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Changing Susceptibility of Enterococcus Species in Urinary Isolates in a Tertiary Care Hospital

Nazish Babar[1], Amjad Mahboob[2], Asim Ali Shah[3], Asfandyar Babar[4]

[1]Associate Professor, Department of Pathology Gajju Khan Medical College, Swabi
[2]Associate Professor, Department of Medicine Gajju Khan Medical College, Swabi
[3]Incharge Microbiology/Virology, Gajju Khan Medical College, Swabi
[4]Student Final year BDS, Shifa College of Dentistry, Islamabad

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[1]Writing the manuscript
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Address of Correspondent
Dr Nazish Babar
Associate Professor, Department of Pathology, Gujju Khan Medical College, Swabi, Pakistan
meon_bunny@yahoo.com

ABSTRACT

Objective: To determine the efficacy of Fosfomycin against the Enterococcus species and if can still be the drug of choice for lower urinary tract infections.

Methodology: This study was conducted from August 2022 to March 2024 at Microbiology Laboratory, MTI- GKMC/BKMC, Swabi, KPK. After seeking permission from the institutional review board, a total of 3000 urine samples were received at Microbiology Department of Bacha Khan Medical Complex, Swabi, KPK in the said time period for culture and sensitivity. Urine specimens were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar (Oxoid, UK) and incubated aerobically at 35°C ± 2 for 18 to 24 hours. After identification of colony morphology, Gram staining and biochemical reactions were carried out to confirm if it was Enterococcus species, antimicrobial susceptibility testing was carried out. Demographic data of the patients was also recorded.

Results: Out of the total 3000 urine samples received at Microbiology Department of BKMC, Swabi, 95 samples yielded the growth of Enterococcus species out of which 22% were Vancomycin resistant and showed 51% resistance to Fosfomycin.

Conclusion: An increased rate of fosfomycin resistance against Enterococcal isolates was observed in our study, which urges cautious prescription of Fosfomycin and it is necessary to continuously monitor fosfomycin resistance in future as well.

Key words: Antimicrobial resistance, Enterococcus, Fosfomycin, Urinary pathogens.

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Introduction

As highlighted by WHO, it was first in the list of ESKAPE pathogens including Enterococci spp., Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp., responsible for causing nosocomial and antibiotic-resistant infections.[1] A highly resilient organism, found ubiquitously in soil, water, food, sewage and plants. Enterococcus, is also found on human skin, oral cavity, and large intestine, constituting less than 1% of the total microbiome.[2]

Not only that they are intrinsically resistant to a list of antibiotics including cephalosporins, clindamycin, aminoglycosides, and trimethoprim-sulfamethoxazole,[3] they also have the ability to acquire and transfer mobile genetic elements which are related to resistance using different routes like plasmids, conjugation, and transposons.[4] Absence of CRISPR-Cas gene loci, which usually limits invading harmful DNA is the major reason by which they acquire and transfer resistance. Their presence on the hands of the healthcare workers makes the transmission quite eaiser.[5] Even after the availability of treatment options against Vancomycin resistant Enterococcus (VRE) the associated morbidity and mortality is still on the rise, with prolonged hospitalization.[6]

As a consequence of the reported increase in antibiotic resistance and the accompanying side effects with

commonly used antibiotics like ciprofloxacin and trimethoprim/sulfamethoxazole, fosfomycin has become the most commonly used antibiotic against uncomplicated UTI.[7] Fosfomycin is a broad spectrum antibiotic effective against both gram positive (Enterococcus) and gram negative organisms (Escherichia coli, Klebsiella spp., Enterobacter spp., and Proteus mirabilis),[7] by inhibiting cell wall synthesis and reducing the capability of bacteria to adhere to uroepithelial cells.

Globally the urinary tract infections are the commonest of all and those due to Enterococcus species account for 5% of the CAI and rated as the third leading cause of hospital acquired urinary tract infections. Not only this, Enterococcus species are also held responsible for 30% of the CAUTIs,[8] with Enterococcus fecalis being the predominant and more virulent species.[9]

Although Fosfomycin is reported to have shown good in vitro activity against Enterococcus species including the Vancomycin resistant enterococcci,[10] the continuous increase in the use of this antibiotic has led to the development of different mechanisms of resistance especially when used alone. [11]

Methodology

This study was conducted from August 2022 to March 2024, at Bacha Khan Medical complex, Swabi, KPK after seeking permission from the Institutional review board (IRB), F. No. 6305/Ethical Board/GKMC.

A total of 3000 urine samples from both OPD and IPD patients were received at Microbiology Department of Bacha Khan Medical Complex, Swabi, KPK in the said time period for culture and sensitivity. All the patients were instructed to collect clean-catch midstream urine aseptically into a wide mouth, sterile screw-capped container with proper labeling and transported to the laboratory for analysis.

Nonprobability convenience sampling was done. All other urinary isolates and repeated samples of the same patient were excluded from the study.

Urine specimens were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar (Oxoid, UK) and incubated aerobically at 35°C ± 2 for 18 to 24 hours. After identification of colony morphology, the isolates were identified by a positive Gram reaction, a negative catalase test and a positive bile esculin hydrolysis test followed by their serological confirmation by agglutination with Group-D antiserum.

According to CLSI guidelines, inoculum of bacterial suspension (0.5 McFarland standard) was inoculated on Mueller-Hinton agar (Oxoid, UK) followed by antibiotic susceptibility testing of the bacterial isolates which was carried out by Kirby Bauer's disc diffusion method using the discs (Oxoid, UK) Ampicillin (10 µg), Vancomycin (30 µg), Linezolid (30 µg), doxycycline (30 µg), Ciprofloxacin (5 µg), Nitrofuratoin (300 µg), and Fosfomycin (200 µg), and incubated aerobically at 35°C ± 2 for 18 to 24 hours. The zones of inhibition were measured as per zone sizes recommended by CLSI after 24 hours of incubation at Mueller Hinton agar plates.

Results

A total of 3000 urine samples were received at the Microbiology Department of Bacha Khan Medical Complex, Swabi. Out of these 3000 urine samples, 1478 showed no growth, 505 showed mixed growth and 1017 showed growth on CLED agar of all the uropathogens in the said time duration of study including the 95 isolates which yielded the growth of Enterococcus, which is an overall culture positivity of 33.9%.

Out of the 95 samples which yielded the growth of Enterococcus species, 62 (65.26%) were from females whereas the remaining 33 (34.73%) were from males.

Table I shows the age wise distribution of the samples with gender distribution revealing maximum number of patients suffering from urinary tract infection being females, with maximum number of patients falling in the age group of 51 to 60 years and minimum number of females in the age group pf 10 to 20 years.

Table I: Age groups of the patients with the gender distribution. (n=95)

AGE GROUPS	FEMALES (n= 62)	MALES (n= 33)
10 – 20 years	01 (1.61%)	03 (9.09%)
21 – 30 years	20 (3.22%)	06 (18.18%)
31 – 40 years	11 (17.74%)	05 (15.15%)
41 – 50 years	03 (4.83%)	04 (12.12%)
51 – 60 years	12 (19.35%)	06 (18.18%)
61 – 70 years	10 (16.12%)	05 (15.15%)
71 – 80 years	03 (4.83%)	04 (12.12%)
81 – 90 years	02 (3.22%)	00 (00%)

The distribution of males into different groups was different from the females with 6 males each in the age groups 10 to 20 and 51 to 60 years of age with no males affected above 80 years.

Table II: Species wise distribution of the organism based on Vancomycin resistance. (n=95)

	Vancomycin resistant	Vancomycin intermediate	Vancomycin sensitive
Enterococcus fecalis			80
Enterococcus fecium	14	01	

Table II shows the species wise distribution of the organism, where maximum i.e. 14.73 % were Vancomycin resistant. Out of the 95 positive samples maximum patients were from IPD and the remaining from the OPD as shown in Table III.

Table IV shows the resistance pattern of Enterococcus against the recommended antibiotics showing maximum resistance against Ciprofloxacin, followed by Doxycycline, Ampicillin and then Fosfomycin in which 51% of all the isolates were found resistant to Fosfomycin. Linezolid showed 100% susceptibility against all the 95 Enterococcus isolates.

Table III: Department wise distribution of the received samples (n=95)

	Number
OPD	36 (37.89%)
IPD	59 (62.10%)

Table IV: Antimicrobial resistance (%) profile of Enterococcus species (n=95)

	AMP	VA	LNZ	DO	LEV	CIP	FOS	F
URINE	67	14	00	81	79	90	51	46

Discussion

Enterococcus fecalis is reported as the leading cause of hospital acquired UTI.[13] However, the identification of an enterococcal isolate from a urine culture may often represents colonization with unclear clinical significance.[13]

Although, fosfomycin is being used increasingly for treating uncomplicated UTI, its antimicrobial susceptibility testing is not being performed routinely in most of the laboratories and keeping in mind the scarcity of the data, this study was conducted to analyze the antibiotic susceptibility of Enterococcus species in particular, obtained from urinary tract infections and to highlight the increasing, but less highlighted development of resistance against fosfomycin.

Variable results have been quoted in the past regarding the culture positivity in different studies on urinary pathogens. As reported in our study, the positivity of growth on culture was reported to be 33.9%, which included all the different types of urinary pathogens isolates in that time period including the Enterococcus species. It is higher than the culture positivity reported by Sharmin et.al.[14]

As reported from different parts of the world fosfomycin resistance is increasing gradually. There was a time when Fosfomycin resistance was not detected amongst the *Enterococcus* spp. isolates as quoted in a study by Mosime et.al, [7] and it may still be the same in those parts of the world where it is not being used as an empirical antibiotic for treating uncomplicated lower urinary tract infections like Bangladesh[14], but resistance in Enterococcus species especially in VRE is being reported worldwide.

As quoted in a study the fosfomycin resistance rate of around 2% was reported in *Enterococcus species* in South America.[15] and a much higher resistance rate, reaching 16.0%.[16] has been reported from a study from North America.

Published in an antibiotic resistance related systematic review on Enterococcus in which median resistance from four studies was reported to be 21.5 %.[17,18]

Regarding the gender wise distribution of the patients in our study maximum number of the patients i.e. 65% were females which is in accordance with many other studies conducted worldwide like Sharmin et.al.,[14] and another study conducted in Canada.[16]

Results in our study were aligned with the studies in the past quoting the VRE being a hospital acquired pathogen. [16,19] Our study also showed 62% of the organisms to be hospital acquired out of which 14 were Vancomycin resistant.

Important point of concern in the antibiotic susceptibility testing was the highly increased resistance against Fosfomycin, which was considered a drug of choice showing no resistance at all in the Enterococcus species as quoted above. [7]

Linezolid being the only antibiotic approved by FDA against VRE,[20] still proves to be of utmost significance and Enterococcus isolates in our study also showed 0% resistance against Linezolid which is similar to many other studies like,[16] showing 100% sensitivity of the Enterococcus isolates in their study to Linezolid. They reported the resistance against Fosfomycin to be 5%, which is way higher in our study i.e. 51%, which is comparatively very high not reported as such from anywhere, as of the literature reviewed so far.

Enterococcus, a conditionally pathogenic organism, which in addition to urinary tract infections, can also cause soft tissue infections, and even bacteremia. [21] The point of concern is that although vancomycin was the drug of choice for treating severe enterococcal infections, but its misuse has led to the development of Vancomycin resistant strains of enterococci (VRE)[22] which is associated with very high morbidity and mortality. [23]

Favourable susceptibility rates reported in the past which labelled Fosfomycin to be the most reliable oral treatment option for the treatment of lower UTIs caused by Enterococcus species, now seem to change, and the results declared in our study definitely, together with all these factors urge the need to find newer antibiotic combinations to treat Vancomycin resistant *Enterococcus* faecium related infections.

Conclusion

An increased rate of fosfomycin resistance against Enterococcal isolates was observed in our study, which urges cautious prescription of Fosfomycin and it is necessary to continuously monitor fosfomycin resistance in future as well.

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