DIAGNOSTIC EVALUATION OF NORFLOXACIN, FOSFOMYCIN AND NITROFURANTOIN IN URINARY ISOLATES CAUSING UTI

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ABSTRACT

Objective: Diagnostic evaluation of Norfloxacin, Fosfomycin and Nitrofurantoin in urinary isolates causing UTI

Material and Methods: It was Comparative Cross-sectional study. It was conducted at Department of Pathology at THQ hospital Pindigheb. The study was completed (including data compilation & analysis) in 1 year period from 28 August 2021 to 27 August 2022. Two hundred and seventy-five urinary isolates from urine specimens received for culture and sensitivity in the department of pathology at THQ hospital Pindigheb, were included in the study, as approved in the Internal Review Board. Non-probability consecutive sampling technique was used. Isolates were identified as urinary isolates by colonial morphology, Gram stain and biochemical tests. The zones of clearance around filter paper disc of Norfloxacin (10 µg), Fosfomycin (200µg) and Nitrofurantion (300µg) impregnated over lawn of bacterial isolate on Mueller-Hinton agar by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines were recorded.

Results: Out of total 275 samples 180(65.45%) samples yielded significant growth of Enterobacteriaceae. The females were 44.43% and males were 55.55% in indoor and outdoor patient respectively. 0ut of 180 samples 100(55.55%) samples yielded growth of *Escherichia coli* followed by 50 (27.77%) were *klebsiella pneumoniae* and 30 (16.67%) were *Enterobacter spp.* The sensitivity pattern of *Escherichia coli* was noted to norfloxacin, Fosfomycin and nitrofurantoin {(50%,30%)},and {(6%, 2%)} resistance and sensitive respectively. *klebsiella pneumonia* and *Enterobacter cloacae* showed resistance and sensitive pattern {60%,10%)}(1), {(33.33%,16.67%)}, {(2%,10%)},{(10%,6.7%)}and {(4%,2%)},{(13.33%,20%)} against norfloxacin, Fosfomycin and nitrofurantoin respectively.

Conclusion: Our study revealed high frequencies of norfloxacin, Fosfomycin and nitrofurantoin resistance among uropathogen at THQ hospital Pindigheb. A strong liaison between clinicians and microbiologists is recommended to keep abreast with the changes in antimicrobial susceptibility of uropathogens in order to suggest a suitable empiric therapy. Infection control measures are recommended, so that appropriate management can be instituted and spread of these resistant organisms curtailed.

Key Words: Norfloxacin resistance, *Escherichia coli*, Urinary tract infection. Urinary isolates, Fosfomycin.

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INTRODUCTION

Urinary tract infection (UTI) is a serious health issue affecting a large number of people worldwide [1, 2]. UTIs can be caused by Gram negative bacteria such as *Escherichia coli*, Klebsiella species, Enterobacter species, Proteus species and Gram-positive bacteria like Enterococcus species, and *Staphylococcus saprophyticus*. *E.coli* is the most common organism causing both community as well as hospital acquired UTI [3] *E. coli* accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs [2,4]. Various factors like age, gender, immunosuppression and instrumental intervention, either diagnostic or therapeutic, may affect the prevalence of UTIs [5]. Because of the uncomfortable symptoms, the treating physicians usually prescribe antibiotic treatment prior to obtaining the culture results [5].

Detection of UTI causing pathogens and resistance of these pathogens to commonly prescribed antibiotics in clinical set ups is essential and helpful in improving the efficacy of empirical treatment [7, 8].

The resistance rates of uropathogens to various antibiotics have been reported from Brazil and Pakistan as beta-lactams (57.4%), co-trimoxazole (48.5%), gentamicin (58.2%), amikacin (33.4%), cefuroxime (56%), nalidixic acid (77.7%), nitrofurantoin (10%), fosfomycine (6%) and fluoroquinolones (74.5%) [10,11].

This study will document the susceptibility of *E.coli, k.pneumoniae* and *Enterobacter* isolated from

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urine samples of UTI cases to norfloxacin, Fosfomycin and nitrofurantoin at THQ hospital Pindigheb bearing in mind that no such study had been carried out before. It will establish a treatment protocol in this health care setting and will limit inappropriate antimicrobial usage. The objective of this study was to compare and evaluate resistance of Norfloxacin, Fosfomycin and Nitrofurantoin in urinary isolates causing UTIs at THQ hospital Pindigheb. Data was analysed using SPSS version 2024

MATERIAL AND METHODS

Study design was Comparative Cross-sectional study. The study was conducted at Department of pathology at THQ hospital Pindigheb. The study was completed (including data compilation & analysis) in 1 year period from 28 August 2021 to 27th August 2022. Sample size was calculated by WHO calculator using the formula; N=z2P (1-p/e; where z=confidence interval (CL), p= prevalence and e= error in CL. Two hundred and seventy-five urinary isolates from urine specimens received for culture and sensitivity in the department of pathology at THQ hospital Pindigheb, were included in the study, as approved in the IRB. 50 samples were contaminated 15 samples yielded mixed isolates 30 samples were yielded no growth. Non-probability consecutive sampling technique was used. The urine samples of either gender, age 18 ~ 75 years, presenting with symptoms of UTI (e.g., increased urinary frequency along with urgency and dysuria) and yielding growth of urinary isolates were included. Duplicate samples of same patient and samples of patients already on antibiotics were not included. After obtaining institutional ethical committee's approval and informed consent, the urine specimens were collected from both indoor and outdoor patients. Five ml of clean voided midstream urine was collected in a sterile leak-proof container after cleansing the genital area with gauze sponges soaked in non-bacteriostatic saline. Urine was collected by sterile aspiration of catheter with needle and syringe catherized patients. The urine cultures were made semi quantitatively, so that bacteria per ml of urine be estimated. A bacteriuri test strip (MAST DIAGNOSTICS) was dipped in the urine upto a defined mark (the strip picks 0.2µl of urine). The strip carrying urine was inoculated on Cysteine lactose electrolyte deficient agar (CLED agar) and incubated at 35-37˚C under aerobic condition for 24 hours. After overnight incubation, the CLED agar plates were examined and colonial count of 20 colonies (10^5/ml) on the inoculated area were taken as significant growth. The organisms were identified based on colonial morphology, Gram staining and biochemical tests· confirmation to the species level were carried out by using API 20E.Bacterial suspensions were made in normal saline, matching 0.5 McFarland turbidity standards. This was done by touching at least five to ten colonies from a pure growth with a straight loop and mixing in normal saline. The suspension was matched with 0.5 McFarland standard and turbidity adjusted accordingly. For preparation of 0.5 McFarland standard, 0.5 ml of 1% $BaCl_2$ was added to 99.5 ml of 1% H_2SO_4 with constant stirring. The standard was distributed into screw capped bottles and stored in dark at room temperature. Control used as *Escherichia coli* ATCC 25922 [12]. According to CLSI guidelines, inoculums (0.5 McFarland) of bacterial suspensions of isolates were plated on Mueller-Hinton agar (Oxoid, Basingstoke, UK). The surfaces of the agar were allowed to dry for 5 minutes. Concurrent quality control testing was performed. Bacterial suspension of control strains were prepared as described above and matched with 0.5 McFarland standard. These were inoculated on separate Mueller-Hinton agar plates.

Using sterile forceps, Norfloxacin (10µg) disc (Oxoid, Basingstoke, UK), nitrofuration (300µg) and Fosfomycin (200µg) were placed on the inoculated plates [12]. Within 30 minutes of applying the discs, the plates were incubated aerobically at 35±2˚C for 16 – 18 hours. After overnight incubation, the control and test plates were examined. The diameter of each zone of inhibition around the antimicrobial Disc was measured by using a ruler on the underside of the plate. The susceptibility testing results were interpreted according to recommendations of CLSI [13].

RESULTS

The mean age groups and the distribution of patients in urinary isolates had shown in Table-I. Among 275 urine samples sent for culture and sensitivity testing to the pathology department at THQ hospital Pindigheb from28 Aug 2021 to 27 Aug 2022, out of total 275 samples 180 (65.45%) samples yielded significant growth of Enterobacteriaceae. 0ut of 180 samples 100 (55.55%) samples yielded growth of *Escherichia coli* followed by 50 (27.77%) were *klebsiella pneumoniae* and 30 (16.67%) were *Enterobacter cloacae.* The *proteus, staphylococcus sapropyticus* and enterococcus isolates not isolated in our setup. The sensitivity pattern of *Escherichia coli* was noted to norfloxacin, Fosfomycin and nitrofurantoin {(50%,30%)}, and {(6%, 2%)} resistance and sensitive respectively. *klebsiella pneumonia* and *Enterobacter cloacae* showed resistance and sensitive pattern {60%,10%)}, {(33.33%,16.67%)}, {(2%,10%)}, {(10%,6.7%)}and {(!4%,2%)}, {(13.33%,20%)} against norfloxacin, Fosfomycin and nitrofurantoin respectively.

The API 20 E was used for detection of Enterobacteriaceae isolates however the only three isolates were clearly detected. The other common isolates were *staphylococcus saprophyticus, proteus* and *enterococcus* were contaminated and mixed growth.

Table-1: Frequency of age groups in urinary isolates.

Age Groups	No of Patients (%)
<21	12(9.3%)
21~30	56(8%)
31~40	19(12%)
41~50	15(20%)
51~60	18(24%)
61~70	33(17.3%)
>70	27(9.3%)
Total	**180(100%)**

Table-II: The gender distribution of patients in urinary isolates had shown.

Gender distribution	Indoor patients	Outdoor patients	Total
Males	60(33.33%)	40(22.22%)	100(55.55%)
Females	50(27.77%)	30(16.66%)	80(44.43%)

Table-III: sensitivity pattern of urinary isolates causing UTI.

Urine isolates	Norfloxacin Resistance		Fosfomycin resistance		Nitrofurantoin resistance		Total	P-value
	Yes	Not resistant	Yes	Not resistant	Yes	Not resistant		
Escherichia coli	50 (50%)	30 (30%)	2 (2%)	10 (10%)	6 (6%)	2 (2%)	100	≤0.001
klebsiella pneumoniae	30 (60%)	5 (10%)	1 (2%)	5 (10%)	7 (14%)	2 (4%)	50	
Enterobacter	10 (33.33%)	5 (16.67%)	3 (10%)	2 (6.7%)	4 (13.33%)	6 (20%)	30	

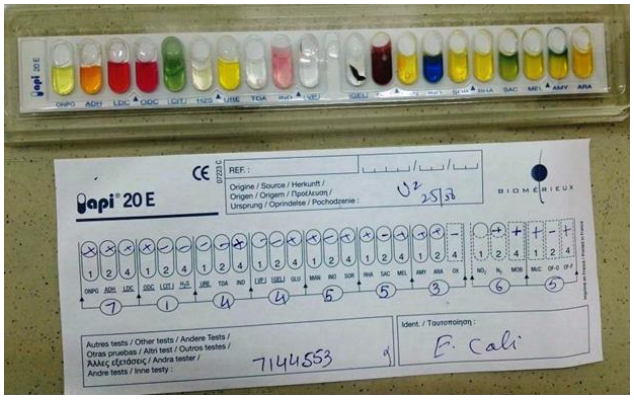

Figure-I: API 20E.

DISCUSSION

Microbial drug resistance is an inevitable consequence of indiscriminate and injudicious utilization of antimicrobial therapy. Antibiotic resistance surveillance has a key role among all strategies to manage the problem of antibiotic resistance. Fluoroquinolones being broad spectrum antibiotics and effective against most of the uropathogens are widely used for the treatment of UTIs [14]. Nevertheless, in recent years, anti-microbial resistance has increased to uropathogens due to irrational use of antimicrobials. The recent literature postulates a fluoroquinolone sparing protocol; however, if more than 20% of uropathogens are resistant to an antimicrobial, it cannot be used empirically [15]. The concerns today are that the fluoroquinolones which now serve as the alternative agents in our setup are continuously losing their clinical application and rapidly following similar trends with agents that have lost their chemotherapeutic relevance [16].

Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are concerned about antibiotic resistance in *E. coli* isolated from UTIs. It is because of the increasing incidence of such infections and injudicious antibiotic consumption. There is tremendous variability of antimicrobial resistance not only in different geographic regions, but also over timeframe in specific areas of a region. This phenomenon makes continuous surveillance of the extent and trends of antimicrobial resistance, essential for guiding effective empirical therapy in every continent, country, city, hospital or even health care unit. This study has provided an overview of the current situation regarding norfloxacin resistance among *E.coli* causing UTIs in THQ hospital Pindigheb with a focus on their frequency. In our study, the frequency

of norfloxacin resistance among *E.coli* causing UTIs was 77.33% [17].

Sabir et al has revealed frequency of norfloxacin resistance in uropathogenic *E.coli* as 11.2% from Lahore [18] Whereas, Zaheer et al has reported frequency of norfloxacin resistance as 42.5% from Islamabad [19] These findings were in contrast to our results which showed increased frequency of Norfloxacin, Fosfomycin and Nitrofurantoin resistance causing UTIs. Uropathogen causing UTIs were of hospital origin. In our setup the frequency of norfloxacin Fosfomycin and nitrofurantoin for *E.coli*, *klebsiella pneumoniae and enterococcus* were {(50%,30%) (2%,10%) (6%,2%)}, {(30%,5%) (1%,5%) (7%,2%)} and {(10%,5%) (3%, 2%) (4%,6%)} respectively.

Comparison with studies which were conducted in other parts of the world revealed that the global resistance to fluoroquinolones was approximately 12 % at Sao Paulo, Brazil[8] and rate of norfloxacin, Fosfomycin and Nitrofurantoin resistance had been reported 47.1 %,2%and 10% from turkey [20]. Comparison of this study with other conducted in India showed Fosfomycin and nitrofurantoin resistance were 6%and 10% respectively [21]. Considering the grave scenario of antibiotic resistance in our country, clinicians should re-formulate the antibiotic policies to avoid injudicious use of antibiotics.

CONCLUSION

Our study revealed high frequencies of norfloxacin, Fosfomycin and nitrofurantoin resistance among uropathogen at THQ hospital Pindigheb. A strong liaison between clinicians and microbiologists is recommended to keep abreast with the changes in antimicrobial susceptibility of uropathogens in order to suggest a suitable empiric therapy. Infection control measures are recommended, so that appropriate management can be instituted and spread of these resistant organisms curtailed.

AUTHOR CONTRIBUTION

Saira Salim: Literature search and article writing
Naila Iqbal: Data collection
Lubna Ghazal: Drafted the study design
Firdous Iqbal: Overall supervision of the study
Abdul Rehman: Statistical analysis
Nayab Ali: Proofreading

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