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Invitro Activity of Fosfomycin Alone and in Combination with Other Antibiotics against Multi Drug Resistant – *E. coli* for Treatment at a Tertiary Care Hospital of Karachi

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ABSTRACT:

Background: MDR *Escherichia coli* (MDR *E. coli*) has emerged as a serious public health issue in Pakistan and other countries throughout the world, causing treatment failure and a significant health burden.

Aim: The goal of this study was to assess the prevalence and susceptibility of MDR *E. coli* isolated from patients at a tertiary care hospital of Karachi.

Method:*E. coli* (n = 439) was isolated from clinical specimens, identified, and tested for antibiotic susceptibility using conventional procedures between June and August 2021.

Results: Of the 439*E.coli* isolates, the majority were from urine (55%). MDR *E. coli* were present in 176 (40.1%). Of these, the resistance percentage were recorded to: Gentamicin 60%, Moxifloxacin 15%, Ciprofloxacin 40%, Amoxicillin 20%, Fosfomycin 10% and Ceftolozane/Tazobactum 5%. Female isolates were more resistant than male isolates. The antibacterial drug combination with Fosfomycin demonstrated a significant difference (p- 0.002).

Conclusion: Drug resistance monitoring and epidemiologic analysis of patient data are required on a regular basis and can provide useful information for antimicrobial resistance management.

Keyword*: E.Coli*, Antibiogram, Fosfomycin, Ceftolozane/Tazobactam, UTI

INTRODUCTION:

Antibacterial drugs are the primary therapeutic approach in medicine for treating a wide range bacterial infections(1). The recent developments in antibiotics is considered to be among the most significant advances in contemporary science. Millions of lives have been saved as a result of antibiotics. However, resistance to antimicrobials is on the rise and is one of the world's most serious threats(2). The increased use, and repeatedly misuse, of antibacterial drugs has resulted in the occurrence of bacteria that no longer respond to therapeutic interventions (3).

Urinary tract infections (UTIs) are frequently caused by *Escherichia coli*, and they account for both communal and hospital acquired UTIs(4). Approximately 150-250 million cases are reported worldwide each year(5). The UTI affects approximately 40–50% of women and 5% of men at some point in their lives(6). *E. coli* is the most communal facultative anaerobic species in the gastrointestinal tract. It is characteristically a harmless type of bacteria, but it's a medically

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important bacteria that causes the majority of diseases(7).Antibiotic resistance among *E. coli* isolates has increased significantly in recent years, and rising resistance rates are becoming a serious issue in both developed and developing countries(8).

Multi-drug resistance (MDR) occurs when bacteria develop resistance to two or more antibiotics at the same time due to a variety of mechanisms, such as the production of antimicrobial enzymes, changes in bacterial proteins that bind to penicillin, efflux pump extrusion, or mutations in genes that affect other bacteria via mobile genetic elements(9). Emerging MDR organisms and their associated complications in developing countries are currently a major source of concern among medical and clinical practitioners. These circumstances make intervention more difficult and, in some cases, endanger the patients' lives(10).Since,*E. coli* is responsible for the majority of UTI cases, as well as the rising use of antibiotics against it has resulted in growing bacterial resistance and the emergence of MDR strains, therefore, it appears necessary to undertake regional research into the bacteria's resistant strains(11).

Current research on the antimicrobial resistance pattern of MDR *E.coli* is needed to assess the organism's sensitivity to commonly prescribed medicines. This would help researchers and clinicians in optimizing the already available successful therapy options (12).

The existing resistance rate of *E. coli* to Fosfomycin is assessed to be between 5% to 10% among extended spectrum β-lactamase (ESBL) producers worldwide (13, 14). Although, Fosfomycin has a high sensitivity rate, it has been shown that resistance to the drug has risen over time, prompting the development of a treatment with a higher sensitivity rate than Fosfomycin.

The purpose of this study is to examine into and determine the MDR patterns of pathogenic *E. coli* strains against Fosfomycin, as well as to see if Fosfomycin has a synergistic effect with fifth-generation Cephalosporin. The findings of this study, may aid in the development of more effective UTI treatment options in the region, hence lowering MDR infection spread.

MATERIAL AND METHODS:

It was a pre-clinical trial that was conducted in a laboratory setting. The research was carried out at the Microbiology lab of the Tertiary care hospital of Karachi. The ethics committee at the hospital gave their approval for the study. 900 specimens of pus, wound swabs, blood and urine

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were processed for culture and sensitivity testing according to established guidelines between May 2021 and October 2021.

On MacConkey agar (Oxoid) and Blood agar, specimens were inoculated with normal culture media (Oxoid). The plates were incubated for 24 hours at 37° Celsius. Standard microbiological procedures were used to identify all gram negative, catalase, and oxidase positive colonies up to species level.

Kirby Bauer's disc diffusion method was used to test antibiotic susceptibility. A bacterial inoculum lawn was created on a 150 mm Mueller Hinton Agar plate using this procedure (Oxoid UK). Antibiotic disc ofAmoxicillin 20 µg, Fosfomycin200 µg,Ciprofloxacin 5 μg, Moxifloxacin µg, Gentamicin10 μg and Ceftolozane/tazobactam (30/10 μg)were placed on agar plate. Plates were incubated at 35°C for 16-24 hours before results were determined. According to CLSI recommendations (2018), the zones of growth inhibition around each antibiotic disc were quantified and designated as either sensitive or resistant.

E. coli isolates were detected by double disc synergy method using Fosfomycin and other antibiotics. The zones of growth inhibition around each antibiotic disc were quantified and designated as either sensitive or resistant.

E. coli Biochemical Characterization and Confirmatory tests:

Biochemical testing was used to examine the suspected microorganisms. Assays for carbohydrate fermentation include the TSI (triple sugar iron agar slant), Indole, Methyl red, Vogus Proskaur, Catalase, Urease, Simmon citrate utilisation test, Oxidase, and Carbohydrate fermentation tests.

Statistical Analysis:

The Statistical Package for Social Sciences (SPSS) version 21 was used to analyse the data. For numerical variables, descriptive analyses were expressed as Mean with Standard Deviation. For categorical variables, frequencies and percentages were determined. The Chi square test was used to evaluate the relationship between drug sensitivity and resistance patterns, with a *p* value less than 0.05 considered significant.

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RESULTS:

Four hundred and thirty nine*E.coli* strains were isolated from 900 specimens on the basis of identifying methods. Out of the 439 samples 176(40.1%) were MDR *E.coli*and 263(59.9%) were non MDR *E.coli*. Looking over the gender wise frequency, the MDR *E.coli* were predominant in females that were 63.8% as compared to males, which was 36.2% as shown in **Table I.**

Table-I: Total samples of E.Coli.

Total Sample	MDR	Non- MDR
439	176 (40.1%)	263 (59.9%)
Male 160	64 (36.4%)	96 (36.5%)
Female 279	112 (63.6.1%)	167 (63.5%)

As shown in **Table-II**, the majority of the isolates were acquired from urine (54.7%) followed by blood sample (17.1%), vaginal swab (15.0%), and wound (13.2%).

Table-II: Frequency of MDR E.Coli. in Specimen.

Source	Total Samples 439	MDR 176 (40.1%)	Non- MDR 263 (59.9%)
Urine	240 (54.7%)	72 (40.9%)	168 (63.9%)
Blood	75 (17.1%)	43 (24.4%)	32 (12.2%)
Vaginal swab	66 (15.0%)	36 (20.5%)	30 (11.4%)
Wound	58 (13.2%)	25 (14.2%)	33 (12.5%)

Outdoor patient departments isolated the organism at a higher rate than inpatient departments, with 54% and 46%, respectively. As demonstrated in **Table-III**, the majority of *E.coli*.strains were isolated from the departments of gynecology, general medicine, surgery and ICU. In these departments, there was a high incidence of *E.coli* positive cultures. Gynecology had the highest

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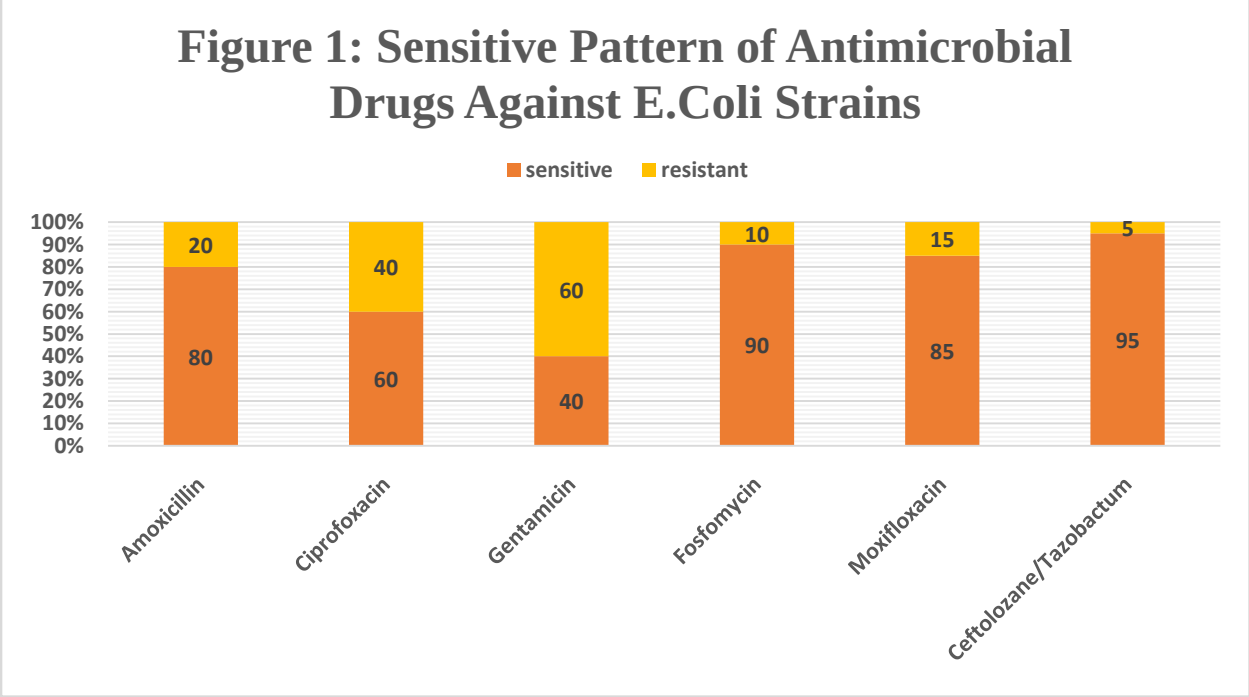
number of instances (176 or 39.9%). General Medicine came in second with (109 or 24.8%), Surgical ward came in third (84 or 19.1%), and ICU came in fourth (70 or 15.9%).

Table-III: Percentage of MDR isolates in different Departments..

Department	**Total Samples 439**	**MDR 176 (28.3%)**	**Non- MDR 263 (71.7%)**
Gynecology ward	176 (39.9%)	70 (39.8%)	106 (40.3%)
Medicine ward	109 (24.8%)	47 (26.7%)	62 (23.6%)
Surgery ward	84 (19.1%)	37 (21.0%)	47 (17.9%)
ICU	70 (15.9%)	22 (12.5%)	48 (18.2%)

All the drugs showed resistance in MDR *E.cColi*. Gentamycin has the highest resistance level (60%). Ciprofloxacin resistance is 40%, Amoxicillin is 20%, Moxifloxacin is 15% and Fosfomycin is 10%. As revealed in **Fig. 1**, Ceftolozane/tazobactam is 95% sensitive and 5% resistant against all MDR *E.coli*isolates.



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As shown in **Table IV**, the combination of Fosfomycin and Amoxicillin showed 92 percent sensitivity, while the combination of Fosfomycin and Gentamicin showed 95 percent sensitivity and the combination of Fosfomycin and Ceftolozane/tazobactum showed 99 percent sensitivity. A significant difference was discovered when all three combination groups were compared.

Table-IV: Sensitive pattern of Synergistic Drug against MDR E.Coli

Drug Combination	**Sensitive**	**Resistant**	***p*-value**
Amoxicillin plus Fosfomycin	92%	8%	0.002
Gentamicin plus Fosfomycin	95%	5%	
Ceftolozane/tazobactum plus Fosfomycin	99%	1%	

DISCUSSION:

Antibiotic treatment is the primary and most important treatment for UTI, as it controls the invasive agents. As a result, a link between antibiotic usage and the growth of resistance bacteria appears natural(15, 16).Antimicrobial resistance in *E. coli* has risen globally, and susceptibility patterns reveal significant geographic heterogeneity as well as population and environmental variables(17).

In this investigation, 48.8% of *E. coli* isolates were found from the 900 samples. The *E.coli* 48.8% isolates came from outpatient departments (54%) and hospitalized patients (42%) respectively. These findings are consistent with a recent study by Rasool *et al*., who found *E.coli* to be abundant in the isolated sample. In the study by Rasool *et al*, *E. coli* was identified in the urine of 58% of outdoor patients and 42% of indoor patients, indicating that *E. coli* is common in community-acquired UTIs(18).

In our study, we found females were shown to be more resistant to *E.coli* in our investigation than males. Mitra *et al*. discovered a female majority, with females accounting for 30 (58.8%) and males accounting for 21 (41.2%), respectively(19).The possible explanation for the high prevalence in females is that females have open genitalia, which predisposes them to faecal

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contamination, as opposed to males, who have close genitalia, which prevents *E.*coli from establishing itself. *E.coli* spreads through the vaginal passage due to faecal contamination, invading and colonizing the urinary tract, resulting in infection (20).

In our research, we discovered a very significant increase of multi-drug resistance (40.1%). This alarming condition is most likely owing to our setup's indiscriminate use of antibiotics.

The current study's findings showed a complex microbial community (bacterial, moulds, and fungi) that was purified for diagnosis before *E. coli* was transferred to media. *E. coli* appeared in clinical specimens such as urine, blood samples, vaginal swabs, and wounds. Previous studies with Iraqi samples have yielded a similar findings, with some recoding *E. coli* in clinical samples(21).

In our research, the number of UTI patients related with the Gynecology department was greater. As previously mentioned that women were in greater numbers as men therefore during pregnancy, the odds of developing a urinary tract infection (UTI) increase. This research is backed up by Pallet's earlier research that females are more prone to UTI than females (22).

Semisynthetic penicillin with or without beta-lactamase inhibitors, Gentamycin, Ciprofloxacin and Moxifloxacin are the antibacterial drugs most commonly recommended in the treatment of UTIs all over the world.The antibiogram sensitivity pattern in the current study samples revealed that all E coli isolates were sensitive to 95 percent of Ceftolozane/Tazobactam, 90% of Fosfomycin, and then Moxifloxacin and Amoxicillin (85% and 80%, respectively). In terms of resistance patterns, 60% of the isolates tested positive for Gentamycin, followed by 40% for ciprofloxacin (**Fig 1**).The findings of Gentamicin resistant pattern is in harmony with the findings of the study by Jafri *et al*., which reveal that gentamicin resistance is extremely high. The extensive and unregulated use of antibiotics in our environment may be the cause of this significant resistance to routinely used antibiotics (23). In addition, the high resistance rate of ciprofloxacin against*E.coli* in this study backs up similar findings from other researchers. This has been connected to the use of fluoroquinolones in patients who should not be taking them(24). Long-term usage of low-dose fluoroquinolones like ciprofloxacin has also been demonstrated to be the most major risk factor for resistance development(25).

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The occurrence of *E. coli* isolates with distinct MDR phenotypes, involving co-resistance to three or more unrelated antimicrobial agent families have been previously described, and this is regarded a severe health risk(26, 27). Others researcher also have reported similar findings (27, 28).The recent investigation found increasing resistance different antibiotic classes that were previously found to be sensitive. Almost all of our MDR *E. coli* isolates showed antibiotic resistance, including amoxicillin, ciprofloxacin, and gentamicin. These resistance profiles were prevalent, and a number of known acquired resistance genes could account for them(29).

Amoxicillin, Gentamicin, Ceftolozane/tazobactum when given in combination with Fosfomycin was found to be 92%, 95% and 99% effective against MDR strains of *E.coli*. in our research. Moreover, when we look at the results Ceftolozane/ tazobactum and Fosfomycin together was found to be the most sensitive antibiotic against*Pseudomonasaeruginosa*in another investigations conducted in Pakistan and other countries (30-32).

Hassan *et al*, reported highest resistance of MDR *E.coli* against, 94% to ampicillin, 85% to ciprofloxacin and Augmentin and 60% to gentamicin (33). In Pakistan, MDR *E.coli* strains are becoming more prevalent. The fact that the irrational use of these medications in secondary and tertiary medical centers, now has resulted in an increase in resistance in our community. There is a causative association between the usage of antimicrobials and the developing resistance, according to mainstream chosen theory.

LIMITATION:

It was a single-centered research project. It is strongly suggested that this study be expanded to include more clinical settings around the country in order to acquire more accurate antibiotic susceptibility patterns against MDR *E.coli*, which will aid in infection control and be useful for better infectious disease management.

CONCLUSION:

E.coli resistance has increased during the previous few decades. In the current investigation, MDR strains were found to be extremely resistant to commonly used therapeutic medications. The medicine with the best anti-*E.coli* activity was ceftolozane/tazobactum. Given the foregoing, it is reasonable to advocate for the use of broad-spectrum antibiotics only when absolutely necessary. In the light of developing resistance to various antibiotics among *E.coli* isolates

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around the world, it is also vital to investigate novel combinations and stewardship approaches. Furthermore, in both the community and hospital settings, antimicrobial susceptibility should be checked on a frequent basis. Despite their limitations, these findings could be useful in guiding future in vivo research.

AUTHORS DECLARATION:

Conflicts of Interest: None

We now confirm that all of the manuscript's Figures and Tables are our own.

Ethical Clearance: The project was approved by the University local ethical council.

AUTHORS CONTRIBUTION STATEMENT:

TS designed and directed the project; SAZ performed the experiments; MT and LF analyzed the data and interpreted the results; UZ and SI developed the theoretical framework and wrote the article. All authors reviewed the results and approved the final version of the manuscript.

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