ejpmr, 2021,8(10), 281-285

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS ISOLATES FROM CLINICAL SAMPLES AT A TERTIARY CARE CENTRE IN WESTERN MAHARASHTRA, INDIA

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Article Received on 27/07/2021 **Article Revised on 17/08/2021** **Article Accepted on 07/09/2021**

ABSTRACT

Introduction: *Enterococci* are normal commensals of the gastrointestinal tract. They are one of the leading causes of nosocomial urinary tract infections. They are opportunistic pathogens harbouring antibiotic resistance genes which may lead to treatment failures. **Aim**: To determine the prevalence and antibiotic susceptibility pattern of Enterococcus isolates in clinical samples. **Materials and Methods:** Consecutive, non-repeat *Enterococcus* isolates (264), from clinically relevant samples (28,945 of them) received at a tertiary care centre during the study duration (01 May 2018 to 31 Oct 2020) were included in this retrospective laboratory-based study. Identification of positive cultures was done by phenotypic and automated methods by Vitek 2 Compact Bacterial Identification System (BioMerieux). The antibiotic susceptibility testing was done by automated and Kirby-Bauer disk diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** A total of 264 *Enterococcus* isolates were identified during study duration. The distribution of these isolates in different clinical samples was; urine (1.42%), pus (1.01%), blood (0.18%) and miscellaneous samples including body fluids (0.79%). The species were *Enterococcus faecalis* (46.2%), *Enterococcus faecium* (33.3%) and unspeciated *Enterococci* (19.3%). 57.6% of these isolates were obtained from ward patients, and 13.6% and 28.8% from Intensive Care Unit (ICU) and Outpatient Department (OPD) patients respectively. A maximum number of isolates were susceptible to Teicoplanin (93.0%) and Linezolid (92.0%), Fosfomycin (88.4%), Vancomycin (84.2%), Nitrofurantoin (77.9%), and High-Level Aminoglycosides (HLA); Gentamicin (55.2%) Streptomycin (57.5%). **Conclusion:** *Enterococcus* strains were found to be least resistant to Teicoplanin. Hospital wards were the commonest place of spread of infection. Pan-resistant, multidrug resistant and glycopeptide resistant *Enterococci* were noted in this study.

KEYWORDS: *Enterococci,* Antibiotic susceptibility, Glycopeptides.

INTRODUCTION

The genus *Enterococcus* are Gram positive, ovoid shaped cocci, arranged in short chains or pairs. *Enterococci* are widely distributed in nature and are usually part of the mixed flora commonly found in gastrointestinal tract.[1] Extensive use of broad-spectrum antibiotics is responsible for *Enterococci* emerging as opportunistic nosocomial pathogens. One health continuum study sequencing the whole genome of enterococcal isolates from human, cattle and environmental samples revealed antibiotic resistance genes across the spectrum.[2] The high prevalence of multidrug resistant (MDR) *Enterococcal* infection in a tertiary care set up is due to excessive and indiscriminate use of broad-spectrum antibiotics.[3] The most common nosocomial infection caused by *Enterococci* are urinary tract infections (UTI) followed by surgical site infections (SSI). The antimicrobial therapy of *Enterococcal* infections is challenging because of the inherent resistance of *Enterococci* to several common antibiotics such as cephalosporins, low level aminoglycosides, clindamycin and cotrimoxazole. This problem is amplified because of their acquired resistance to all currently available antibiotics including glycopeptides, that leaves very limited treatment options. Vancomycin resistant *Enterococcus faecium* (VREfm) is an emerging pathogen, especially in bacteraemia.[4] Endogenous infections from their natural habitat such as the gastrointestinal tract commonly occurs post abdominal surgery. Within the hospital environment, where these bacteria are exposed to different antibiotics, the

enterococci mutate to become multidrug resistant pathogens.[5]

MATERIALS AND METHODS

Consecutive, non-repeat Enterococcus isolates (264), from clinically relevant samples (28,945 of them) received at a tertiary care centre during the study duration (01 May 2018 to 31 Oct 2020) were included in this retrospective laboratory-based study. The study was carried out at Department of Microbiology at a tertiary care centre in Western Maharashtra. Identification of positive cultures was done by smear microscopy, catalase test, bile esculin hydrolysis test, sugar fermentation tests and by Vitek 2 Compact Bacterial Identification System (BioMerieux). Antibiotic susceptibility tests were done by the Kirby-Bauer disk diffusion method using antibiotic discs; Ampicillin (10µgm), Tetracycline (30µgm), Ciprofloxacin (5µgm), Nitrofurantoin (300µgm), Fosfomycin (200µgm), Vancomycin (30µgm), Linezolid (30µgm), Teicoplanin (30µgm), and High-level aminoglycosides resistance (HLAR) was done by using Gentamycin (120µgm) for which zone size of ≤6mm was regarded as resistant. Antibiotic susceptibility tests done by Vitek2 Compact (BioMerieux) were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, for vancomycin ≤4 µg/ml is interpreted sensitive, 8-16 µg/ml as intermediate and ≥32 µg/ml as resistant.

RESULTS

A total of 28,945 clinical samples were processed for various clinical indications, 264 *Enterococcal* isolates (0.91%) were identified over a period of 30 months from 1 May 2018 to 31 Oct 2020. The distribution of enterococcal isolates in the various clinical samples received were as follows- 188 isolates from 13,174 urinary specimens (1.42%), 25 isolates from 2,460 pus specimens (1.01%), 16 isolates from 8,907 blood cultures (0.18%) and 35 isolates from 4404 (0.79%) other miscellaneous samples that consisted predominantly of high vaginal swabs, placental membranes, seminal fluid, ascitic fluid, cerebrospinal fluid and bone or tooth fragment.

The species isolated in decreasing order of frequency were *Enterococcus faecalis* (122/264) 46.2%, *Enterococcus faecium* (88/264) 33.3%, *Enterococcus durans* (2/264) 0.7%, *Enterococcus avium* (1/264) 0.3%, and remaining unspeciated *Enterococci* (51/264) 19.3%. Blood cultures in comparison to other samples harboured more of *Enterococcus faecium* than other clinal specimens which had *Enterococcus faecalis*.

The *Enterococci* isolated from various clinical samples were from urinary specimens 188 (71.2%), pus; 25 (9.5%), blood; 16 (6%) and miscellaneous body samples were 35 (13.3%) of the total positive isolates (Fig 1).

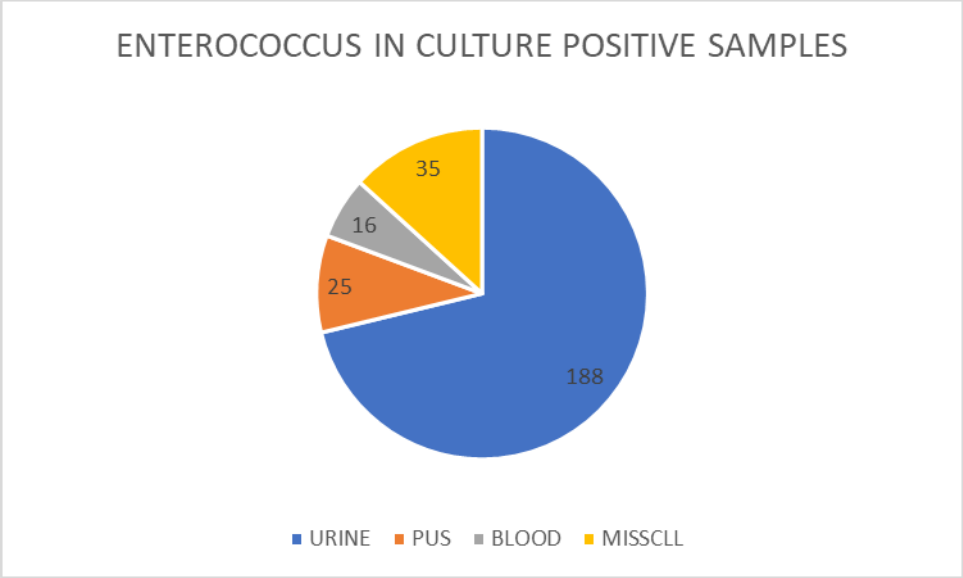

Fig. 1: Pie diagram showing distribution of different clinical samples positive for Enterococci.

These specimens were received from clinical settings of ICU in (36/264) 13.6%, wards (152/264) 57.6% and from OPD (76/264) 28.8% patients **(**Fig 2).

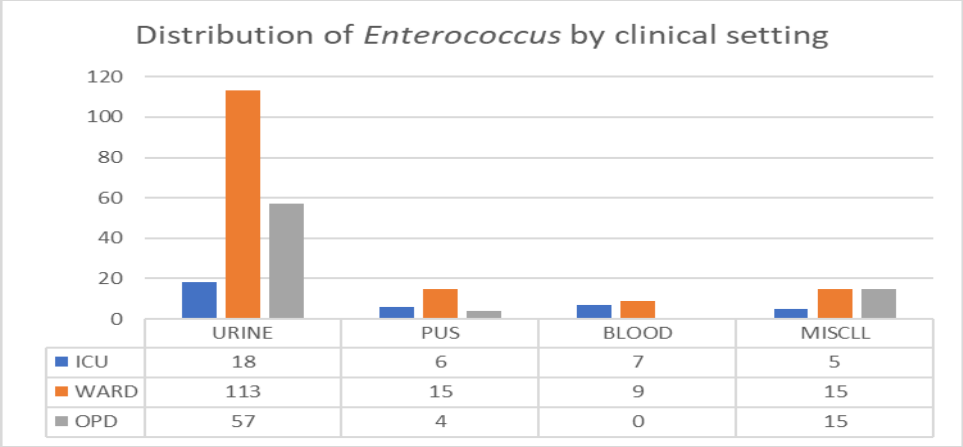

Fig. 2: Distribution of Enterococci in different clinical samples from ward, ICU and OPD.

The percentages of *Enterococcus faecalis* and *faecium* isolated from various samples (Fig 3).

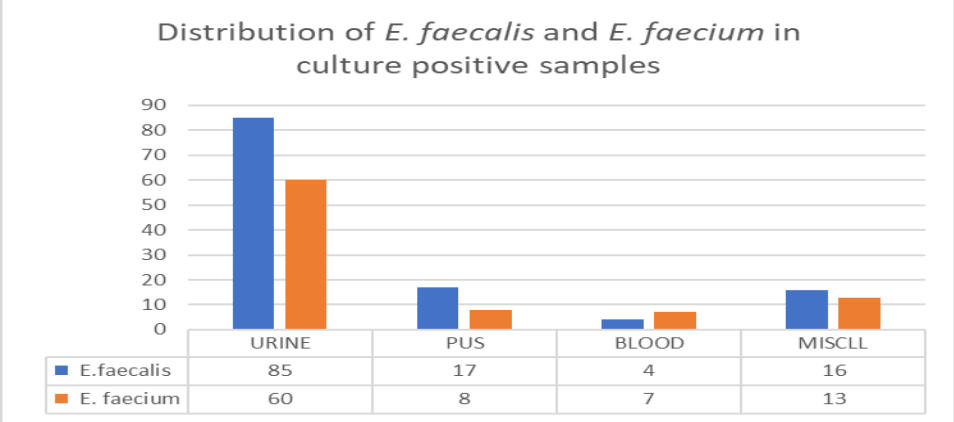

Fig. 3: Distribution graph of clinical samples positive for Enterococci faecalis and Enterococcus faecium in different samples.

Antibiotic susceptibility pattern for the isolates revealed that maximum number of isolates were susceptible to Teicoplanin (93.0%), Linezolid (92.0%), Fosfomycin (88.4%), Vancomycin (84.2%), Nitrofurantoin (77.9%), and High-Level Aminoglycosides (HLA); Gentamicin (55.2%) Streptomycin (57.5%) (Fig 4).

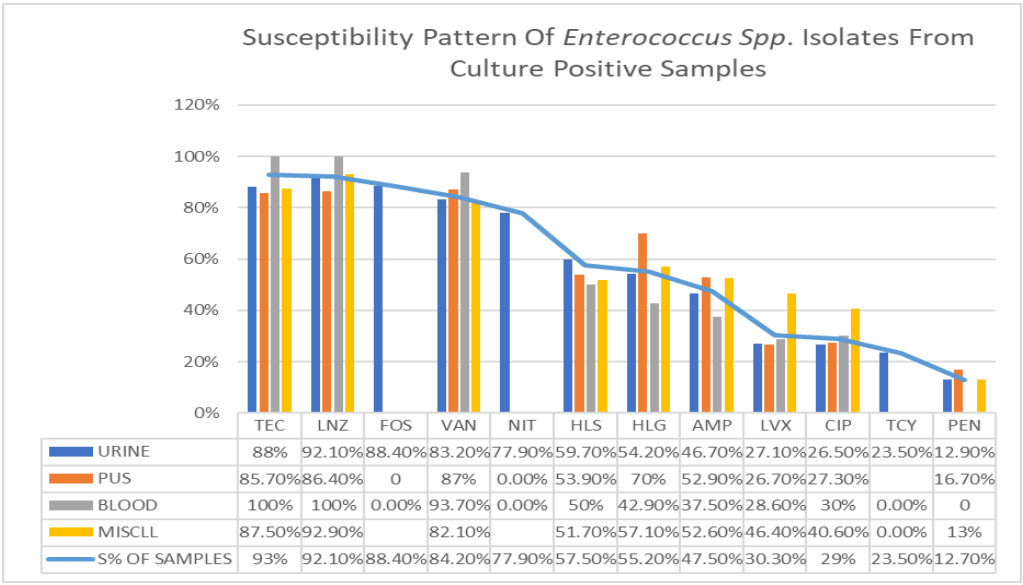

Fig. 4: Antibiotic susceptibility pattern of Enterococci.

DISCUSSION

The findings of our study are similar to the findings of different studies from India.[6-9] The prevalence ranges from 1-11% from different centres. In our study *Enterococci* were mainly isolated from urinary specimens, this has been the trend seen in other studies.[10,11] The isolation rates of Enterococcus from different clinical samples in our study was; urine (1.42%), pus (1.01%), blood (0.36%) and other miscellaneous samples (0.39%). This is similar to findings of another study that had overall (2.5%), urine (1.97%), exudates (5.54%) and blood (1.4%).[12] More than half the isolates were obtained from ward patients, exhibiting broad spectrum antibiotics resistance and having undergone interventions such as placement of urinary or peripheral vascular catheters. This may be due to the ability of this bacteria to form biofilms.[13] This supports the previous studies incriminating induced selection and spread of virulent strains in hospitals and patient's microbiota.[14] Similar to findings in another study, isolated more *Enterococcus faecium* from the blood cultures as compared to *Enterococcus faecalis* isolates.[15]

The antibiotic susceptibility profile of Enterococcus as seen in our study included the most susceptible drugs such as; Teicoplanin 93% and linezolid 92%. Vancomycin susceptibility was 84%. high level aminoglycoside 52.2%, AMP 47.5%, Nitrofurantoin is found effective in three fourth of urinary isolates. The efficacy of traditional treatment of *Enterococcal* infection with ampicillin synergistically with a high-level aminoglycoside still holds but is effective in only about half the infections.[16]

In OPD settings, Enterococcal UTI is managed with Nitrofurantoin, or a fluoroquinolone like Ciprofloxacin. In indoor settings, Penicillin, Ampicillin or Vancomycin in combination with like Gentamicin or Streptomycin for synergistic action, is used. In case of glycopeptide resistance, Linezolid is used, or for *Enterococcus faecium* only, Quinopristine-Dalfopristine is used.

Enterococcus faecium was isolated more than *faecalis* from blood cultures. This observation was also reported by other studies.[17,18]

Infections caused by VRE often involve intra-abdominal sites, urinary tract, bloodstream, surgical sites and often vascular catheter sites and tend to occur in seriously ill hospitalised patients. Overall VRE were found to be 26% in our study, which was comparable to data from other tertiary care centres.[19,20]

CONCLUSION

Teicoplanin and Linezolid were found to be least resistant drugs to *Enterococcus*, followed by Vancomycin. Hospital wards were found to be the commonest place of infection spread. Pan-resistant, multidrug resistant and glycopeptide resistant *Enterococci* were noted in this study. This can only be curtailed by increasing awareness regarding drug resistance and use of more appropriate antimicrobials.

ACKNOWLEDGEMENTS

Nil.

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