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Evaluation Sensitivity of Uropathogenic E. coli and Gram Positive Cocci to Fosfomycin and Amoxyclav

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Abstract: Background: Urinary Tract Infection (UTI) is one of the most common bacterial infections both in community and hospital settings. Urine samples constitute a major proportion of the samples tested in routine diagnostic laboratories. Fosfomycin used to treat UTI for both gram positive and negative bacteria. The aim of the study was to evaluate the resistance of some uropathogens to fosfomycin because antibiotic resistance is growing with time, so continuous evaluation of antibiotic is urgent.

Materials and methods: Mid-stream urine samples were collected from patients suspected with UTI. All these samples cultured aerobically at 37°C for overnight. A total of 100 bacteria of both *E. coli* and gram positive cocci were diagnosed by general diagnostic bacteriological methods. Disc diffusion method used for antibiotic sensitivity test; two discs were tested, fosfomycin 200 µg and Amoxyclav 30 µg. All the statistical analysis was done by using SPSS 26 software and Excel app.

Results: From the total of 100 positive specimens, female was high prevalence with 70 specimens (70%), while males were just 30 specimens (30%) significantly (P< 0.0001). fosfomycin in *E. coli* showed high sensitivity rate 87.5% compared to Gram positive cocci (G+ ve cocci) that was 100 % sensitive to fosfomycin with significant differences (P= 0.02). For Amoxyclav, both *E. coli* and Gram positive cocci revealed different rate of sensitivity 31.3 % and 66.7 % respectively with significant differences (P= 0.001).

Conclusion: Fosfomycin is effective in both *E. coli* and G+ ve cocci, but it is more effective against G+ ve cocci 100% than gram negative bacteria (*E. coli*) 87.5% significantly (P=0.02). Amoxyclav showed high activity against G+ ve cocci 66.7% compared to *E. coli* 31.3% significantly (P= 0.001).

Keywords: *Fosfomycin, E. coli, Uropathogens, Gram positive cocci, Amoxyclav, Antibiotics sensitivity, Urinary tract infection.*

Introduction

Urinary Tract Infection (UTI) is one of the most common bacterial infections both in community and hospital settings, highest incidence is in children especially in girls due to short urethra and close proximity of genitourinary and anal opening [1-2]. Early treatment is essential because delay in treatment leads to severe morbidity and mortality [3-4]. Early detection of urinary tract infection in laboratory by dipstick, microscopy or urine culture is important because failure to diagnose UTI can have serious complications, especially in pregnant women [5-7].

In both the outpatient and inpatient settings, urinary tract infections (UTIs) are among the most common illnesses [8]. UTIs were assessed to be the third most frequent illness after surgical site infections and pneumonia in the latest European point-prevalence study of health-care-associated infections in acute-care hospitals from 2011 to 2012, accounting for 19 percent of cases [9]. Despite the implementation of particular preventive initiatives, it appears that rates are rising. In the United States, 93,300 UTIs were reported in hospitalized patients over the course of a year [10].

Clinically the diagnosis of UTI can be difficult as symptoms are non-specific. The only way to reliably exclude a urinary tract infection is by the laboratory examination of a urine specimen [11]. Many prompt diagnostic methods are available including wet mount microscopy, Gram stain, dipstick and automated assays, but gold standard method for diagnosis of UTI is quantitative urine culture [12].

Fosfomycin, originally named phosphonomycin, was discovered in Spain in 1969. It has a broad spectrum of activity against a wide range of Gram-positive and Gram-negative bacteria. It is highly active against Gram-positive pathogens such as *Staphylococcus aureus* and Enterococcus, and against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [13].

This study may be helpful in establishing empiric therapy guidelines to prevent the emergence of further resistance and to contribute data to larger and more extensive surveillance

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programs.

Materials and methods:

Patients (Study group):

A total number of 100 positive culture patients (subjects) were enrolled in this cross sectional study during the period 7/8/2022 to 15/2/2023.These patients from the urology department in AL-Shomali general hospital, each patient suffering from complaint of frequent urge to urinate and painful, burning feeling in the bladder or urethra during urination. The number and date of projects approval were 8492 in August 05, 2022.

Sample collection:

To reduce the risk of contamination, participants were informed to clean their hands with water and their genital area with swab soaked in normal saline before collection of the clean catch mid-stream urine samples. After the urethra is properly cleaned, the collection may begin by discarding the initial stream of urine into the toilet. Then, 10-15 milliliters (ml) of urine collected in the provided sterile specimen cup by directly urinating into the cup. Once an adequate amount is collected, then the remaining urine should be voided in the toilet. For men, the opening of the urethra (tip of the penis) should be wiped clean with a cleansing wipe before the collection is begun. The collected urine sample should be analyzed soon within 1 hour after collection.

All isolates were identified based on their morphology, Gram staining, biochemical tests, and culture on selective media (EMB agar) for E. coli and (Mannitol salt agar) for Staph species. The isolates were identified at first by standard microbiological and biochemical tests [14].

Antibiotic sensitivity test (Disc diffusion method):

The disc diffusion method, also known as the Kirby-Bauer method, is a widely used technique to determine the sensitivity of bacteria to different antibiotics by using a sterile swab to transfer bacteria from the plate to a sterile saline solution. Adjust the bacterial suspension to match the turbidity of a 0.5 McFarland standard. The method involves placing a small paper disc containing a specific antibiotic on a culture of the bacteria and observing whether the antibiotic inhibits the growth of the bacteria [15]. Two discs were tested, fosfomycin 200 µg and Amoxyclav 30 µg (Sigma Aldrich Corporation, USA).

Data analysis:

All the statistical analysis was done by using SPSS 26 software and Excel app. For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. T test was used to examine measurement data. Chi- square was used for non- parametric variables, P value < 0.05 considered significant. P value < 0.05 was taken into account to denote statistical significance additionally, Spearman correlation test used.

Results:

Figure 1: Showed the distribution of genders in the current study, from the total of 100 samples that were collected, female was high prevalence with 70 specimens (70%), while males were just 30 specimens (30%) significantly (P< 0.001).

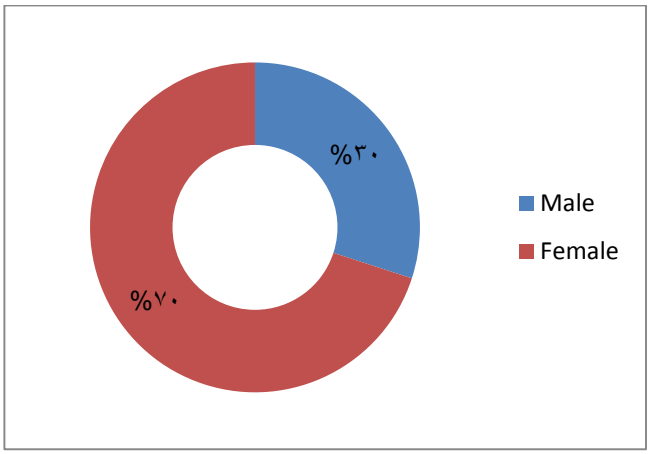

Fig. 1: Distribution of genders in this study

Antibiotic sensitivity of fosfomycin in *E. coli* showed high sensitivity rate 87.5% compared to Gram positive cocci that was 100 % sensitive to fosfomycin with significant differences (P= 0.02). [Table 1]

For Amoxyclav, both *E. coli* and Gram positive cocci revealed different rate of sensitivity 31.3 % and 66.7 % respectively with significant differences (P= 0.001) [Figure 2]

Table 1: Antibiotic sensitivity of isolated bacteria to various antibiotics

Antibiotics		*E. coli* No (64)	Gr + cocci No (36)	P value
Fosfomycin	Count	56	36	**0.027**
	%	87.5 %	100%	
Amoxyclav	Count	20	24	**0.001**
	%	31.3%	66.7%	

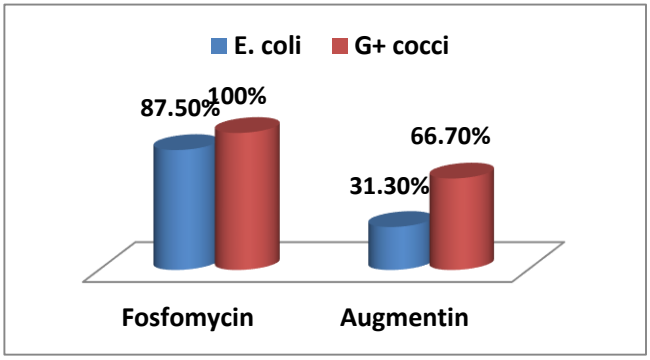

Fig. 2: Antibiotic sensitivity among different isolated bacteria

There is no significant correlation between Amoxyclav and fosfomycin (r= - 0.03, P= 0.72) [Table 2]

Table 2: Correlation between fosfomycin and Amoxyclav

Antibiotic		Amoxyclav
Fosfomycin	Correlation Coefficient	- 0.036-
	Sig. (2-tailed)	0.725

Discussion:

Urinary Tract Infections (UTIs) are infections of the genitourinary tract, which extends from the renal cortex of the kidney to the urethral meatus. UTIs are one of the most common bacterial infections that affect people of all ages and stages of life. UTIs result in more than eight million visits to clinicians and more than two million hospitalizations in emergency rooms in the United States each year. Urinary tract infection (UTI) caused by P. aeruginosa is a serious public health problem that affects millions of people each year, and catheterization of the urinary system is one of the most common predisposing factors to such infections [16].

This study showed that women are more prone to UTIs than men as 70% and 30% respectively, because women tend to have UTI more often than man due to the short and wider female urethra and it's travelling from the anus to the urethra. Furthermore, women lack the bacteriostatic properties of prostatic secretions that have bactericidal activity against bacteria [17].

Urinary tract infection is less common in men than in women because the male urethra is long, making it difficult for bacteria to spread to the bladder .Women are more prone to UTIs than men because the urethra is much closer to the anus and is shorter than in males; furthermore, women lack the bacteriostatic properties of prostatic secretions. Among the elderly, UTI frequency is roughly equal in women and men. This is due, in part, to an enlarged prostate in older men. As the gland grows, it obstructs the urethra, leading to increased frequency of urinary retention [18].

In the current study, antibiotic sensitivity of fosfomycin by disk diffusion methods in *E. coli* showed high sensitivity rate 87.5% compared to Gram positive cocci that was 100 % sensitive to fosfomycin with significant differences (P= 0.02).

Another study also used disk diffusion methods in order to determine the in vitro susceptibility of Gram-positive and Gram-negative bacteria to fosfomycin [13].

Other studies found the most frequently isolated uropathogens were *E. coli* (70/116); 60.3% followed by *Klebsiella spp*. (31/116); 26.7% [19-21]. Fosfomycin showed a high level of inhibitory effect on most of tested isolates; *E. coli* revealed low resistance rate of 4.2%, while *Klebsiella spp*, *Pseudomonas aeruginosa* and *Acinetobacter baumani* showed resistance rate of 16%, 36%, and 50%, respectively. A total of 72 (62.1%) isolates was ESBL producers, of which 92% isolates were Fosfomycin sensitive, while 25(22%) isolates were MBL-positive, of which 88% were sensitive to Fosfomycin [22].

Another study mentioned that overall susceptibility for fosfomycin against the gram-positives was 98.6%. There were 37.9% multidrug-resistant Enterobacteriaceae (MDRE) isolated during the study period. Fosfomycin displayed activity against 94.4% of extended-spectrum β-lactamase (ESBL) producers and 90.7% for carbapenem-resistant Enterobacteriaceae (CRE). None of the methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococcus isolates tested was fosfomycin resistant. The overall in vitro susceptibility was significantly higher for fosfomycin (p = 0.0001) compared to amoxyclav acid, cephalexin, cefuroxime, ciprofloxacin, trimethoprim/sulfamethoxazole and nitrofurantoin [23].

Conclusion:

Fosfomycin is effective in both *E. coli* and Gram positive cocci (G+ ve cocci), but it is more effective against G+ ve cocci 100% than gram negative bacteria (*E. coli*) 87.5% significantly (P=0.02). Amoxyclav showed high activity against G+ ve cocci 66.7% compared to *E. coli* 31.3% significantly (P= 0.001).This study may be helpful in establishing empiric therapy guidelines to prevent the emergence of further resistance.

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