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Research Article

INCIDENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROCOCCAL INFECTIONS IN TERTIARY CARE HOSPITAL

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Abstract- Introduction: *Enterococci* have become second most common cause of hospital acquired Urinary tract infections [HA-UTIs] and wound infections and third leading cause of bacteremia. In recent years; *Enterococci* recognized with increasing frequency as common cause of Intra abdominal and pelvic infections, post surgery wound infections, and endocarditis. Most infections are caused by two enterococcal species *i.e., Enterococcus faecalis* and *Enterococcus faecium*. Early detection of resistant strains will help in the institution of appropriate therapy and also helps to lessen treatment failure, selection and spread of resistant strains.

Materials and methods: The study was a Cross sectional study and was conducted in the Department of Microbiology at a tertiary care health centre in Western Maharashtra over a period of one year from July 2015 to June 2016. *Enterococci* were isolated on Blood agar plate as non-haemolytic 0.5–1mm size streptococci-like colonies; and on CLED agar as small yellow colonies from fermentation of lactose. Identification was done by standard conventional methods. The susceptibility was determined using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (CLSI, 2007) Identification and isolation with VITEK 2C AUTOMATED SYSTEM were done for *Enterococci*, Daptomycin Epsilometer test (E test) were done to determine MIC.

Results and Observations: A total 141 [100%] *Enterococci* species were isolated from various clinical infections; of which 58.15% strains were from UTIs, 324.11% were from wound and pyogenic infections, 10.63% were from various body fluids while 3.54% were from bacteremia and respiratory infections. Of which 141; 54.6% enterococcal strains were isolated from female patients and 45.4% were from male patients. The age group 20–60 years constitute the largest proportion 85 (60%) followed by age group 1- 12 years 30 (21.3%) and in Elderly [≥ 60yrs] 26 [18.4%]. Of the total 141 clinical isolates of *Enterococci*; 15.60 % isolates were from outdoor patients, 84.39% admitted in hospital; 22% from various ICUs. *E. faecalis* were 48 %, *E. faecium* in 47 %, *E. gallinarum* 3 %, *E. avium* in 1 %. In our study, VRE was seen in approximately 2 %. All *E. faecalis* were sensitive to Vancomycin, Teicoplanin, Linezolid, Tigecycline and Daptomycin.

Conclusion: High level resistance was detected in aminoglycoside, penicillin, quinolones in the present study though the prevalence rate of VRE is low. In recent years; changing pattern of Enterococcus spp. as a causative agent in clinical infections should be consider as *E. faecium* with high level resistance is more prevalent in developing countries.

Keywords- VRE, *Enterococcus faecalis, Enterococcus faecium*, HA-UTI.

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Introduction

Enterococcus are opportunistic pathogens that cause a number of infections predominantly in the immunocompromised patients, elderly patients with serious underlying diseases, patients who have been hospitalized for long duration, use invasive devices and under treatment with broad spectrum antimicrobial therapy [1]. *Enterococci* have become second most common cause of hospital acquired Urinary tract infections [HA-UTIs] and wound infections and third leading cause of bacteremia. In recent years; *Enterococci* is recognized to have increasing frequency as common cause of Intra abdominal and pelvic infections, post-surgery wound infections, and endocarditis. Most infections are caused by two enterococcal species i.e. *Enterococcus faecalis* [*E. faecalis*] and *Enterococcus faecium* [*E. faeicum*] [2]. Most enterococcal infections are due to the patient's endogenous flora however recent studies indicates that vancomycin resistant *Enterococci* [VRE] can be transmitted directly from one patient to another through patient care equipments or via contaminated environmental surfaces [3]. Due to intrinsic resistance; *Enterococci* exhibits deprived activity to several commonly used antimicrobial agents; due to which, recommended therapy for serious infections such as endocarditis, meningitis or other systemic infections require treatment with a combination of cell wall active agent such as β– lactams [penicillin or vancomycin] combined with an aminoglycosides [Gentamicin or Streptomycin].This synergistic bactericidal effect is lost when the isolates become resistant to β – lactams or have high level resistance [HLR] to aminoglycosides [4-6]. Resistance to Vancomycin was first detected in 1986 [5,6]. Since then VRE strains are increasingly reported across the globe. In general, the prevalence of VRE is as low as as 4% in Europe and as high as 33% in North America. In India, the prevalence of VRE ranges from 0 to 30 % [7,8]. As a result, *Enterococci* have emerged as one of the leading therapeutic challenges when identified as a cause of serious; life threatening infections. Hence, the present study was undertaken to determine prevalence and systematic surveillance of *Enterococci* from tertiary care hospital

Materials and methods

The study was a Cross sectional study and was conducted in the Department of Microbiology at a tertiary care health centre in Western Maharashtra over a period.

of one year from July 2015 to June 2016.

Ethics statement: Institutional ethical clearance was obtained.
Demographic and clinical data were obtained from each patient.

Sample collection: Clinical specimen such as blood, urine, wound swabs; sputum and various body fluids were received in the department of microbiology for culture and susceptibility. Aerobic cultures and isolation was done on Blood agar and urine samples were cultured on Cystiene Lactose Electrolyte Deficient [CLED] agar with sterile standard loop and incubated for 24 hours at 37°C.

Isolation/identification of *Enterococci*
Enterococci were isolated on Blood agar plate as non-haemolytic, 0.5–1mm in size streptococci-like colonies; and on CLED agar as small yellow colonies due to fermentation of lactose. The colonies were confirmed as *Enterococci* with Gram stain positivity, negative catalase test, positive bile-aesculin (bile insolubility) test, growth in 6.5% NaCl broth and as *Enterococcus* species by specific sugar glucose, lactose, mannitol, sorbitol and arabinose fermentation reactions [9,10].

Antibiotic susceptibility test
The susceptibility of each *Enterococci* isolate to oxacillin and vancomycin was determined using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (CLSI, 2007) [11] on Mueller-Hinton agar [M-H agar](supplemented with 2% NaCl) with 1µg oxacillin and 30µg vancomycin discs and incubating at 35°C for 24 hours. Oxacillin zone diameter (ZD) of inhibition ≥14mm defined oxacillin susceptibility in *Enterococci* while vancomycin ZD ≥17mm defined vancomycin susceptibility (CLSI, 2007). Susceptibility of each isolate to other antibiotics (ampicillin 10µg, erythromycin 15µg, gentamicin 10µg, cotrimoxazole 25µg, tetracycline 10µg, ceftazidime 30µg and ciprofloxacin 5µg) was performed using the disk diffusion method of Bauer *et al* (1966). ZD for susceptibility to these antibiotics in *Enterococci* was; ampicillin ≥17mm, erythromycin ≥23mm, gentamicin ≥15mm, cotrimoxazole ≥16mm, tetracycline ≥19mm, ceftazidime ≥18mm and ciprofloxacin ≥21mm (CLSI, 2007) [11,12].

Quality control: *E. faecalis* ATCC 29212 usedas susceptible control strain and *E. faecalis* ATCC 51299 as resistant control strain.

Identification with VITEK 2C Automated System: Bacterial strains Inoculum preparation. Suspensions were prepared by emulsifying bacterial isolates in 0.45% saline to the equivalent of a 0.5 McFarland turbidity standard. The same suspension was used for identification and AST for the VITEK 2 system. The test panels (ID-GPC) Card type: GP testing Instrument: 0000148FF364 (8551); were used for identification which contained 46 fluorimetric tests that included pH change tests and also includes 16 fermentation tests (for D-raffinose, amygdaline, arbutine, D-galactose, glycerol, D-glucose, L-arabinose, lactose, D-maltose, D-mannitol, N-acetylglucosamine, salicin, D-sorbitol, D-trehalose, D-melibiose, and D-xylose), two decarboxylase tests (for ornithine and arginine), and six miscellaneous tests (for urease, pyruvate, optochin, novobiocin, polymyxin B sulfate, and 6% NaCl). The card was automatically filled by a vacuum device, sealed and inserted into the VITEK 2 reader-incubator module (incubation temperature, 35.5°C), and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the ID-GPC database, and final results were obtained automatically. All cards used were automatically discarded into a waste container [13].

Antibiotic Susceptibility Testing with VITEK 2C Automated System: The 0.5 McFarland bacterial suspensions was diluted to 1.5 x 10^7 CFU/ml in 0.45% saline. Cards were automatically filled, sealed, and loaded into the VITEK 2 instrument for incubation and reading. Card type: AST-P628 testing Instrument: 0000148FF364 (8551)card used for *Enterococci* contained ampicillin, ampicillin-sulbactam, cefuroxime, ciprofloxacin, clindamycin, erythromycin, high-concentration (HC) gentamicin, imipenem, HC kanamycin, levofloxacin, nitrofurantoin, norfloxacin, ofloxacin, quinupristin-dalfopristin, HC streptomycin, teicoplanin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin [13-15].

Daptomycin Epsilometer test (E test)-It is a unique MIC determination paper strip which is coated with daptomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016 mcg/ml to 256 mcg/ml, for testing against the organism.

Test procedure: Preparation of inoculum-From the pure colony of *Enterococcus spp*., 2-3 colonies were inoculated in the 5 ml tryptone soya broth and were incubated at 35-37^{0}C for 2-4 hrs until moderate turbidity developed. The inoculum turbidity was matched with the turbidity of 0.5 McFarland. Daptomycin Ezy MIC strips are supplemented with calcium ions therefore it can be tested on regular MH agar plate. Place the strip at the desired position on the plate pre-lawned with test organism aseptically. Transfer the plates in the incubator at 35-37^{0}C for 18-24 hrs.

MIC reading: MIC was read where the ellipse intersects the MIC scale on the strip.

Observations and Results
The ubiquitous nature of *Enterococci* however requires caution in establishing the clinical significance of a particular isolates from various clinical infections during study period.
A total 141 [100%] *Enterococci* species were isolated from various clinical infections; of which 82 [58.15%] strains were from UTIs, 34 [24.11%] were from wound and pyogenic infections, 15 [10.63%] were from various body fluids while 5 [3.54%] were from bacteremia and respiratory infections. Of which 141; 77 [54.6%] enterococcal strains were isolated from female patients and 64 [45.4%] were from infections suffered by male patients. The age group 20–60 years constitute the largest proportion 85 (60%) followed by age group 1- 12 years 30 (21.3%) and in Elderly [≥ 60yrs] 26 [18.4%].
Of the total 141 clinical isolates of *Enterococci*; 22 [15.60 %] isolates were from outdoor patients while 119 [84.39%] were from patient's admitted in hospital. Total 31 [22%] isolates were from patients admitted in various ICUs, 26 [18.43%] isolates were from patients hospitalized for longer duration, and 19 [13.5%] isolates were from post operative infections.

Speciation of 141 *Enterococci* isolates by phenotypic methods: *E. faecalis* were identified from 68 samples (48 %), *E.faecium* from 67 (47 %), *E. gallinarum*from 4 (3 %), *E. avium* from 2 (1 %) samples.

Identification and antibiotic susceptibility of *Enterococcus* spp. with the VITEK 2 system
The results of this study indicate that the VITEK 2 system represents an accurate and acceptable means for performing identification and antibiotic susceptibility tests for *Enterococcus* spp.

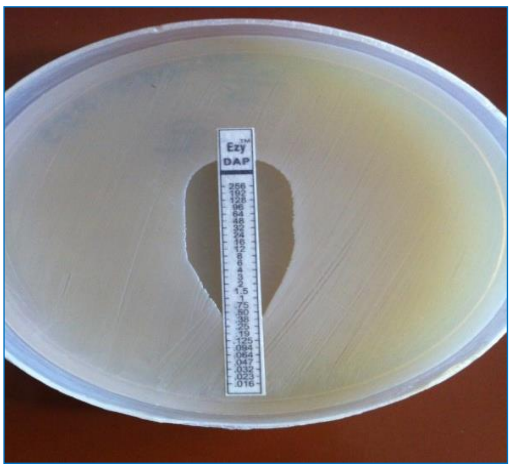

Fig-1 Daptomycin E-test

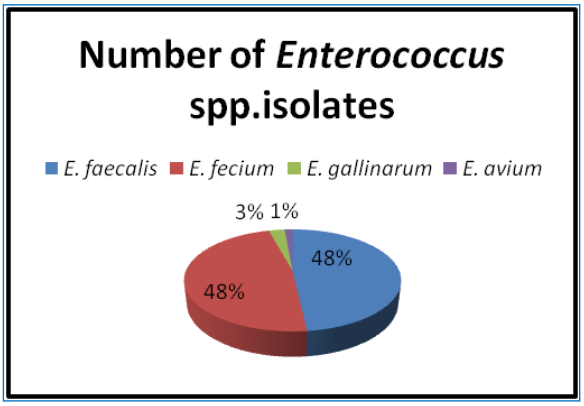

Fig-2 Isolation of *Enterococcus* spp.

Table-1 *Antibiotic susceptibility of Enterococcus spp. from UTIs infections[N=82]*

Antimicrobial agent	*E. faecalis* [n=55]		*E. faecium* [n=27]	
	Susceptibility	Resistance	Susceptibility	Resistance
Ampicillin	9 [16.36%]	46 [86.63%]	0	27[100%]
Gentamycin	12 [21.81%]	43[78.18%]	0	27[100%]
Ciprofloxacin	5 [9.09%]	50 [90.90%]	0	27[100%]
Nitrofurantoin	43 [78.18%]	12 [21.81%]	14 [51.85%]	13 [48.14%]
Erythromycin	9 [16.36%]	46 [83.63%]	0	27 [100%]
Linezolid	55 [100%]	0	26 [96.29%]	1 [3.70%]
Norfloxacin	4 [7.27%]	51 [92.72%]	2 [7.40%]	25 [92.59%]
Vancomycin	55 [100%]	0	26 [96.29%]	1 [3.70%]
Daptomycin	55[100%]	0	27 [100%]	0

Antimicrobial susceptibility of *E. faecalis* isolated from urine samples showed 100 % susceptibility to vancomycin, linezolid and 78.18% susceptibility to nitrofurantoin while 92.72% resistance to norfloxacin, 90.90% resistant to ciprofloxacin , 86.85 resistance to ampicillin and erythromycin, 78.18% to gentamicin. HLR were detected to gentamicin by Vitek 2C AST

Antimicrobial susceptibility *E. faecium* isolated from urine samples showed 100% resistance to ampicillin, gentamicin, ciprofloxacin and erythromycin which higher resistance that the *E. faecalis* strains. Susceptibility to nitrofurantoin was 51.85 %, Linezolid and vancomycin were 96.29% while 92.59% resistance to norfloxacin, 90.90% resistant to ciprofloxacin, 86.85 resistance to ampicillin and erythromycin, 78.18% to gentamicin. HLR were detected to gentamicin by Vitek 2C AST

Of the total clinical 141 isolates; 82 [58.15%] clinical isolates were from UTIs patients urine sample of which 35 [42.68%] urine isolates were from geriatric age groups and 17 [20.73%] were from pediatric age group. 15 [18.3%] *Enterococci* strains were from ICUs patients with invasive devices used.

Table-2 *Antibiotic susceptibility of Enterococcus spp. for other than UTIs infections [N=59]*

Antimicrobial agent	*E. faecalis* [n=36]		*E. faecium* [n=23]	
	Susceptibility	Resistance	Susceptibility	Resistance
Ampicillin	9 [25%]	27 [75%]	0	23[100%]
Gentamycin	30 [83.33%]	6 [16.66%]	0	23 [100%]
Ciprofloxacin	8[22.22%]	28 [77.77%]	0	23 [100%]
Clindamycin	15[41.66%]	21 [58.33%]	14 [60.86%]	9 [39.13%]
Erythromycin	17 [47.22%]	19 [52.77%]	0	23 [100%]
Linezolid	36 [100%]	0	23 [100%]	0
Teicoplanin	4 [11.11%]	32 [88.88%]	21 [91.30%]	2 [8.69%]
Vancomycin	36 [100%]	0	22 [95.65%]	1 [4.34%]
Daptomycin	36 [100%]	0	23 [100%]	0

Discussion: *Enterococci* have been implicated in approximately 10% of all UTIs and 16% of nosocomial UTIs.Enterococcal bacteriuria usually occurs in patients with underlying structural abnormalities and /or in those who have undergone urologic manipulations. Intra-abdominal and pelvic infections are the next most commonly encountered infections. In the present study clinical data revealed; prevalence of *Enterococci* causing UTIs patients was high 82[58.15%] followed by pyogenic infections 34 [24.11%], 20 [14.18%] bacteremia and sepsis while 5[3.54%] respiratory infections [Fig-1]. Sreeja, *et al*., (2012) from Banglore India reported high prevalence of *Enterococci* spp. from pus samples [43%] followed by urine samples [31%] [16], Chakraborty, *et al*., (2015) reported high prevalence in urine samples [66%] followed by pus sample [19.6%] and blood samples [8.50%] [17]. Preeti Srivastava et al 92013), Seema Mittal et al (2016) from India reported high prevalence of *Enterococci* in UTIs [7, 8] [Fig-2]. Pus samples were received from intra-abdominal and pelvic infections, samples were from otitis media of pediatric age group, colorectal cancer, rectal cancer, wound gap; cholecystitis, cholelithiasis, detriment, appendicitis, of the total bacteremia and sepsis patients; approximately 95% blood samples were received from neonatal intensive care unit [NICU] and were from patients with invasive devices. A patient is seven times more likely to acquire an infection in hospital if an invasive device is used. Biofilm formation in *Enterococci* is one of the several mechanisms to evade action of antibiotics and help in persistence of infections especially on indwelling catheters [19].

E. faecalis [48.22%] and *E. faecium* [47.51%] were isolated in almost equal numbers.

All *E.faecium* isolates were resistant to pencillins, aminoglycosides. Total 4 [7.31%] Enterococcus strains from UTIs were susceptible for norfloxacin. Of the total 82 *Enterococci* isolated from UTIs 57 [69.51%] were susceptible to nitrofurantoin. Resistant to nitrofurantoin was detected higher in *E. faecium* as compared to *E. faecalis* strains. Overall HLR was observed more in *E.fecium* strains than *E.fecalis* strains in present study [Table-1]. HLR to gentamycin [MIC ≥2000 µg/ml]was seen in 99 [70.21%] isolates and were detected by Vitek 2CAST automated method. HLR were also reported in study by Horodniceanu T*et al*(1979), Mederski Samorak *et al* (1983) Latika Shah*et al* (2012). PJ Desai et. al. (2001) reported 48.21% of colonization of Foleys catheters by *Enterococcus spp.*was found to be high incidence of UTIs. Nita gangurde et al (2014) reported VRE in *E. faecium* [13.7%] and *E. faecalis* [4.6%][19-22].

All *E.faecalis* were sensitive to vancomycin, teicoplanin, linezolid, tigecycline and daptomycin. All *E.faecium* were sensitive to linezolid, tigecycline [tigecycline was tested in selected strains].Two isolates were resistant to vancomycin and teicoplanin. One isolate of *E. gallinarum* was resistance to vancomycin but sensitive to teicoplanin. Total 59 [41.84%] strains were isolated other than UTIs including wound infections, bacterimia and respiratory infections. Of these 59 *Enterococci* strains; all 23 were *E. fecium* showed 100% resistant to ampicillin, gentamycin, ciprofloxacin, erythromycin [Table-2]. Low resistance was detected by teocoplanin and vancomycin. Tigecycline was not tested for samples received from neonates and pediatric patients, Ventillator associated Pneumonia patients. Tigecycline can be considered a preferred treatment for polymicrobial Intra abdominal infections [IAIs] associated with VRE and in the present study we have not tested tigecycline and also we do not recommend it to be used for VRE bacteremias due to low serum concentrations, and is lack of clinical data to support its use for other indications. No quality studies have been performed to assess the efficacy of tigecycline monotherapy for the treatment of infective endocarditis [IE], but it has been used successfully along with daptomycin for the treatment of IE due to VRE. In the present study, all *Enterococci* spp. were susceptible to tigecycline. Current treatment options including linezolid, daptomycin, quinupristin/ dalfopristin, and tigecycline have shown favorable activity against various vancomycin-resistant *Enterococcus* infections, but there is a lack of randomized controlled trials assessing their efficacy. Daptomycin is a cyclic lipopeptide with rapid concentration-dependent bactericidal activity against many resistant Gram-positive organisms, including VRE *faecalis* and *faecium*. Both linezolid and daptomycin should still be used as first-line options for the treatment of VRE bacteremia, but high-dose daptomycin use should be considered (8–12 mg/kg) [5,22]. Daptomycin is a preferred agent for the treatment of bacteremia, IE, UTI, CNS infection, IAI, and SSSI, but higher doses should be considered for the treatment of serious VRE infections, and synergy with a β-lactam can be attempted for refractory cases[5,23-27]. In the present study; Enterococcus strains resistant to aminoglycoside, quinolones, benzylpenicillin and β-lactams were tested for Daptomycin E-test and all were showed susceptibility to daptomycin. Out of the141 isolates 3[2%] showed resistance to vancomycin and out of these 3 VRE, 2 were *E.fecium* and 1 was *E. gallinarum*. Ebbing Lautenbach

et al (2003) reported steadily increased prevalence of VRE from 17.4% to 29.6% (10 years study from Division of Infectious Diseases and Centre for Clinical epidemiology and biostastisitcs; Philadelphia, Pennsylvana) [5]. In our study, VRE was seen in approximately 2 %, which is much lesser when compared to other studies wherein VRE ranged from 4% to 23% [5,19,25-27]. All *E.fecalis, E. gallinarum and E. avium* were 100% sensitive to Teicoplanin. Only 2 isolates of *E. fecium* (MIC≥ 32) were resistant to teicoplanin. Culture from patients with peritonitis; intra-abdominal abscess; biliary tract infections, surgical site infections, endomyometritis are frequently polymicrobial and role of *Enterococci* in this settings remains controversial [1,2,5]. In our study, the VITEK 2 system demonstrated similar rates of accuracy in identification and AST of *Enterococci*. In the clinical setting, reasons for species identification of *Enterococci* are very limited (serious infections, such as endocarditis, or epidemiological surveillance within hospitals). In general, presumptive identification to the genus level together with determination of susceptibility is considered to be sufficient. Several taxonomy changes have been introduced in the *Enterococcus* genus, mainly involving species other than *E. faecalis*.

Conclusion:

High level resistance was detected in aminoglycoside, penicillin, quinolones in the present study though the prevalence rate of VRE is low. To determine the prevalence of colonization with VRE and to identify risk factors with VRE in health care settings is mandatory for preventive measures. In recent years, changing pattern of *Enterococcus* spp. as a causative agent in clinical infections should be consider as *E. faecium* with high level resistance is more prevalent in developing countries.

Application of the research: Early detection of resistant strains of *Enterococci* will help in the institution of suitable therapy and also helps to reduce treatment failure and increase of resistant strains.

Research category: MDR *Enterococci*

Abbreviation: MDR: Multidrug resistant, VRE: Vancomycin Resistant *Enterococci*

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