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Research Article

High Fosfomycin Susceptibility in *Escherichia Coli* Recovered from Urine in Brazil

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Abstract

Urinary Tract Infections (UTIs) are widespread globally, with a notably higher incidence in women. In 2018, the European Medicines Agency (EMA) endorsed the use of fluoroquinolones for uncomplicated UTI treatment. Following this recommendation, several international agencies adopted similar guidelines. Consequently, older antimicrobial agents like fosfomycin emerged as primary treatment options for UTIs. In this context, our study aimed to evaluate the susceptibility of *Escherichia coli* strains from urine samples to various recommended UTI antibiotics. These strains were collected between January 2017 and July 2020 in São Paulo, Brazil. We utilized the disk-diffusion method for antimicrobial susceptibility testing, interpreting the results according to BRCAST/EUCAST guidelines. Out of the 86,957 urine cultures undertaken during this timeframe, 10,041 yielded *E. coli* isolates. Of these, 8,655 were tested against fosfomycin, with 99.0% (8,572 strains) found to be susceptible. Additionally, susceptibility rates for other drugs were as follows: nitrofurantoin (95.8%), amoxicillin/clavulanic acid (83.9%), ciprofloxacin (65.1%), norfloxacin (65.6%), and levofloxacin (67.7%). Notably, of the 571 ESBL-positive strains, 94.0% were susceptible to fosfomycin. It's important to mention a slight decline in fosfomycin susceptibility observed during this period. This finding underscores the importance of continuous monitoring for fosfomycin resistance and rational usage of the drug.

Keywords: Urinary tract infection; Fosfomycin; Fluoroquinolones; *Escherichia coli.*

Introduction

Urinary Tract Infections (UTIs) represent one of the most prevalent infections globally, impacting approximately 150 million individuals annually [4,20]. These infections can be anatomically categorized into lower (bladder, ureters, and urethra) and upper (kidneys) tracts. Additionally, UTIs can be defined based on symptomatic presence (symptomatic vs. asymptomatic) and the severity of the disease (complicated vs. uncomplicated). Symptomatic UTIs typically manifest with malaise, dysuria, increased urinary frequency, lumbar discomfort, and fever [2,18]. Women are more predisposed to UTIs, a susceptibility attributed to specific anatomical features of the genito-urinary tract. Factors like a shorter urethra and its proximity to the anus enhance the likelihood of urinary tract colonization by gastrointestinal microorganisms. Research indicates that 20-48% of women will experience a UTI at least once in their lives [10]. Of this cohort, 24% are anticipated to contract another UTI within the subsequent six months, with 2-5% presenting recurrent infections [3]. UTIs significantly affect morbidity; symptoms persist for an average of 6.1 days, leading to approximately 2.4 days of restricted activity and 0.4 days bedridden [18].

Bacteria, predominantly *Escherichia coli*, are the primary etiological agents behind UTIs (accounting for nearly 80% of cases) [6,7,14]. For years, fluoroquinolones were the preferred treatment for UTIs. However, growing concerns over bacterial resistance and documented adverse reactions led to recommendations against its use for uncomplicated UTIs, prompting the emergence of alternative therapeutic guidelines [1,5]. Fosfomycin-trometamol now stands as the primary recommendation for treating uncomplicated UTIs [3]. Fosfomycin impedes bacterial cell wall synthesis by irreversibly binding to the MurA enzyme, critical for generating bacterial cell wall components [9,12]. Given the evolving UTI treatment landscape and the surge in fosfomycin prescriptions, it's noteworthy that resistance rates to this drug in *E. coli* remain notably low [13,17,19]. Given these developments, it's imperative to conduct epidemiological studies detailing *E. coli* susceptibility to fosfomycin. This research seeks to elucidate the susceptibility patterns of *E. coli* strains obtained from urinary cultures associated with UTIs in São Paulo from January 2017 to July 2020.

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Materials and Methods

Collection of Isolates

Urine samples were obtained from outpatients at a clinical laboratory located in the northwest region of São Paulo state. Between January 2017 and July 2020, a total of 86,957 urine cultures were carried out using CPS chromogenic agar. Subsequent bacterial identification was achieved through biochemical testing methods.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the isolates was determined using the Kirby-Bauer disk-diffusion method, according to the guidelines set by BRCAST (BRCAST, 2020). In brief, an inoculum standardized to 0.5 McFarland was swabbed onto the surface of Mueller-Hinton (MH) agar. Disks of different antimicrobial agents were then applied. The presence of the Extended Spectrum Beta-Lactamases (ESBL) phenotype was verified via the disk-approximation test. This utilized disks of ceftazidime, ceftriaxone, cefepime, cefotaxime, and amoxicillin/clavulanic acid. The formation of a "ghost-zone" was indicative of a confirmed ESBL phenotype (EUCAST, 2017).

Statistical Analysis

To evaluate the trend in antimicrobial susceptibility of the isolates over the 43-month period, time series analysis employing a linear regression model was used. The normality of each model was tested. Furthermore, a multinomial logistic regression was executed to compare the susceptibility rates among isolates for various agents: fosfomycin, ciprofloxacin, levofloxacin, norfloxacin, nitrofurantoin, and amoxicillin/clavulanic acid. All statistical analyses were conducted using Jamovi 1.8 software. For these analyses, a 95% confidence interval was established, and p-values of 0.05 or lower were deemed statistically significant.

Results

Out of the 86,957 urine cultures conducted, 10,041 *E. coli* clinical isolates were identified. Of these, 8,655 were assessed for susceptibility to fosfomycin, with 99.0% (8,572 isolates) showing susceptibility. The susceptibility rates for other tested antimicrobials, such as nitrofurantoin, amoxicillin/clavulanic acid, ciprofloxacin, norfloxacin, and levofloxacin, were 95.8%, 83.9%, 65.1%, 65.6%, and 65.7%, respectively, as visualized in Figure 1. Table 1 enumerates the susceptibility percentages for all the antimicrobials examined. Intriguingly, out of the 571 *E. coli* isolates identified as ESBL-positive, a notable 94.0% were susceptible to fosfomycin.

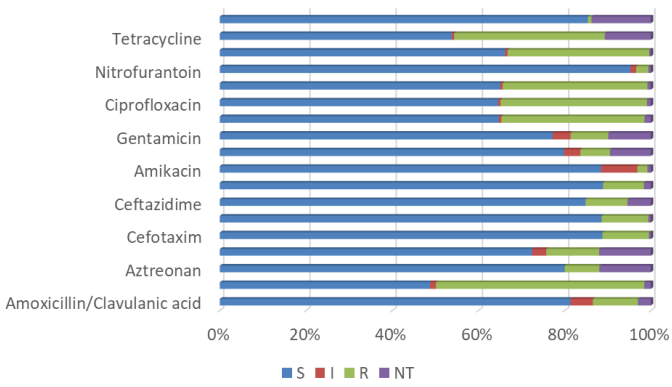

Figure 1:

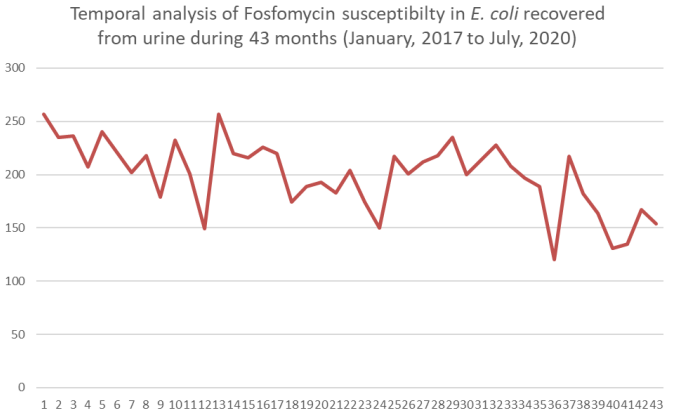

Figure 2:

Table 1: Susceptibility rates of *E.coli* to different antimicrobial agents.

Antimicrobial agent	S	S %	I	I %	R	R%	NT
Amoxicillin/clavulanic acid	8.166	83.9	522	5.4	1049	10.8%	304
Ampicillin	4.895	49.5	139	1.4	4851	49.1%	156
Aztreonan	8.030	90.8	3	0	806	9.1%	1202
Cefazolin	7.272	82.3	331	3,7	1231	13.9%	1207
Cefotaxime	8.910	89.2	3	0	1080	10.8%	48
Ceftriaxone	8.891	89.1	2	0	1087	10.9%	61
Ceftazidime	8.515	89.7	3	0	976	10.3%	547
Cefepime	8.919	90.3	0	0	951	9.6%	164
Amikacin	8.880	89.2	844	8.5	235	2.4%	82
Tobramicin	7.669	84.0	367	4.0	658	7.2%	914
Gentamicin	8.308	92.6	444	4.9	939	10.5%	1066
Levofloxacin	6.499	65.7	59	0.6	3332	33.7%	151
Ciprofloxacin	6.481	65.1	59	0.6	3409	34.3%	92
Norfloxacin	6.530	65.6	59	0.6	3370	33.8%	82
Nitrofurantoin	9.563	95.8	134	1.3	286	2.9%	58
Sulfametoxazole/trimetroprim	6.642	66.4	61	0.6	3299	33.0%	39
Tetracyclin	5.402	60.2	55	0.6	3506	39.1%	1075
Fosfomycin	8.572	99.0	0	0	83	1.0%	1386

S, susceptible; R, resistant; I, susceptible increasing the exposure; NT, not tested.

Table 2: Time series analysis of susceptibility rates to fosfomycin, amoxicillin/clavulanic acid, nitrofurantoin, ciprofloxacin, norfloxacin and levofloxacin.

Antimicrobial agent	β	p-value
Fosfomycin	-1.460	<0.001
Amoxicillin/clavulanic acid	- 1.030	<0.001
Nitrofurantoin	- 0.752	0.048
Ciprofloxacin	- 0.586	0.041
Norfloxacin	- 0.614	0.030
Levofloxacin	- 0.600	0.032

Utilizing a linear regression within a time series analysis model, we noted a downward trend in the susceptibility rates for all the examined antimicrobials, including fosfomycin. A multinomial logistic regression was carried out to contrast the susceptibility rates of fosfomycin with other antimicrobials. The data suggest that the likelihood of isolates being resistant compared to susceptible was significantly lower for fosfomycin than for the other antimicrobials. Relative to fosfomycin, the probability of resistance was 54.31 times greater for ciprofloxacin (logOR=3.99; p-value<0.001), 53.29 times for norfloxacin (logOR=3.98; p-value<0.001), 52.94 times for levofloxacin (logOR=3.97; p-value<0.001), 13.26 times for amoxicillin/clavulanic acid (logOR=2.59; p-value<0.001), and 3.08 times for nitrofurantoin (logOR=1.13; p-value<0.001). These findings are comprehensively laid out in Table 3.

Table 3: Probability of occurring resistant to susceptible isolates of each antimicrobial comparing to the same ratio obtained for fosfomycin.

	Predictor (Antimicrobial agente comparator -fosfomycin)	Estimate (logOR)	p-value	Odds Ratio (OR)	95% Confidence Interval	
					Lower	Upper
Resistant - Susceptible	Amoxacillin/clavulanic acid – Fosfomycin	2.59	< .001	13.26572	10.58741	16.6216
	Ciprofloxacin – Fosfomycin	3.99	< .001	54.31795	43.58669	67.6913
	Levofloxacin – Fosfomycin	3.97	< .001	52.94524	42.48282	65.9843
	Nitrofuratoin – Fosfomycin	1.13	< .001	3.08843	2.41470	3.9501
	Norfloxacin – Fosfomycin	3.98	< .001	53.2945	42.7646	66.4172

OR: Odds Ratio; p-value = < 0.05

Discussion

Fosfomycin has recently re-emerged as a viable therapeutic alternative for various infections, particularly uncomplicated UTIs. As we grapple with the escalating rates of antimicrobial resistance, the use of older antimicrobial agents has become an alternative option for treatment (Gardiner et al., 2019). Therefore, it's essential to conduct surveillance studies to gauge resistance levels and determine the continued viability of these agents in clinical settings. This study provided insights into the resistance patterns of *E. coli* isolates from São Paulo against multiple antimicrobials, with fosfomycin displaying a resistance rate of merely 1%.

UTIs are a prevalent community infections. For years, fluoroquinolones were the primary treatment choice. However, following the European Medicines Agency's 2019 advisory against their use for uncomplicated UTIs due to severe adverse effects and escalating bacterial resistance [5], there has been a decline in their prescription. This paved the way for alternatives like fosfomycin and nitrofurantoin [1]. A collective Brazilian recommendation, encompassing multiple medical societies, suggested fosfomycin-trometamol and nitrofurantoin as the primary treatment for cystitis, with cefuroxime or amoxicillin-clavulanate as secondary options [3].

In our study, we noted an impressive fosfomycin susceptibility rate of 99.0% the highest among all tested agents. Even among ESBL isolates, a 94.0% susceptibility to fosfomycin was observed. Comparable findings have been reported in Brazil and Switzerland, underscoring fosfomycin's potential as a frontline UTI treatment, especially given the minimal resistance observed even in ESBL-positive isolates [15,17].

However, it's worth noting a gradual decline in fosfomycin susceptibility (β=-1.46; p-value <0.001) in our data. This decline, albeit slower than other agents, reaffirm findings by Martínez et al. (2020), who linked increased fosfomycin consumption to rising resistance. The increased fosfomycin use, drove by changing UTI treatment guidelines [3,5], likely precipitated this trend. This diminishing susceptibility trend is evident across all antimicrobials in our study, even with different β-value, underlining the mounting challenge of antimicrobial resistance. This emphasizes the pressing need for vigilant surveillance and rational antimicrobial use. Comparatively, the likelihood of resistance in fosfomycin-treated isolates was the lowest among all agents evaluated, underscoring its current efficacy. Furthermore, single-dose prescriptions improve patient adherence, and a recent meta-analysis affirmed the equivalency of single-dose fosfomycin-trometamol with other UTI treatments concerning microbial and clinical outcomes in women [22].

Conclusion

This study underscores the pivotal role of fosfomycin in treating uncomplicated UTIs, particularly given the persistently low *in vitro* bacterial resistance to this drug. While various studies have attested to fosfomycin's clinical efficacy in managing uncomplicated UTIs, our findings specifically emphasize its superior standing in terms of resistance, displaying the lowest resistance rates among the evaluated agents. The gradual decline in fosfomycin susceptibility among *E. coli* isolates, coupled with limited Brazilian data on the topic, accentuates the urgent need for continuous surveillance of its susceptibility trends and rational application in the Brazilian medical landscape.

Author Statements

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