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Evaluating the Potential of Nitrofurantoin and Fosfomycin for *E. coli* UTIs: A Susceptibility Study

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Abstract: This study was designed to find the susceptibility of Nitrofurantoin and Fosfomycin among urinary isolates of *Escherichia. coli*. Four hundred (400) urine samples were collected for susceptibility of nitrofurantoin and fosfomycin among urinary isolates of *E. coli*. All indoor and outdoor patients' urinary samples yielded growth of *E. coli*. Mid-stream urine specimens were inoculated on blood agar and CLED agar and incubated at 35 ± 2 ℃. Growth was observed, and *Escherichia coli* was identified by Gram staining, Catalase, Motility test and API 20E (Bio murex) as per standard procedure. Antimicrobial susceptibility testing of isolates for nitrofurantoin and fosfomycin was carried out by the modified Kirby-Bauer disc diffusion method according to CLSI guidelines ATCC 25922. *E. coli* was used as a quality control strain. A total of 400 samples were tested susceptibility of nitrofurantoin and fosfomycin among urinary isolates of *E. coli* during this period. A total of 400 samples yielded the growth of *E. coli*, out of which 178 (44.5%) were male and 222 (55.5%) were female samples. Among males, 18 (10%) were tolerant to nitrofurantoin, and 2 (1.1%) were tolerant to fosfomycin. Among females, 9 (4.09%) were susceptible to nitrofurantoin while 6 (2.72%) were susceptible to fosfomycin. Among age groups below 45 years old, 6 (4.76%) were tolerant to nitrofurantoin, and 2 (1.58%) were sensitive to fosfomycin. Between 46–66 years old, 4 (2.81%) were sensitive to nitrofurantoin, and 3 (2.11%) were sensitive to fosfomycin. Between 67–90 years old, 17 (12.87%) were sensitive to nitrofurantoin, and 4 (3.03%) were tolerant to fosfomycin. Fosfomycin and nitrofurantoin showed good susceptibility in urinary isolates of *E. coli* and can be used empirically in our setup.

Keywords: *E. coli*; Fosfomycin; Nitrofurantoin; Susceptibility; Urinary isolates

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1. Introduction

Escherichia coli (*E. coli*) is a coliform bacterium of the genus Escherichia, a facultatively anaerobic, gram-negative, and rod-shaped bacteria that is mostly found in the lower intestine of the endotherm. They are not dangerous and harmful in most cases, but some strains of *E. coli* can lead to serious food poisoning in the host. As a

result, several studies have found food contamination due to such strains of *E. coli*. Our gut comprises healthy flora and is useful to its host organisms by the synthesis of vitamin K1. Such normal flora of *E. coli* hinders the colony formation of pathogenic bacteria in the host intestine [1].

Escherichia coli and facultative anaerobes consist of about 0.1% gut flora and the majority of disease-causing bacteria get into the body through fecal-oral transmission [2]. However, an increasing amount of studies have targeted physiologically lasting *E. coli* that could thrive for lengthy periods without a host. Given the minute peptidoglycan coating and outer membrane that make up their cell wall, particular types of *Escherichia coli* are gram-negative. A broad variety of bacterium called *Escherichia coli* exists normally in the digestive tracts of all humans and some other kinds of animals [3]. The identification of six distinct *E. coli* pathotypes that cause enteric/diarrheal, enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) significantly improved our knowledge of harmful *E. coli*. The most significant violent component of *E. coli* that is frequently separated in UTIs is pyelonephritis-associated P fimbriae (pap) [4]. The main function of P fimbriae was adherence. The two subunits of pap fimbriae are the short fimbrial tip consisting PapG and the big subunit PapA. It develops on blood agar as well as differential media that produce pink colonies by fermenting lactose, such as MacConkey's agar or EMB agar. *E. coli* colonies show a distinctive green sheen on EMB agar. An outdated, wide-ranging antibiotic called fosfomycin prevents the bacterial cell from synthesizing [5]. Compared to urinary clinical isolates with comparatively significant amounts of antibiotic resistance, fosfomycin demonstrates a notably substantial degree of antibacterial properties [6].

As a result, an oral single-dose medication called fosfomycin tromethamine was recently recommended to treat acute, simple cystitis. Fosfomycin is mostly used in clinical settings based on its proven *in vitro* effectiveness against the isolated pathogen, which is ascertained by using the minimum inhibitory concentration (MIC) standards outlined by the Clinical and Laboratory Standards Institute (CLSI) for *E. coli*. Furthermore, there is a suggestion that the immunomodulatory efficacy of fosfomycin could interact with its antibacterial efficacy against ESBL-producing *E. coli* [7]. Urinary tract infections (UTIs) are primarily caused by *Escherichia coli*. Uropathogenic *E. coli* (UPEC) is the name given to *E. coli* that causes UTIs because of its numerous virulence characteristics and capacity to acquire antibiotic resistance quickly. According to reports, nosocomial and community-acquired infections rank highest for UPECs in the community. It is widely recognized that one of the major manufacturers of beta-lactamases is *Escherichia coli*. By hydrolyzing the beta-lactam antibiotics, the bacteria that make the beta-lactamase enzyme have developed a resistance strategy [8].

About 50% of UTIs acquired in hospitals and 85% of UTIs acquired in the community are caused by *E. coli*. Several variables, including age, gender, immunosuppression, and urological equipment can influence the incidence of UTIs. One of the most serious health dangers is catheter-associated UTIs, which account for 34% of infections related to healthcare. Cephalosporin and ciprofloxacin's empirical usage is of concern due to an increase in extended-spectrum beta-lactamases [9]. Recombination of foreign DNA in bacterial chromosomes, horizontal gene transfer, and genetic material change are some of the ways that microorganisms exploit to generate antibiotic tolerance. Microorganism resistance trends differ between countries, states, big and small hospitals, and hospitals and communities. Antibiotic abuse and misuse in Pakistan are exacerbating the issue of drug resistance. Antibiotic resistance is not systematically monitored nationally, and there is not enough data available to accurately estimate the issue. Finding the bacteria that cause UTIs and their resistance to routinely administered antibiotics in the clinical setting is crucial for enhancing the effectiveness of empirical therapy [10]. The issue is becoming more and more significant in countries where the appearance and spread of CTX-M enzymes in this strain resulted in a significant spike in the community-level incidence of ESBL-producing *E. coli*.

Our research aims to investigate the fosfomycin and nitrofurantoin susceptibility of urine isolates of *E. coli*. The goals seemed to establish the nitrofurantoin and fosfomycin susceptibility for urinary isolates to give physicians information about present trends of susceptibility, which would help them choose suitable empirical therapies for UTIs.

In the field of microbiology, it is a need of time to frequently assess the changing trend of pathogenic and opportunistic pathogens which otherwise results in increasing mortality, morbidity and huge economic loss of resources. Antimicrobial resistance has sequentially emerged to traditional first-line drugs, posing patient treatment challenges. So, keeping these alarming factors in mind, a descriptive study was designed to assess the antibiogram in our clinical setting. Various studies are available for the assessment of the susceptibility of nitrofurantoin and fosfomycin among urinary isolates of *E. coli* in the local population and their association with age and gender in patients who were referred to AFIP for testing of UTIs. Data about the spectrum of susceptibility of nitrofurantoin and fosfomycin will help clinicians regarding the diagnosis of UTIs in suspected patients.

2. Methodology

It is a cross-sectional study. All urinary clinical samples belonging to all age groups yielding the growth of *E. coli* are included in the study. Duplicate isolates from the same patient, improperly collected samples and samples yielding mixed growth are excluded from the study. All indoor and outdoor patients' urinary samples yielded growth of *E. coli* at AFIP, CMH Complex, Rawalpindi. A total of 400 samples were recruited during the study period. Data was coded and entered in Microsoft Excel 2010 and Statistical Package for Social Sciences (SPSS version 24). All the components in the questionnaire including socio-demographic & additional questions about β-thalassemia, were coded for analysis. Data was analyzed using descriptive cross-sectional statistics such as prevalence, frequency and percentages of all the variables.

The urine sample was collected in the container after taking proper patient information. Age, gender and lab IDs were recorded of all the patients whose samples were collected for culture. Techniques that were used during our study were gram staining, catalase test, motility test, AP1 20 E, and antimicrobial susceptibility testing. Samples of mid-stream urine have been incubated at 35 ± 2 °C after being treated on blood and CLED agar. Propagation was recognized, and using the normal protocol, *E. coli* was recognized by gram staining, catalase, the motility test, and API 20E (Bio murex). In accordance with CLSI recommendations ATCC 25922, the modified Kirby-Bauer disc diffusion method was used to evaluate the isolates' antimicrobial resistance to nitrofurantoin and fosfomycin. A strain of *E. coli* has been used as a screening check.

Gram-negative bacteria become decolourized and absorbed by the colour of counterstain, appearing pink under an oil immersion lens microscope, but gram-positive bacteria stain with crystal violet and are not decolourized with acetone. Produce a smear first, then quickly move the slide over the flame, coating it with crystal violet for 30 seconds, pouring it out, washing it, and then wrapping it with the mixture of gram iodine and leaving it to act for another 30 seconds. Subsequently, the iodine solution was taken out and carefully cleaned with water. The slide was then placed under a microscope using an oil immersion lens, coated with acetone iodine for 15 seconds, and counter-stained with diluted carbol fuchsin for 30 seconds.

A very poisonous substance to bacteria, hydrogen peroxide, is an oxidative by-product of the aerobic decomposition of carbohydrates. By catalyzing breaking apart hydrogen peroxide into nontoxic compounds of oxygen and water, the catalase enzyme generated by some bacteria renders this hazardous product useless. Fill a test tube with 2–3 mL of brand-new hydrogen peroxide solution. A substantial part of the test organism's development should be removed using a sterilized wooden or glass rod, and it should be submerged in hydrogen

peroxide solution. Seek out instant bubbling, as this signifies the creation of oxygen.

The organism's potential for movement is determined by the motility test, which identifies it. In a test tube, create a semisolid agar medium. Utilizing a straight wire, inoculate the tube by making a single stab down its centre, approximately halfway down the medium's depth. After a period of favourable circumstances for motility, incubate at 37 ℃. With the help of a few standard tests, the API 20E rapid detection system may identify a small number of Gram-negative Enterobacteriaceae or non-Enterobacteriaceae. Twenty tiny response tubes, complete with substrates, are used for preserving the test systems. A test organism population is combined with regular saline to create a bacterial suspension that is then added to these tubes for inoculation. Colour variations caused by metabolism during incubation can either be natural or result from the addition of chemicals. The reading table is then used to evaluate the responses, and the list of profiles is used to determine identity.

Table 1. API20E (Test reading table)

Tests	Negative	Positive
ONPG	Colorless	Yellow
ADH	Yellow	Red/Orange
LDC	Yellow	Red/Orange
ODC	Yellow	Red/Orange
CIT	Pale-green/Yellow	Blue-green/Blue
H2S	Colorless	Black deposit/Thin line
URE	Yellow	Red/Orange
TDA	Yellow	Reddish brown
IND	Colorless	Pink
VP	Colorless	Pink/Red
GEL	No diffusion	Diffusion of black pigment
GLU	Blue-green/Blue	Yellow
MAN	Blue-green/Blue	Yellow
INO	Blue-green/Blue	Yellow
SOR	Blue-green/Blue	Yellow
RHA	Blue-green/Blue	Yellow
SAC	Blue-green/Blue	Yellow
MEL	Blue-green/Blue	Yellow
AMY	Blue-green/Blue	Yellow
ARA	Blue-green/Blue	Yellow

2.1. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) of the isolate was carried out using Kirby-Bauer disk diffusion method on Muller-Hinton as recommended by Clinical Laboratory Standard Institute (CLSI) guidelines 2018. The inoculation was prepared by touching the top of the colonies of the isolates with a sterile wire loop and suspending 0.5 mL of saline (Equal to 0.5 MacFarland's standards). After making the inoculum suspension, dip a sterile, cotton swab into the suspension. Inoculate the dry surface of a Muller-Hinton agar plate by streaking the swab over the entire agar surface. By using sterile forceps, or a multidisc dispenser, place appropriate disks. The

disks should be about 15 mm from the edge of the plate and no closer than about 25 mm from disk to disk. The plates were incubated at 35 °C for 24 hours. After 24 hours of incubation, by using a ruler zone of inhibition was measured in mm.

3. Results

Table 2. Mean and standard deviation of demographic variables for *E.coli*

Demographic variable	Mean ± SD
Age	53.16 ± 2.218

A total of 400 samples yielded the growth of *E. coli* from July 2018 to December 2018, out of which 178 (44.5%) were male and 222 (55.5%) were female samples. The age distribution of patients ranges from 3 to 95 years old with a mean age of 53.16 and a standard deviation of 1.109. Two (1.1%) and eighteen (10%) males proved susceptible to fosfomycin and nitrofurantoin, respectively. Nine females (4.09%) and six (2.72%) had susceptibility to fosfomycin and nitrofurantoin, respectively. Six (4.76%) and two (1.58%) of the age groups under 45 years old were susceptible to fosfomycin and nitrofurantoin, respectively. Four (2.81%) of the individuals between the ages of 46 and 66 had resistance to fosfomycin, and three (2.11%) to nitrofurantoin. Of those aged 67–90, 4 (3.03%) and 17 (12.87%) were resistant to fosfomycin and nitrofurantoin, respectively.

Among 400 urinary samples, ESBL-producing *E. coli* were more isolated from male samples as 130 (73%) out of 178 whereas female samples were 105 (47%) out of 222 were ESBL positive. The percentage of antimicrobial susceptibility of urinary samples showed a sensitivity of 97% with fosfomycin and 92% with nitrofurantoin.

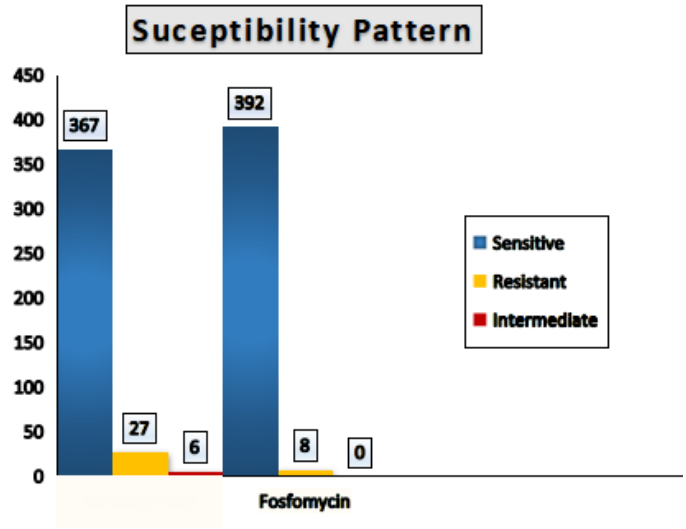

Figure 1. Susceptibility pattern of both drugs.

Table 3. Sensitivity of *E. coli* against nitrofurantoin and fosfomycin in male and female patients

Gender	Total	Sensitivity	
		Nitrofurantoin	Fosfomycin
Male	178	158	196
Female	222	209	216

Table 4. Association of gender with ESBL

Gender	ESBL results		*p*-value
	Positive	Negative	
Male	*n* = 120 (67%)	*n* = 58 (33%)	0.001
Female	*n* = 105 (47%)	*n* = 117 (53%)	

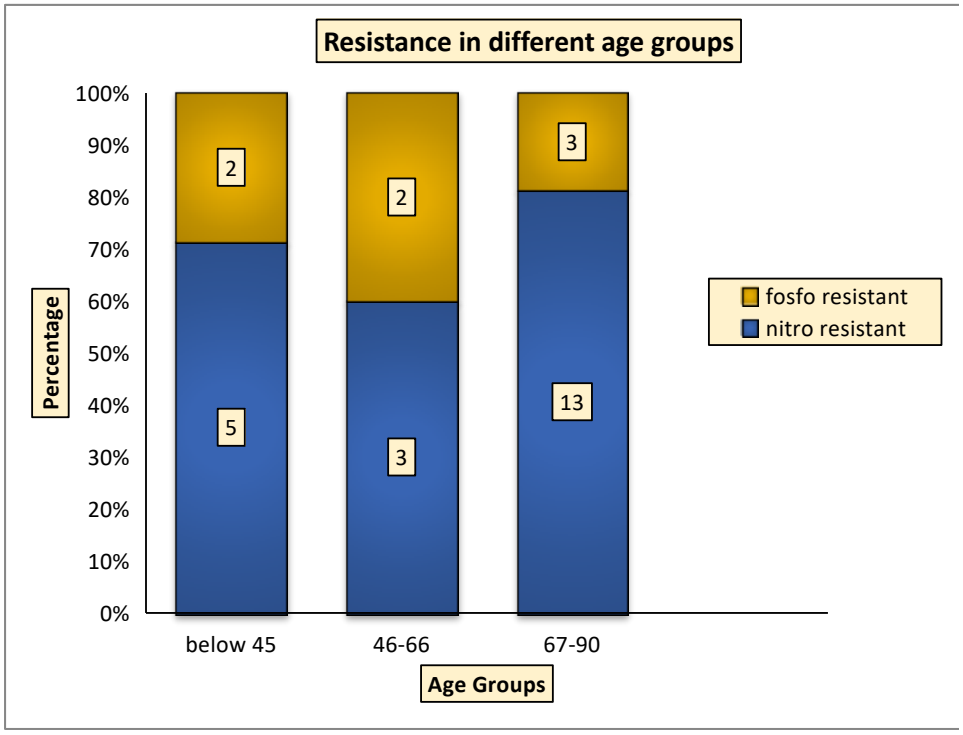

Figure 2. Resistance in age groups.

4. Discussion and conclusion

Escherichia coli is a particularly prevalent bacteria associated with UTIs, which affects at least 250 million people worldwide each year. The kind of *E. coli* that causes UTIs is known as uropathogenic *E. coli* (UPEC) [11]. According to reports, UTIs rank highest among community-acquired and nosocomial infections. It is well known that *E. coli* is a significant generator of beta-lactamases. The beta-lactamase-producing bacteria have a potent resistance mechanism that uses hydrolysis to deactivate beta-lactam antibiotics [12]. In the present study, 400 urinary samples were taken, yielding the growth of *E. coli*, out of which 178 (44.5%) were male and 222 (55.5%) were female samples, as the majority of patients were female. The result of our study was comparable with Paykoc EI *et al.* (2018)'s conducted study on "*Investigation of P Fimbriae Presence in Escherichia coli Strains Isolated from Urine Samples in Human, and Their Antibacterial Resistance"* [13]. In our study, *E. coli* was commonly isolated from elderly patients in the age group of 46–66 years old, where 5 patients were resistant and in the age group of 67–90 years old, 18 patients were resistant showing that resistance was more common in the older age group. It was in good agreement with Sabir S *et al.* (2014) in "*Isolation and Antibiotic Susceptibility of E. coli from Urinary Tract Infections in a Tertiary Care Hospital*" [14].

Nitrofurantoin appears to have good clinical and microbiological efficacy for UTIs caused by common uropathogens and susceptibility against *E. coli* was tested in our study, which shows 367 (91.8%) were sensitive, whereas 33 (8.3%) were resistant out of 400 isolates showing the sensitivity of drug. Our study was in agree-

ment with Huttner A *et al.* (2015) in *"Nitrofurantoin Revisited: A Systematic Review and Meta-analysis of Controlled Trials"* [15]. Fosfomycin had good clinical outcomes against *E. coli* and susceptibility were tested. Our study shows 392 (98%) were sensitive and 8 (2%) were resistant out of 400 isolates. Our study is similar to the study conducted by Lai B *et al.* (2014) in *"In vitro Susceptibility of Escherichia coli Strains Isolated from Urine Samples Obtained in Mainland China to Fosfomycin Trometamol and Other Antibiotics: A 9-year Surveillance Study"* [16].

Our results are in agreement with previous studies. Seo MR *et al.* (2014) conducted a study on *"Susceptibility of Escherichia coli from Community-Acquired Urinary Tract Infection to Fosfomycin, Nitrofurantoin, and Temocillin in Korea in 2015"* [17]. Antimicrobial susceptibilities of the strains to both nitrofurantoin and fosfomycin were 91% and 98% respectively and comparable with the above study showing the efficacy of drugs. Our results are in contradiction with Singh S *et al.* (2015) *"Prevalence of Drug Resistance in ESBL-producing Escherichia coli Causing UTI in Rural Tertiary Care Hospital from Haryana, India"* [18]. In our study, males were more ESBL producers than females but in this study, females were more ESBL producers. Results are limited to isolates only cultured at one centre. So, results of the study cannot be generalized to the overall population. The method that was used for antibiotic susceptibility was Kirby Bauer disk diffusion. Further studies may be carried out using MIC by broth agar dilution which is a standard procedure.

It is concluded that *Escherichia coli* is a common pathogen associated with UTI and other infections. It is common emerging as another pathogen thus becoming more resistant to routinely used antibiotics. UTI can be reduced by the use of these two drugs: (1) nitrofurantoin and (2) fosfomycin by adopting strict infection control methods. Due to the changing pattern of antibiotics, a cross-sectional study was designed. To avoid resistance in clinical isolates local antibiotic policies must be made and followed to control the spread.

Authors contribution

Conceptualization: Usama Ahmed, Muhammad Zubair, Baqa ur Rehman, Hafiz Muhammad Sultan
Investigation: Usama Ahmed
Formal analysis: Usama Ahmed
Writing – original draft: Usama Ahmed
Writing – review & editing: Usama Ahmed, Muhammad Zubair, Baqa ur Rehman, Hafiz Muhammad Sultan

Disclosure statement

The author declares that they have no conflict of interest.

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