A Study on Susceptibility Pattern of Nitrofurantoin in Clinical Isolates of Enterococcus

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Abstract: Introduction: Recent years have witnessed a resurgence of interest in enterococci due to increasing resistance to antibiotics in term of both multiplicity of resistance & level of resistance to particular drugs. In such scenario nitrofurantoin may be the effective option specially in case of urinary tract infection cause by enterococcus. Methodology: This is a hospital based study, conducted in Microbiology Department of an medical college in M.P. Antibiotic susceptibility performed on enterococci isolated from urine samples in the study period of one and half year Result: Enterococcal species isolated mainly E.faecalis (86.62%) and E.faecium (15.18%). In Enterococcus, 89.4% isolates showed resistance to Penicillin, 58.8% to Ampicillin, 61.1% to High Level Gentamicin (HLG) and 54.1% to High Level Streptomycin (HLS) Nitrofurantoin (24.7%), Ciprofloxacin (61.2%), Levofloxacin (63.5%), Norfloxacin (64.7%), Tetracycline (54.1%). Discussion: Our study showed that only 40% E.faecalis and 10% of E.faecium were resistant to nitrofurantoin. Similar result published from Nagpur by Rahangdale et al. According to them enterococci showed only 22.76 % resistant to nitrofurantoin. Conclusion: Enterococcus show very good susceptibility for nitrofurantoin as compare to other commonly use antibiotic for treatment of enterococcal infection. [Saurabh J NJIRM 2017; 8(3):53-56]

Key Words: Enterococci, Antibiotic susceptibility testing, Nitrofurantoin

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Introduction: Recent years have witnessed a resurgence of interest in enterococci due to increasing resistance to antibiotics in term of both multiplicity of resistance & level of resistance to particular drugs. Enterococci particularly E.faecium, always have a high intrinsic level of resistance to antimicrobial agents. As enterococci are present as commensal flora in GIT, enterococcal infection is thought to be endogenous, arising from patient own flora. Enterococcus is a leading cause of nosocomial bacteremia, surgical wound infection, urinary tract infection etc. The mainstay in the treatment of serious enterococcal infection is the synergistic effect of Penicillin/Ampicillin (or Vancomycin) & aminoglycoside. However high level resistance i.e. MIC ≥2000 µg/ml to Gentamicin & other aminoglycoside is seen with increased frequency.[1] In the recent years, enterococci resistant to Vancomycin are also isolated.[2,3] Vancomycin Resistant Enterococci (VRE) commonly have Penicillin/Ampicillin & high level aminoglycoside resistance resulting in bacterial strain that may be untreatable with currently available antibacterial agents.

In such scenario nitrofurantoin may be the effective option. The major strength of Nitrofurantoin is its action at multiple sites and levels. This includes inhibition of bacterial enzymes involved in carbohydrate synthesis and in higher concentration DNA, RNA, and total protein synthesis by the non-specific attack on bacterial ribosomal proteins.[4,5] Nitrofurantoin is metabolized in renal tissue and rapidly excreted in the urine through both glomerular filtration, as well as tubular secretion, with a plasma half-life of 0.5–1 h. With this rapid excretion, the urinary concentration of nitrofurantoin is more than 100 µg/mL (up to 250 µg/mL). High concentration achievable in urine makes it an ideal choice for treatment of urinary tract infection (UTI). Komp Lindgren et al. demonstrated rapid 99% killing was seen among Vancomycin Resistant Enterococcal (VRE) and non-VRE Enterococcus strains at 24 h.[6]

Nitrofurantoin is usually well tolerated. Side-effects occur at rates <0.001%.[7] Macrocrystal formulations reduce gastrointestinal effects such as nausea and vomiting. Haemolytic anaemia can occur in patients with glucose-6-phosphate deficiency. Serious adverse effects are rare and occur only with prolonged medication (>6 months).[4] This includes chronic pulmonary reactions and interstitial fibrosis, peripheral neuropathy and hepatic injury. Nitrofurantoin also has good safety profile for use in pregnancy (pregnancy category B).[8] Nitrofurantoin is contraindicated in patients with renal failure with creatinine clearance rate of 60 mL/min. However, recent studies indicate the use of nitrofurantoin can be expanded to creatinine clearance as low as 40 mL/min for infection with susceptible isolates.[9]

Aims : The aims of present study is to know the susceptibility pattern of Nitrofurantoin in clinical isolates of Enterococcus.

Objectives: (1) Isolation & Speciation of enterococci. (2) To study antimicrobial resistance pattern of enterococci .

Methodology: Current study done in Microbiology Department in a period of one and half year from January 2011 to June 2012. Midstream clean catch urine sample was collected from patient admitted in various wards as well as patient attending O.P.D. and transport to laboratory.[10]

Every urine specimen received in the Microbiology laboratory was processed according to the recommended procedures for the isolation and identification of bacterial isolates.[10] Enterococci were selected by colony morphology from the primary isolation plates. Suspected colonies of the genus Enterococcus on blood agar were small (0.5-1mm) size, semitransparent, smooth, low convex discs.[10,11] It showed no hemolysis, sometimes showed α or β haemolysis. On gram staining enterococci appear as pairs of oval cocci, the cells in a pair arranged at an angle to each other. These colonies of enterococci are catalase negative. Enterococci were identified on the basis of their ability to hydrolyse of L-pyrrolidonyl-b-naphthylamide (PYR), salt-resistant growth (6.5% NaCl), and growth resistant to 40% bile with esculin hydrolysis.[10] Antibiotic susceptibility testing done for each enterococcal isolates by DDT of Kirby Bauer on Muller Hinton Agar according to CLSI guideline.[12] Antimicrobial agents viz β-lactams (Penicillin, Ampicillin), Aminoglycosides (High level Gentamicin and High level Streptomycin), Glycopeptides (Vancomycin), Oxazolidinones (Linezolid), Nitrofurantoin, Ciprofloxacin, Levofloxacin, Norfloxacin, Tetracycline tested for enterococcal isolates from urine.

Result and Observation: A total of 60 Enterococci were isolated in study period. Mainly 2 enterococcal species E.faecalis (86.62%) and E.faecium (15.18%) isolated. Enterococci were mostly isolated from female patient. Resistant patterrn for enterococcal isolates were shown in table 1. In E.faecalis, resistance was low (40%) to nitrofurantoin as compare to Penicillin (90.2%), Ampicillin (64.7%), High Level Gentamicin (HLG) (62.7%), High Level Streptomycin (HLS) (50.9%), Ciprofloxacin (84.3%), Levofloxacin (88.2%), Norfloxacin (88.2%), Tetracycline (74.5%). Similarly In E.faecium, resistance was low (10%) to Nitrofurantoin as compare to Penicillin (88.9%), Ampicillin (22.2%), High Level Gentamicin (HLG) (55.6%), High Level Streptomycin (HLS) (66.7%), Ciprofloxacin (100%), Levofloxacin (100%), Norfloxacin (100%), Tetracycline (90%). There were no resistance to Vancomycin & Linezolid for enterococcus.

Table 1: antibiotic resistance pattern in clinical isolates of Enterococci

Antibiotic	% Resistance in E.faecalis(n=51)	% Resistance in E.faecium(n=9)	% Resistance in Enterococcus (N=60)
Penicillin-G	46 (90.2)	8 (88.9)	54 (90)
Ampicillin	33 (64.7)	2 (22.2)	35 (58.3)
High level gentamicin (HLG)	32 (62.7)	5 (55.6)	37 (61.7)
High level streptomycin (HLS)	26 (50.9)	6 (66.7)	32 (53.3)
Nitrofurantoin	20 (40)	1 (10)	21 (35)
Ciprofloxacin	43 (84.3)	9 (100)	52 (86.7)
Levofloxacin	45 (88.2)	9 (100)	54 (90)
Norfloxacin	45 (88.2)	9 (100)	54 (90)
Tetracycline	38 (74.5)	8 (90)	46 (76.7)
Vancomycin	0 (0)	0 (0)	0 (0)
Linezolid	0 (0)	0 (0)	0 (0)

Discussion -Two enterococcal species E.faecalis (86.62%) and E.faecium (15.18%) were identified & Other enterococcal species not isolated in present study. A study done by Bose et al reported similar finding.[13] Study from Sevagram and Nagpur also isolated two species of Enterococci namely E.faecalis (most common) and E.faecium.[14,15] Recently from Manipal (Sikkim) a study done by Adhikari et al isolated additionally three more species of Enterococci, namely E.casseliflavus, E.durans, E.dispar. In their study E.faecium was 3rd most common.[16] Similarly Agarwal et al also isolated E.avium, E.dispar, E.cecorum, E.hirae from various clinical samples.[17] E. gallinarum (2.44%) and E. raffinosus (0.81%) were isolated from Nagpur.[18] E.faecium was the most common (80.77%) species of enterococci in the study

of Karmarkar et al.[19] In our study resistance to Penicillin-G was very high 90% in enterococci. Similar finding was observed in study from Nagpur where 89.43% isolates of enterococci were resistant to penicillin-G.[18] Titze-de-Almeida et al from Brazil found E.faecium which was less resistant (11.1%) than E.faecalis (27.6%).[20] Karmarkar et al found E.faecium (71.43%) to be more resistant to PenicillinG as compared to E.faecalis (40%).[15] Similarly finding are also reported from Nagpur.[15]

In this study resistance to Ampicillin in E.faecalis was 64.7% and in E.faecium 22.2%. A study done in Mumbai also find similar finding.[19] Rahangdale et al found 43.9% enterococci were resistant to Ampicillin.[18] In a study from Lucknow, E.faecium (61.11%) was found to be more resistant to Ampicillin than E.faecalis (16.67%).[17] Similar result were also published by Agrarwal et al. Exact reason for this difference with our study is not known but this may be due to geographic variation.[18]

In our study HLGR in E.faecalis was 62.7% and in E.faecium 55.6%. A study done by Rahangdale et al, which showed 49.59% high level resistance to Gentamicin.[18] But in study from Lucknow lower prevalence of HLGR was noted in E.faecalis (10.53 %) as well as in E.faecium (6.45%).[17] Similarly Salem Bekhit et al also found low prevalence of HLGR in E.faecalis (22.3 %) as well as in E.faecium (18.5%) as compared to our study. [21] In contrast, high prevalence of HLGR was noted in E.faecalis (100%) as well as in E.faecium (85.71%) in Mumbai.[19]

In this study HLSR in E.faecalis was 50.9% and in E.faecium 66.7%. Observation close to our study was reported from Nagpur.[18] But a study from Lucknow showed low prevalence of HLSR in E.faecalis (21.05%) as well as in E.faecium (22.22%).[17] Recently Salem Bekhit et al and study from Sevagram also found low prevalence as compared to our study.[14,21] Vancomycin resistance was not detected in enterococcus. Resistance to Vancomycin is widely variable. Agrarwal et al, Titze-de-Almeida et al, Rahangdale et al, did not get any VRE in their study. [15,19,20] But in a study from Mumbai 10% Vancomycin resistance in E.faecalis and 28.57% in E.faecium was noted.[19] Similarly, Salem Bekhit et al found 1.8% Vancomycin resistance in E.faecalis and 18.5% in E.faecium.[21] Agarwal et al from Lucknow found 9.52% Vancomycin resistance in E.faecalis but did not find any Vancomycin resistance in E.faecium.[17] Although, at present, VRE is not a problem in our set up, its routine monitoring is essential, since it appears to be an emerging pathogen in India.[14] Tetracycline is effective drug in the enterococcal isolates of urine only. In this study resistance to tetracycline in E.faecalis was 74.5% and in E.faecium 90%. Salem Bekhit et al also found 62.7% tetracycline resistance in E.faecalis and 85.2% in E.faecium.[21] Similar result are also published from Brazil.[20] In a study from Lucknow, E.faecalis was more resistant to tetracycline showing 76.19% resistance as compared to E.faecium (72.73%).[15]

In our study resistance to Ciprofloxacin in E.faecalis was 84.3% and in E.faecium 100%. Agarwal et al from Nagpur also found just similar resistance profile to Ciprofloxacin in E.faecalis (64.3%) and in E.faecium (95.2%).[15] But some authors found decreased resistance to both species of enterococci as compare to this study. Like in a study from Saudi Arabia, 49.4% resistance in E.faecalis and 51.9% in E.faecium was found.[21] Similarly, some authors also found that E.faecalis was more resistance to Ciprofloxacin then E.faecium. Agarwal et al found 64.29% resistance in E.faecalis and 51.51% resistance in E.faecium.[17] Similar finding showed in a study from Brazil and by Karmarkar et al.[19,20]

Our study showed that only 40% E.faecalis and 10% of E.faecium were resistant to Nitrofurantoin. Similar result published from Nagpur by Rahangdale et al.[19] According to them enterococci showed only 22.76 % resistant to nitrofurantoin. Nitrofuratoin is a reserve drug for treatment of enterococccal urinary tract infection as shown in our observation. In a study of Canada, it was found that all VRE were susceptible to Nitrofurantoin.[22]

Conclusion: Enterococcus show very good susceptibility for nitrofurantoin as compare to other commonly use antibiotic for treatment of enterococcal infection. Vancomycin & Linezolid are also a good option inspite of emerging of VRE strain worldwide, but on the basis of pharmokinetic & dynamic Nitrofurantoin is the better option. Similarly for UTI in pregnancy nitrofurantoin is safe & effective option.

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