurantoin susceptibility of ESBL gram negative isolates from patients with urinary tract infection (UTI) in a rural teaching hospital of Telangana

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Abstract

Introduction: Urinary tract infections (UTI) are the most common bacterial infections affecting humans throughout their lifetime. Escherichia coli are the most common pathogen in UTIs. Extended Spectrum Beta lactamase (ESBL)-producing E. coli-related UTI is an emerging problem in many parts of the world. Nitrofurantoin has been used for a long time, but the emergence of antibiotic resistance and the decline in newly developed antibiotics has increased interest in treatment of bacterial UTI with this antibiotic. **Materials & Methods:** A descriptive analysis of culture results of urine samples was performed at Microbiology department of MediCiti Institute of Medical Sciences from October 2014 to September 2015. Clean-catch midstream morning urine specimens were collected using sterile wide mouth disposable plastic container. The specimens were inoculated on nutrient agar, blood agar, and MacConkey agar plates and incubated aerobically at 37°C for 24-48 hours. Culture plates with colony counts of $\geq 10^5$ colony forming units (CFU) were considered positive for UTI. Antimicrobial susceptibility was performed according to CLSI guidelines. **Results:** A total of 2078 cases were included in the study, out of which 485 (23.33%) were males and 1593 (76.66%) females. Male to female ratio was 1:3.2. Majority of the cases (67.2%) belonged to age group 20-40 years. Escherichia coli (E.coli) (60.58%) was the most common pathogen among Gram negative isolates. 153 ESBL'S (49.83%) were reported in this study. Among these 153 isolates of ESBL producer's nitrofurantoin resistance was seen in 44 (28.75%) isolates. **Conclusion:** E.coli remained the predominant isolate among gram negative organisms, more commonly in females presenting to our hospital. The in vitro activity of Nitrofurantoin found in the present study suggests that this drug would provide adequate fluoroquinolones sparing alternative therapy at places where Cotrimoxazole use is no longer prudent because of high rates of resistance. This study shows that nitrofurantoin can be used even for drug resistant strains.

Keywords: Urinary tract infection, Extended Spectrum Beta Lactamase, Nitrofurantoin

Introduction

Urinary tract infections (UTI) are the most common bacterial infections affecting humans throughout their lifetime. They are the frequent cause of morbidity in outpatients as well as most frequently involved in the cause of nosocomial infection in many hospitals [1]. Compounded by a diminishing number of new agents entering clinical practice, resistance is widely recognized as a major threat to public health sectors. Escherichia coli are the most common pathogen in

UTIs. Extended Spectrum Beta Lactamase (ESBL) producing E.coli related UTI is an emerging problem in many parts of the world. In serious cases the major treatment choices are carbapenems, which usually require hospitalization and are associated with higher antibiotic costs [2].

Nitrofurantoin has been used for decades as an alternative treatment of uncomplicated UTI's. Additionally, nitrofurantoin has retained a high prevalence of sensitivity to most uropathogens and has a favorable side-effect profile [3]. Recently there has

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been a renewed interest in reviving older antimicrobial agents, particularly those with activity against multi-drug resistant gram negative bacilli. Nitrofurantoin is a broad spectrum bactericidal antibiotic that, through a complex mode of action which is not completely understood, affects both gram negative and positive bacteria.

Nitrofurantoin has been used for a long time, but the emergence of antibiotic resistance and the decline in newly developed antibiotics has increased interest in treatment of bacterial UTI with this antibiotic. Resistance to nitrofurantoin remained virtually unchanged since its discovery.

In this new microbiological era characterized by multi-drug resistant pathogens, nitrofurantoin is crucial. Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made.

Materials and Methods

Study design- A descriptive analysis of culture results of urine samples was performed at Microbiology department of MediCiti Institute of Medical Sciences from October 2014 to September 2015. The sex and age of patients, the organism isolated and the antimicrobial susceptibility profiles were collected from the registration records using a standard data collection form.

The samples were collected from both inpatients and outpatients and included male and female patients between 0 to 65 years of age who attended the hospital. Samples were collected from patients with clinical diagnosis of uncomplicated urinary tract infection and who have not received antibiotics within 3 days of presentation to the hospital. Patients demographics including age, sex and previous antibiotic usage and relevant medical history were collected from information provided to the laboratory and from patients clinical notes. Exclusion criteria for the study included pregnant and lactating women, patients who had genito-urinary tract disease or abnormalities that may preclude evaluation of therapeutic response or those who had gastrointestinal tract conditions that might affect adequate drug absorption.

Culture and Identification: As the standard operation procedures show clean-catch midstream morning urine

specimens were collected using sterile wide mouth disposable plastic container. Pyuria was considered if there was ≥ 10 leucocytes/ml of centrifuged urine sample. Only one isolate per patient was processed to avoid strain duplication. Samples were processed on the same day and when there was delay; samples were stored at 2°C- 4°C until being processed. A semi quantitative method was adopted for primary isolation of organisms using a calibrated loop of 4 mm diameter which delivers 0.01ml of urine [4].

The specimens were inoculated on nutrient agar, blood agar, and Mac Conkey agar plates and incubated aerobically at 37°C for 24-48 hours. Culture plates with colony counts of $\geq 10^5$ colony forming units (CFU) were considered positive for UTI.

Cultures that showed no growth in 24 to 48 hours indicated absence of infection. From positive cultures, uropathogens were identified according to the standard biochemical reactions [5]. A significant bacterium was considered if urine culture yielded $\geq 10^5$ CFU/ml [6].

Antimicrobial susceptibility testing: According to the standard operational procedures, in vitro antimicrobial susceptibility testing was done on Mueller-Hinton agar (Hi-Media Lab Ltd, India) using Kirby-Bauer disc diffusion method. A suspension of test organism was made in sterile normal saline and turbidity adjusted to 0.5 McFarland standards.

The test organism was uniformly seeded over the surface of Mueller Hinton agar plates. The plates were allowed to dry for 10 minutes before application of antibiotic impregnated discs. The plates were incubated at 37°C for 16-18 hours. After incubation clear zones around the antibiotic discs were measured with a ruler and recorded in millimeters.

The antimicrobial agents tested were: Cefazolin (30µg), Nitrofurantoin (300µg), Amoxycylav (30µg), Gentamicin (10µg), Amikacin (30µg), Cipro floxacin (5µg), Norfloxacin (10µg), Cefuroxime (30µg), Cefotaxime (30µg), and Cotrimoxazole (25µg) (Hi-Media Lab Ltd, Mumbai). Resistance data was interpreted according to Clinical laboratory Standards Institute guidelines⁷

The screening for extended spectrum beta lactamase (ESBL) was done using cefpodoxime (≤ 17 mm), ceftazidime (≤ 22 mm), aztreonam (≤ 27 mm), cefotaxime (≤ 27 mm), and ceftriaxone (≤ 25 mm). If the organisms showed a zone of inhibition lower than the

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minimum for any antibiotic disc, ESBL positivity was suspected. The phenotypic confirmation was done by testing the strain against ceftazidime (Ca) and ceftazidime / clavulanic acid. A >5-mm diameter of the zone of inhibition for ceftazidime / clavulanic acid in comparison to ceftazidime was considered indicative of

ESBL production. *Escherichia coli* ATCC 25922 was used as an ESBL-negative and *Klebsiella pneumoniae* 700603 was used as an ESBL-positive reference strain [7].

Results

A total of 2078 cases were included in the study after obtaining consent from them. Out of which 762 (36.66%) were from OP and 1316 (63.33%) from IP. 485 (23.33%) were males and 1593 (76.66%) females. Male to female ratio 1:3.2. Majority of the cases (67.2%) belonged to age group 20-40 years. (Table 1)

Table-1: Age wise distribution of cases.

Age group	number	percentage
neonates	33	1.58
paediatric	197	9.48
20-40 years	1397	67.2
41-60 years	252	12.1
>60years	199	9.57

The culture positivity rate observed in this study was 19.1% (397 out of 2078 samples). Gram negative isolates were 307 (14.77%), 84 (4.04%) were Gram positive isolates and 6 (0.28%) candida species.

There was no polymicrobial growth. *Escherichia coli* (E.coli) (60.58%) were the most common pathogen among Gram negative isolates followed by *Klebsiella* spp. (25.7%) (Table 2). *S.aureus* (23.8%) was the most common pathogen among Gram positive isolates.

Table-2: Frequency of gram negative isolates reported from urine samples.

S.No.	Gram negative isolate	Number	Percentage
1.	<i>Escherichia coli</i>	186	60.58
2.	<i>Klebsiella</i> spp.	79	25.7
3.	<i>Enterobacter</i> spp.	4	1.3
4.	<i>Citrobacter</i> spp.	17	5.53
5.	<i>Proteus</i> spp.	11	3.58
6.	<i>Pseudomonas</i> spp.	8	2.6
7.	<i>Flavobacterium</i> spp.	1	0.3
8.	<i>Weeksiella virosa</i>	1	0.3

Carbapenems had the least resistance (3.9%), followed by amikacin (28.0%), and nitrofurantoin (29.9%). A high rate of resistance was recorded against quinolones (74.1%) and co-trimoxazole (100%).

153 ESBL'S (49.83%) were reported in this study. Among these 153 isolates of ESBL producer's nitrofurantoin resistance was seen in 44 (28.75%) isolates. Among non ESBL producers nitrofurantoin resistance was observed in 48(31%) isolates

There was no much difference in nitrofurantoin resistance observed among ESBL and non ESBL strains emphasizing that nitrofurantoin can be used even among multidrug resistant organisms.

Discussion

With the increases in antibiotic resistance among *E. coli* and other Enterobacteriaceae over the past several decades, surveillance data have become critical for appropriate empiric selection of antibiotic therapy. U.S. guidelines specify that TMP/SMX should be avoided for empiric treatment of uncomplicated acute cystitis or pyelonephritis in populations where non-susceptibility to this agent exceeds 20% in uropathogens. Antibiotic treatment is typically selected empirically, based on the patient clinical presentation, medical history and local patterns of antibiotic susceptibility [8].

In the present study gram negative pathogens (14.7%) outnumbered gram positive organisms (4.33%) which is comparable to study done by Khoshbakht R et al 2013 who reported predominance of gram negative bacilli (83.17%) while gram positive organisms as 21.73% [9]. Among gram negative uropathogens *E. coli* remained predominant isolate (60.58%) which is in agreement with the findings of Khoshbakht R et al 2013, Shalini et al 2011 and Kibret M & Abera B 2014 who also reported *E. coli* as most predominant pathogen isolated from urine samples with prevalence of 66.08%, 64.33% and 63.6% respectively [9,10,11].

Majority of *E. coli* isolates were susceptible to Nitrofurantoin (87.12%), with resistant isolates only 12.89%, which is similar to results documented by Shalini et al 2011, Kibret M & Abera B 2014 and Rijal A et al 2012, in which sensitive isolates were 93.48%, 96.2% and 96.5% with only 6.52%, 3.8% and 3.5% resistant isolates [10,11,12].

The drug of choice as depicted by the findings of present study remains Nitrofurantoin as 70.1% isolates were sensitive, with only 29.9% isolates resistant. The consistent and high-level susceptibility of gram negative isolates to nitrofurantoin may be influenced by Nitrofurantoin's narrow spectrum of activity, limited indication, narrow tissue distribution, and limited contact with bacteria outside the urinary tract [13].

Conclusion

E. coli remained the predominant isolate among gram negative organisms, more commonly in females presenting to our hospital. In developing countries, self-medication because of on counters availability of antibiotics and secondly the unwanted prescriptions of antimicrobials by physicians make the situation worst. The in vitro activity of Nitrofurantoin found in the

present study suggests that this drug would provide adequate fluoroquinolones sparing alternative therapy at places where Cotrimoxazole use is no longer prudent because of high rates of resistance. This study shows that nitrofurantoin was found to be susceptible even resistant strains. As it is a sparing drug it should be used judiciously. A review of antibiotic policy pertaining to treatment of urinary tract infections is necessary, which shall require interactions of various departments.

A common working policy has to be formulated using local surveillance data to guide the empiric selection of antibiotic therapy as well as prevention of development of resistance.

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