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(RESEARCH ARTICLE)

Excellent fosfomycin and linezolid susceptibility among multidrug resistant urinary Enterococcus isolates in Eastern India

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Abstract

Urinary tract infection (UTI) is one of the most common infectious diseases. Enterococcus spp. is an important causative agent of UTI. Emergence of resistance is high among enterococcus isolates. Fosfomycin has emerged as a novel oral therapeutic option for urinary isolates. A prospective study was done in Medical College, Kolkata for a period of 6 months (from January to June 2018). After obtaining ethical clearance from the Institutional Review Board (IRB) urine samples with significant growth of enterococcus were included in the study. Identification of Enterococcus species was preliminary done by Gram stain and conventional biochemical tests along with automated identification by VITEK 2 Compact. These isolates were tested for antimicrobial susceptibility to different antibiotics like ampicillin/ penicillin, tetracycline, ciprofloxacin, levofloxacin, vancomycin, teicoplanin, linzolid, fosfomycin and nitrofurantoin by Kirby Bauer disc diffusion method and minimum inhibitory concentration (MIC) by VITEK 2 Compact. MIC for fosfomycin was done by E-test method. Interpretation of susceptibility was done according to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines. During the period of 6 months (from January to June 2018) there were 194 isolates of Enterococcus spp. from 2732 urine samples showing a prevalence of 7.1%. Among these 194 isolates there were 148 *Enterococcus faecalis*, 40 *Enterococcus faecium*, 2 *Enterococcus casseliflavus*, 2 *Enterococcus gallinarum* and 2 *Enterococcus avium*. All isolates were highly susceptible to vancomycin, teicoplanin, linezolid and fosfomycin. There were total of 8 (4.12%) isolates of vancomycin resistant enterococcus. Resistance to ampicillin and high level gentamicin was significantly related in *Enterococcus faecium*. Fosfomycin is a highly susceptible for urinary enterococcus isolates. However, larger prospective in vivo studies are required to determine the efficacy.

Keywords: Antimicrobial susceptibility; Resistance; Urine; Fosfomycin; Linezolid

1. Introduction

Urinary tract infection (UTI) is one of the most common clinical entities encountered by the medical practitioners. The most frequent pathogens associated with UTI are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* spp., *Pseudomonas aeruginosa*, Enterobacter spp., and Staphylococcus spp. (1). The genus enterococcus consists of Gram-positive, facultatively anaerobic organisms that are ovoid in shape, arranged in short chains or in pairs. They were earlier classified as Group D Streptococcus (2). The emergence of resistance to the most common anti-enterococcal antibiotics which include the β-lactam antibiotics like ampicillin, high level aminoglycosides besides being inherently resistant to many others like cephalosporins and clindamycin has made the treatment of these infections a real

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challenge for clinicians (3). Enterococcus has intrinsic and acquired resistance to many antimicrobials. They have intrinsic resistance against a number of antimicrobials due to carrying several resistance genes as well as acquired resistance against several antibiotics like macrolides, vancomycin, tetracycline, and fluoroquinolones, resulting from either DNA mutation or acquisition of new genes through gene transfer (4). Multidrug resistant isolates are those isolate which are resistant to three or more different classes of antimicrobials (5).

Glycopeptide antibiotics are used in the treatment of infections caused by resistant enterococcus. Six types of glycopeptide resistance have been described in enterococci that can be distinguished on the basis of the sequence of the structural gene for the resistance ligase (vanA, vanB, vanC, vanD, vanE, and vanG) (6). In a study by Phukan *et al*, it was found that 24% of the enterococcus isolates were resistant to vancomycin (7). The emergence of high level resistance to aminoglycosides has made the therapeutic combination of penicillin and gentamicin ineffective. In a study done in Assam, high level gentamicin resistance (HLGR) and high level streptomycin resistance (HLSR) were found to be 53.76 and 33.33 per cent respectively (8).

Fosfomycin is a bactericidal antibiotic agent. It inhibits an enzyme-catalyzed reaction in the first step of the synthesis of the bacterial cell wall. Fosfomycin interferes with the first cytoplasmic step of bacterial cell wall biosynthesis, the formation of the peptidoglycan precursor UDP *N*-acetylmuramic acid (UDPMurNAc). This inhibitory action takes place at a step earlier than the action of beta-lactams or glycopeptides. Fosfomycin reduces adherence of bacteria to urinary epithelial cells. Fosfomycin is considerably active against both Gram negative and Gram positive pathogens. Specifically, fosfomycin is considered active against *Enterococcus* spp. (9).

The aim of this study was to determine the prevalence, identification, characterization and susceptibility pattern of enterococcus isolates from urine samples.

2. Material and methods

A prospective study was done in Medical College, Kolkata for a period of 6 months (from January to June 2018). The study was done after obtaining ethical clearance from the Institutional Review Board (IRB) of the college.

The uncentrifuged urine samples received in the laboratory were examined microscopically for presence of pus cells, bacteria, casts and other sediments. Semi quantitative urine culture was done for all samples as per standard criteria. After culture, colony count and identification of the significant urinary isolates were done by using standard microbiological techniques. The urine samples with significant growth of enterococcus were included in the study. Identification of enterococcus species was preliminary done by Gram stain, non-fastidious growth and conventional biochemical tests like catalase test, growth on 6.5% NaCl, MacConkey agar, bile esculin agar and arginine hydrolysis for genus identification and fermentation of mannitol, arabinose, sorbitol and growth on tellurite agar and automated identification by VITEK 2 Compact (BioMerieux Inc., France) for species identification.

Antimicrobial susceptibility test - These isolates were further tested for antimicrobial susceptibility to different antibiotics like ampicillin (10µg), tetracycline (30µg), ciprofloxacin (5µg), levofloxacin (5µg), vancomycin (30µg), teicoplanin (30µg), linzolid (30µg), fosfomycin (200 µg) and nitrofurantoin (300µg) by Kirby Bauer disc diffusion method using standard microbiological techniques on Mueller Hinton agar plates. Minimum inhibitory concentration (MIC) was tested by VITEK 2 Compact (BioMerieux Inc., France) for penicillin, tetracycline, ciprofloxacin, levofloxacin, vancomycin, teicoplanin, linzolid, and nitrofurantoin. Disc diffusion for fosfomycin was carried out on Mueller Hinton agar supplemented with 25µg/ml G6P with 200µg discs. Minimum inhibitory concentration (MIC) for fosfomycin was tested by E-strip (BioMerieux Inc., France) on the same medium with drug concentrations ranging from 0.064 - 1024µg/ml. All interpretation of susceptibility pattern was done according to the Clinical and Laboratory Standards Institute (CLSI) version 2017 guidelines. Susceptibility to high level gentamicin (120µg) was done by Kirby Bauer disc diffusion method and interpretation was done by using EUCAST guidelines version 2016. The quality control for antimicrobial susceptibility testing was done with *Staphylococcus aureus* ATCC 25923 for disc diffusion and *Enterococcus faecalis* ATCC 29212 for dilution method.

All data were entered in the excel spreadsheet (Microsoft Office, Redmond, Washington, USA). The geometric mean (GM) and the standard deviation (SD) were calculated using excel spreadsheet. The statistical analysis of the data was done using STATA version 20. The data were summarized using mean along with standard deviation for continuous variables, and frequency along with percentages for categorical variables. Chi square test was used to check the categorical variables association and p value <0.05 was taken as significant.

3. Results

A total of 2732 urine samples were collected in the Department of Microbiology, Medical College, Kolkata during the period of 6 months (from January to June 2018). There were 194 isolates of *Enterococcus* spp. showing a prevalence of 7.1%. Among these 194 isolates there were 148 (76.29%) *Enterococcus faecalis*, 40 (20.62%) *Enterococcus faecium*, 2 (1.03%) *Enterococcus casseliflavus*, 2 (1.03%) *Enterococcus gallinarum* and 2 (1.03%) *Enterococcus avium*. Among the antimicrobial agents tested, 45 (23.19%) isolates were susceptible ampicillin, 52 (26.8%) isolates were susceptible and 6 (3.09%) intermediately susceptible to ciprofloxacin, 54 (27.83%) isolates were susceptible and 12 (6.18%) intermediately susceptible to levofloxacin, 113 (58.25%) were susceptible to high level gentamicin, 152 (78.35%) were susceptible to nitrofurantoin and 37 (19.07%) were susceptible to tetracycline (table 1). There were total of 8 (4.12%) isolates of vancomycin resistant enterococcus which constitutes of 2 *Enterococcus casseliflavus*, 2 *Enterococcus gallinarum* 2 *Enterococcus faecium* and 2 *Enterococcus faecalis*. Only 4 isolates were resistant to teicoplanin. There was only 2 isolates resistant to fosfomycin. Only one isolate was intermediate susceptible to linezolid. Table 2 shows the relation between resistance and species of enterococcus. Resistance to ampicillin and high level gentamicin was significantly related in *Enterococcus faecium*. There were 140 (72.16%) multidrug resistant (MDR) enterococcus which were resistant to three different classes of antimicrobials. All these MDR isolates were highly susceptible to linezolid and only 2 isolates were resistant to fosfomycin.

Table 1 Shows the antimicrobial susceptibility of *Enterococcus* spp. (n=194)

Antimicrobial agent	***Enterococcus spp.* (n=194)**	**MIC range µg/ ml**	**MIC_{90} µg/ ml**	**MIC_{50} µg/ ml**
Ampicillin/ Penicillin	45 (23.19%)	<=2 - >=32	32	32
Ciprofloxacin	52 (26.8%)	<=0.5 - >=8	8	8
Levofloxacin	54 (27.83%)	<=0.5 - >=8	8	8
High level gentamicin	113 (58.25%)	-	-	-
Nitrofurantoin	152 (78.35%)	<=16 - 256	128	16
Vancomycin	186 (95.87%)	<=0.5 - >=32	1	0.5
Teicoplanin	190 (97.94%)	<=0.5 - >=32	1	0.5
Linezolid	193 (99.48%)	<=0.5 - 4	1	0.5
Tetracycline	37 (19.07%)	<=0.5 - >=16	16	16
Fosfomycin	192 (98.97%)	<=0.5 - 512	8	2

Table 2 Shows the relation of antimicrobial resistance to *E. faecalis* and *E. faecium*

Antimicrobial agent	***E. faecalis* (n=148)**	***E. faecium* (n=40)**	**P value**
Ampicillin	110	39	**0.0007**
Ciprofloxacin	104	32	0.318
Levofloxacin	98	30	0.342
High level gentamicin	57	24	**0.019**
Nitrofurantoin	15	9	0.058
Vancomycin	2	2	0.199
Teicoplanin	2	2	0.199
Tetracycline	125	32	0.48
Fosfomycin	1	1	0.381

4. Discussion

In a study done by Chakroborty *et al* in Kolkata in 2011 there was a prevalence of 7.3% enterococcus isolates from all clinical samples (10). In this study it was found that there is a prevalence of 7.1% among urine samples which is very similar to the previous finding.

In a study done in Assam, speciation of 93 enterococcus species by Vitek 2 automated system was similar to that by conventional biochemical tests. *E. faecalis* was the commonest species (81.72%) isolated, followed by *E. faecium* (12.9%) , *E. raffinosus* (3.23%, n=3), *E. avium* (1.08%, n=1) and *E. gallinarum* (1.08%, n=1) (8). In this study, among these 194 isolates there were 148 (76.29%) *Enterococcus faecalis*, 40 (20.62%) *Enterococcus faecium*, 2 (1.03%) *Enterococcus casseliflavus*, 2 (1.03%) *Enterococcus gallinarum* and 2 (1.03%) *Enterococcus avium*. Similar results were seen in another study in Uttar Pradesh where it was found that out of 100 Enterococcus strains 47 were *E. faecalis*, 51 were *E. faecium*, 2 were *E. gallinarum* and 1 was E. *casseliflavus* (11).

Glycopeptide resistance in enterococci is associated with diverse phenotypes. Intrinsic low-level vancomycin resistance is characteristic of *Enterococcus gallinarum* and *Enterococcus casseliflavus/flavescens.* The VanC-1 ligase is specific for *E. gallinarum*, and the VanC-2/3 ligase is specific for *E. casseliflavus/flavescens*. The VanC enzymes participate in the synthesis of pentapeptide peptidoglycan precursors ending in D-alanyl-D-serine, which display reduced affinity for vancomycin. Organisms with resistance to VanC remain susceptible to teicoplanin. This naturally occurring vancomycin resistance has not been shown to be transferable, and the related genes are chromosomally encoded in the members of these species (12). In this study there were two isolates each of *E. gallinarum* and *E. casseliflavus* which were resistant to vancomycin but susceptible to teicoplanin.

In this study the prevalence of multidrug resistant enterococcus was 72.16%. This is similar to the finding of *Bhatt et al* where the prevalence of multidrug resistance among enterococcus was found to be 63% among 200 clinical isolates (13). In a study done by Praharaj and colleagues out of 367 isolates of enterococcus 32 (8.7%) were found to be resistant to vancomycin. None of the Enterococcus isolates were resistant to linezolid (14). However in this study there were 8 (4.12%) isolates of vancomycin resistant enterococcus. This finding is less in comparison to the findings in Mangalore where out of 150 total isolates, 13 (8.6%) isolates showed vancomycin resistance, of which 11(7.3%) had an MIC >8 µg/ml (15). Though the first report of linezolid resistant enterococcus isolate was from Kolkata (16), we did not find any linezolid resistant strain. Only one isolate of *Enterococcus faecium* was intermediate susceptible to linezolid with MIC of 4 µg/ ml. In a study done in Katihar, 2.8% enterococcus isolates were resistant to linezolid (17).

In a study by Adhikari *et al*, the highest resistance to aminoglycoside was observed among *E. faecium*, followed by *E. durans, E. fecalis* and *E. casseliflavus*, both by disk-diffusion and agar-screen methods. The high-level aminoglycoside resistance (HLAR) was significantly (P<0.05) higher in *E. faecium* (18). In this study it was found that resistance to ampicillin and high level gentamicin was significantly related in *Enterococcus faecium*. In this study 81 (41.75%) enterococcus were resistant to high level gentamicin. In a study done in Iran among 53 isolates, high-level gentamicin resistance was observed among 50.9% of the isolates though all of the isolates were MDR (100%) (19). In another study, it was found that out of 100 isolates studied 93% of isolates were resistant to one or more antimicrobial agents, with the most frequent resistance found against tetracycline (86%), ciprofloxacin (73%) and quinupristin-dalfopristin (53%). High level gentamicin and high level streptomycin resistance were detected in 50% and 34% of isolates, respectively (20). There were only two isolates resistant to fosfomycin. Urinary enterococcus showed excellent susceptibility to fosfomycin (98.97%) and linezolid (99.48%) and good susceptibility to nitrofurantoin (78.35%). Hence these drugs are commonly used for management of UTI. Molecular characterization of the vancomycin resistant enterococcus and high level aminoglycoside resistance among the isolates was beyond the scope of this study.

5. Conclusion

Urinary enterococcus isolates are highly susceptible to fosfomycin and linezolid. Resistance to ampicillin and high level gentamicin was significantly related in *Enterococcus faecium*. However, larger prospective in vivo studies are required to determine the efficacy.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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