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Phenotypic Expression and Underlying Genotypic Determinants of Fosfomycin Resistance Among Bacterial Isolates Recovered from Urinary Tract Infections at JK Hospital, Ujjain, Madhya Pradesh

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Abstract:
Urinary tract infections (UTIs) remain among the most common bacterial infections encountered in clinical practice. Fosfomycin is increasingly used for the treatment of uncomplicated UTIs due to its broad spectrum activity and favorable pharmacokinetic profile. However, the emergence of fosfomycin resistance among uropathogens has become a growing concern worldwide. The present study aimed to evaluate the phenotypic expression and underlying genotypic determinants of fosfomycin resistance among bacterial isolates recovered from urinary tract infections in patients attending JK Hospital.
A cross-sectional laboratory-based study was conducted over a period of one year. A total of 70 bacterial isolates were obtained from urine samples of clinically suspected UTI patients. Bacterial identification was performed using standard microbiological methods. Antimicrobial susceptibility testing including fosfomycin susceptibility was determined by Kirby-Bauer disk diffusion and minimum inhibitory concentration (MIC) methods. Polymerase Chain Reaction (PCR) assays were performed to detect resistance genes including fosA, glpT, uhpT, and murA.
Among the 70 isolates, Escherichia coli was the most predominant pathogen followed by Klebsiella pneumoniae, Enterococcus spp., and Pseudomonas aeruginosa. Phenotypic resistance to fosfomycin was observed in 14.3% of isolates. Molecular analysis revealed the presence of fosA gene in 8 isolates, while mutations associated with glpT and uhpT genes were identified in several resistant strains. These genes are known to influence fosfomycin uptake and enzymatic inactivation. Resistance mechanisms often involve mutations in transporter genes or production of fosfomycin-modifying enzymes that inactivate the antibiotic.
The study highlights the emergence of fosfomycin resistance among uropathogens and emphasizes the importance of continuous surveillance and molecular monitoring to preserve the effectiveness of fosfomycin in the treatment of UTIs.

INTRODUCTION

Urinary tract infections (UTIs) represent one of the most prevalent bacterial infections affecting individuals across all age groups. Globally, millions of cases are reported each year, resulting in significant

morbidity and healthcare burden. The majority of UTIs are caused by Gram-negative bacteria, particularly Escherichia coli, followed by Klebsiella spp., Proteus spp., and Pseudomonas spp.

Fosfomycin is a broad-spectrum antibiotic widely used in the treatment of uncomplicated urinary tract infections. It acts by inhibiting the bacterial enzyme MurA, which catalyzes the first step of peptidoglycan synthesis in bacterial cell wall formation. Due to its unique mechanism of action and minimal cross-resistance with other antibiotics, fosfomycin has become an important therapeutic option for multidrug-resistant uropathogens.

Despite its effectiveness, increasing reports of fosfomycin resistance have been documented in recent years. Bacterial resistance to fosfomycin can occur through multiple mechanisms. These include mutations affecting drug uptake transporters such as GlpT and UhpT, modification of the target enzyme MurA, and production of fosfomycin-inactivating enzymes such as FosA.

The transporter proteins GlpT and UhpT facilitate the entry of fosfomycin into bacterial cells. Mutations or reduced expression of these transporters lead to decreased drug uptake and contribute to resistance.

Furthermore, plasmid-mediated genes such as fosA3 can encode enzymes that chemically modify and inactivate fosfomycin, facilitating the spread of resistance in hospital settings.

Understanding both phenotypic resistance patterns and underlying genetic mechanisms is essential for guiding appropriate antibiotic therapy and developing strategies to control antimicrobial resistance.

OBJECTIVES

Primary Objective

To evaluate the phenotypic expression of fosfomycin resistance among bacterial isolates recovered from urinary tract infections.

Secondary Objectives

1. To identify the bacterial species responsible for UTIs in patients attending JK Hospital.
2. To determine the prevalence of fosfomycin resistance among uropathogens.
3. To detect genotypic determinants of fosfomycin resistance including fosA, glpT, uhpT, and murA genes.
4. To correlate phenotypic resistance patterns with genotypic findings.

MATERIALS AND METHODS

Study Design

Hospital-based cross-sectional laboratory study.

Study Site

Department of Microbiology, JK Hospital.

Study Duration

12 months.

Sample Size

70 bacterial isolates obtained from urine samples of patients with suspected urinary tract infections.

Inclusion Criteria

- Urine samples from patients clinically diagnosed with UTI
- Significant bacteriuria (>10^5 CFU/mL)
- Pure bacterial growth

Exclusion Criteria

- Mixed bacterial growth
- Contaminated urine samples
- Samples from patients already on prolonged antibiotic therapy

LABORATORY PROCEDURES

Sample Collection

Midstream urine samples were collected under sterile conditions and transported to the microbiology laboratory.

Isolation and Identification

Samples were cultured on:

- MacConkey agar
- Blood agar
- CLED agar

Identification was performed based on:

- Colony morphology
- Gram staining
- Biochemical tests

Phenotypic Detection of Fosfomycin Resistance

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method following CLSI guidelines.

Fosfomycin susceptibility was tested using fosfomycin (200 µg) with glucose-6-phosphate disks.

Minimum inhibitory concentration (MIC) was determined by broth microdilution.

Genotypic Detection

DNA extraction was performed from bacterial isolates.

PCR was used to detect the following resistance genes:

- fosA
- glpT
- uhpT
- murA

PCR products were analyzed using agarose gel electrophoresis.

RESULTS

Distribution of Uropathogens (n=70)

Organism	Number	Percentage
Escherichia coli	38	54.3%
Klebsiella pneumoniae	14	20%
Enterococcus spp.	8	11.4%
Pseudomonas aeruginosa	6	8.6%
Proteus spp.	4	5.7%

Fosfomycin Resistance Pattern

Category	Number	Percentage
Sensitive	60	85.7%
Resistant	10	14.3%

Genotypic Determinants

Gene	Number Detected	Percentage
fosA	8	11.4%
glpT mutation	6	8.6%
uhpT mutation	5	7.1%
murA mutation	3	4.3%

DISCUSSION

The present study evaluated both phenotypic and genotypic aspects of fosfomycin resistance among urinary isolates. E. coli was identified as the predominant uropathogen, which is consistent with global epidemiological trends of UTIs.

Phenotypic testing revealed that approximately 14.3% of isolates were resistant to fosfomycin, indicating the emergence of resistance in the clinical setting. Although fosfomycin resistance remains relatively low compared to other antibiotics, increasing usage may contribute to selective pressure.

Genotypic analysis demonstrated that several resistant isolates carried the fosA gene, which encodes a glutathione transferase enzyme that inactivates fosfomycin by modifying its epoxide ring.

Mutations in glpT and uhpT genes were also detected in some isolates. These genes encode membrane transport proteins responsible for the uptake of fosfomycin into bacterial cells. Alterations in these transporters reduce antibiotic entry and result in decreased susceptibility.

The coexistence of multiple resistance mechanisms suggests that fosfomycin resistance may arise through both chromosomal mutations and plasmid-mediated gene transfer.

CONCLUSION

The study demonstrates the presence of both phenotypic and genotypic fosfomycin resistance among bacterial isolates causing urinary tract infections at JK Hospital. Although the overall resistance rate remains relatively low, the detection of resistance genes such as fosA, glpT, and uhpT indicates the potential for further spread.

Continuous antimicrobial surveillance, rational antibiotic prescribing, and molecular monitoring are essential to preserve fosfomycin as an effective therapeutic option for UTIs.

LIMITATIONS

- Small sample size (70 isolates)
- Single-center study
- Limited number of resistance genes analyzed

RECOMMENDATIONS

- Implementation of antimicrobial stewardship programs

- Routine monitoring of fosfomycin resistance
- Larger multicenter molecular epidemiological studies

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