BAOJ Microbiology

Niharika Lall and Silpi Basak, BAOJ Microbio 2016 2: 2
2: 007

Research Article

Study of Antibiotic Susceptibility Profile of *Enterococcus* Species

Niharika Lall[1] and Silpi Basak[1*]

[1]Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, India

Abstract

Background

Enterococcus has emerged as one of the common cause of nosocomial infections. Their increasing resistance to commonly used antibiotics poses a great threat for treatment of enterococcal infections. Hence, the present study was undertaken to study the antibiotic susceptibility profile of *Enterococcus* species and to detect the incidence of Multidrug resistant *Enterococcus* species.

Material and Methods

Enterococcus strains were isolated and identified by conventional tests from different clinical samples. Antibiotic susceptibilty test was done by Kirby- Bauer disk diffusion method, Agar dilution method and minimum inhibitory concentration (MIC) was detected by Etest strip (BioMeriux) according to CLSI guidelines.

Result

A total number of 190 *Enterococcus* strains were isolated mainly from urine followed by blood, pus, wound swab etc. *E. faecalis* (55.8%) and *E. faecium* (43.7%), *E. hirae* (0.5 %) were the species isolated. All 190 (100%) *Enterococcus* strains were sensitive to vancomycin and linezolid. High level aminoglycoside resistance (HLAR) was detected in 60.5% strains. The presence of HLAR in *Enterococci* makes the synergism of cell-wall inhibitor and aminoglycoside ineffective. 47.5% enterococcal strains were multiple drug resistant (MDR).

Conclusion

Enterococcus strains which are commonly isolated from different clinical specimens must be screened routinely for HLAR, MDR and vancomycin resistant *Enterococci* (VRE) by all clinical microbiology laboratories to prevent the emergence and spread of this multiple antibiotic resistant organism.

Keywords: *Enterococcus* Species; Antibiotic Resistance; High Level Aminoglycoside Resistance; Multidrug Resistance

Introduction

Enterococcus species were considered as harmless commensals for many years. But incidence of enterococcal infections especially hospital acquired has dramatically increased over the last 25years [1]. The commonly encountered Enterococcal infections are urinary tract infections, acute or sub-acute endocarditis, bacteremia, central nervous system (CNS) infections and soft tissue infections [2]. *Enterococcus* sp. have been reported to be the second most common cause of hospital acquired urinary tract and wound infections and third most common cause of nosocomial bacteriaemias [3]. *E. faecalis* accounts for 80-90% whereas *E. faecium* accounts for 5-15% of all clinical isolates. Other species, *E. gallinarum, E. casseliflavus, E. durans, E. hirae* are isolated less frequently. Emergence of *Enterococcus* species as an important nosocomial pathogen in the past two decades in many respects can be attributed to their resistance to many antimicrobial agents and ease with which they attain and transfer resistant genes [4] . *Enterococcus* is intrinsically resistant to most of the β lactam antibiotics because of low affinity penicillin binding protein. HLAR occurs due to aminoglycoside modifying enzyme (AME) and is defined as streptomycin MIC >2000 µg/ml and gentamicin MIC >500µg/ml[3]. Multidrug resistant (MDR) strain is defined as acquired non- susceptibility to at least one agent in three or more antimicrobial categories [5]. In 1988, isolation of vancomycin resistant *Enterococci* (VRE) was reported from England [6] and then from different parts of world. Hence, the present study was undertaken to study the antibiotic susceptibility profile of *Enterococcus* species and to detect the incidence of MDR *Enterococcus* species.

Material and Methods

The present study was conducted in a tertiary care hospital in central India from 1st July 2011 to 31st August 2013 (2 year period). A total number of 190 *Enterococcus* strains were isolated from different clinical samples e.g. urine, blood, pus and wound swab, different body fluids, urinary catheter tips etc. received from indoor as well as from outdoor patient departments. All the specimens were cultivated on blood agar and MacConkey's agar. The *Enterococcus* species were identified by conventional tests like growth on and blackening of bile-esculin agar (photograph 1), heat test (pohotograph2), growth in the presence of 6.5% sodium chloride and positive PYR (pyrrolidonyl aryl amidase) test etc (3). Further speciation was done by sugar fermentation tests (mannitol, arabinose, lactose, raffinose and sucrose) (photograph 3), arginine

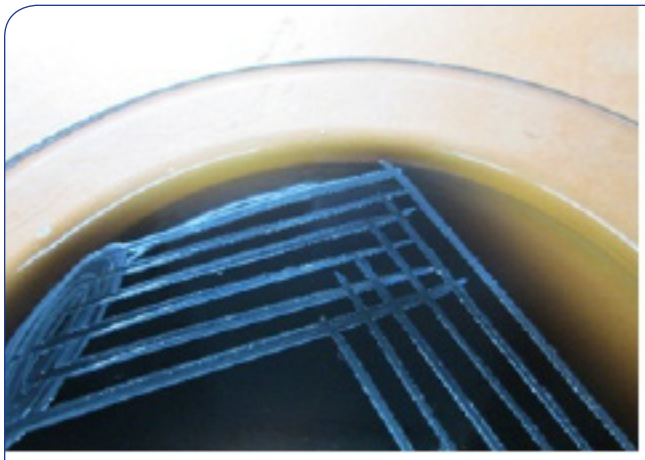
***Corresponding author:** Silpi Basak, Professor, Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (MS)-442004, India, E-mail: drniharikalall@gmail.com

Sub Date: December 28, 2015, **Acc Date:** January 25, 2016, **Pub Date:** January 27, 2016

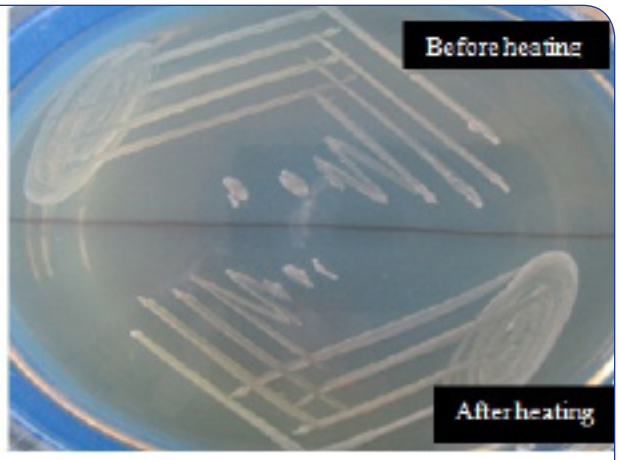
Citation: Niharika Lall and Silpi Basak (2016) Study of Antibiotic Susceptibility Profile of Enterococcus Species. BAOJ Microbio 2: 007.

dihydrolase test (photograph4), motility test (photograph5), pyruvate fermentation test (photograph 6) and yellow pigment production detection etc [7].

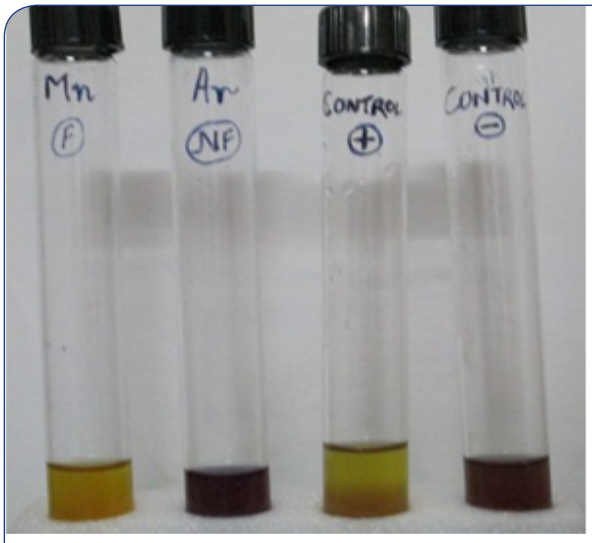
Antibiotic susceptibilty test was done for all 190 strains by Kirby-Bauer disk diffusion method [8], according to CLSI guidelines [9]. Followingantibioticswere tested-ampicillin(10µg),linezolid(15µg),



photograph 1
Bile-esculin Hydrolysis Test: Positive

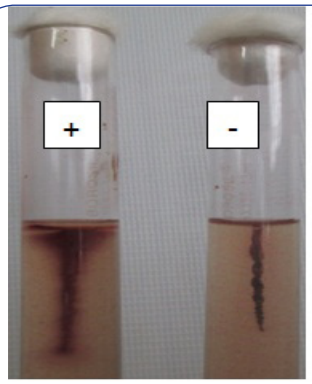


photograph 2
Heat Test: Positive



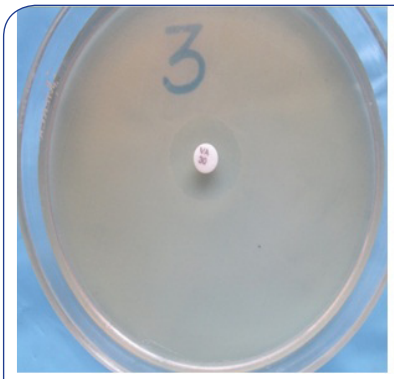
photograph 3
Sugar fermentation tests- E.faecalis

photograph 4
Arginine Dihydrolase test:+ve in E.faecalis



photograph 5
Motility test: -ve in E.faecalis

photograph 6
Pyruvate fermentation test-E.faecalis



photograph 7
Vancomycin disc diffusion test
Zone of Inhibition -16mm
(Intermediate range)

photograph 8
Vancomycin E-Test Vancomycin
MIC: 3µg/ml

quinupristin- dalfopristin (15µg), vancomycin (30 µg), gentamicin (HLG-120µg), streptomycin (HLS-300µg), chloramphenicol (30 µg), erythromycin (15 µg), rifampin (5 µg), tetracycline (30 µg). For urine samples- norfloxacin (10 µg), nitrofurantoin (300 µg) were also tested. HLAR was detected by disk diffusion test using high level streptomycin (HLS - 300 µg) disk and high level entamicin (HLG - 120 µg) disk and agar dilution method taking gentamicin according to CLSI guidelines [9].

As per CLSI guidelines, for detection of HLGR by agar dilution method, gentamicin concentration was taken as 500µg/ml [9]. HLGR was also confirmed by putting the Himedia Ezy MIC strip. MIC range of the strip was from 0.064-1024 µg/ml. VRE detection was done by disk-diffusion test (vancomyin 30 µg) and VRE screen agar (vancomycin concentration 6µg/ml) and vancomycin E test (BioMeriux)[9].

Observations and Results

In the present study, 190 *Enterococcus* strains were isolated from different clinical samples. *Enterococcus* strains were mainly isolated from urine followed by blood, pus & wound swab and others (peritoneal fluid, CSF, ascitic fluid, drain fluid, granulation tissue, catheter tip) (Figure 1). *E. faecalis* (55.8%) and *E. faecium* (43.7%) were the predominant species isolated. One relatively uncommon *Enterococcus* species, *E. hirae* (0.5 %) was also isolated from blood sample. Maximum 64 (33.7%) enterococcal strains were isolated from clinical specimens received from surgery ward followed by pediatrics 32 (16.9%) and medicine 24 (12.7%). 20 enterococcal strains were isolated from all Intensive Care Units (ICUs) i.e. (MICU, PICU, NICU etc) (figure 2). From Out Patient Department (OPD), 23 *Enterococcus* strains were isolated.

All 190 (100%) *Enterococcus* strains were sensitive to vancomycin and linezolid. 14 enterococcal strains (5- *E. faecalis* and 9- *E. faecium*) were in the intermediate range for vancomycin by disc diffusion method (repeated thrice). For presumptive identification of vancomycin resistance as per CLSI guidelines, those 14 strains were inoculated on VRE agar and no strains were grown. For further confirmation, MIC of vancomycin for all those 14 strains was detected by vancomycin E Test (BioMerieux). But all 14 strains showed vancomycin MIC < 4µg/ml, which is in the sensitive range (Photograph 7, 8). Hence, those 14 strains were considered sensitive to vancomycin.

It was observed that out of 190 *Enterococcus* strains, 58 (30.5%) were sensitive to ampicillin (Figure 3). There was a marked difference in the sensitivity to ampicillin between the two species (*E. faecalis* 42% sensitive and *E. faecium* only 14% sensitive) (Table 1). Both the species isolated from urine samples i.e. *E. faecalis* (95%) and *E. faecium* (87%) were highly sensitive to nitrofurantoin. Out of 190 *Enterococcus* strains isolated, HLAR was detected in 115 (60.5%) strains. Amongst 115 HLAR strains 59 (51.4%) strains were *E. faecalis* and 56 (48.6%) were *E. faecium* [10].

Out of total 190 *Enterococcus* strains, 90 (47.5%) were MDR. MDR *Enterococcus* strains were detected on the basis of resistance (acquired) to erythromycin, tetracycline and high level aminoglycosides. Out of 90 MDR strains, *E. faecium* and *E. faecalis* accounted for 55.5% (n=50) and 44.5% (n=40) of MDR strains respectively (Figure 4).

Discussion

Enterococcus strains were mainly isolated from urine followed by blood, pus & wound swab and others. This is comparable with Suresh et al (2013) as in their study majority of the isolates were from urine (62%) followed by blood (10.3%) [11]. Most common

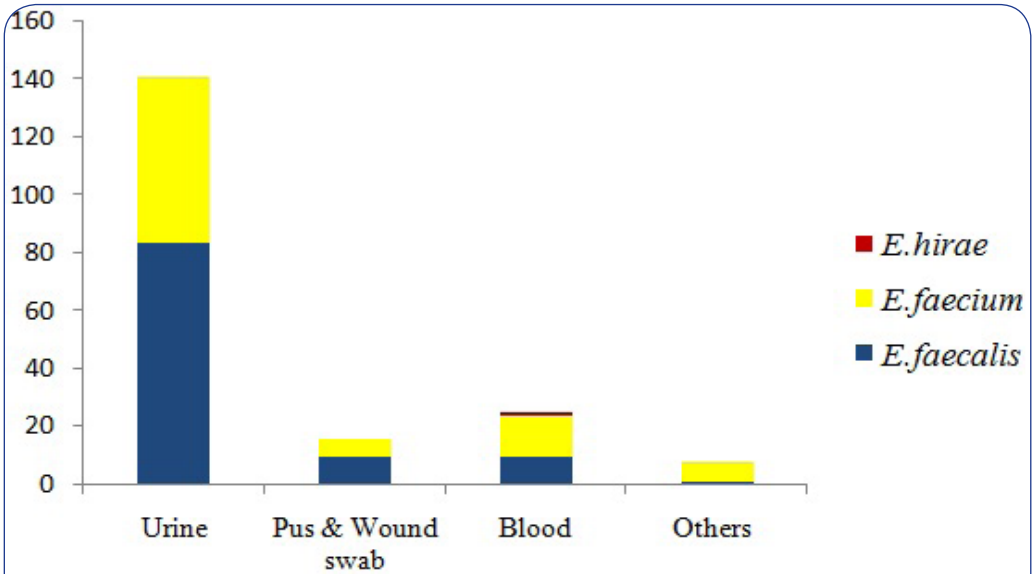


Figure 1: Isoation of *Enterococcus* species from different clinical specimens (n=190) Others include body fluids [CSF (n=2), peritoneal fluid (n=1), ascitic fluid (n=1)], catheter tip (n=1), drain fluid (n=2), granulation tissue (n=1)]

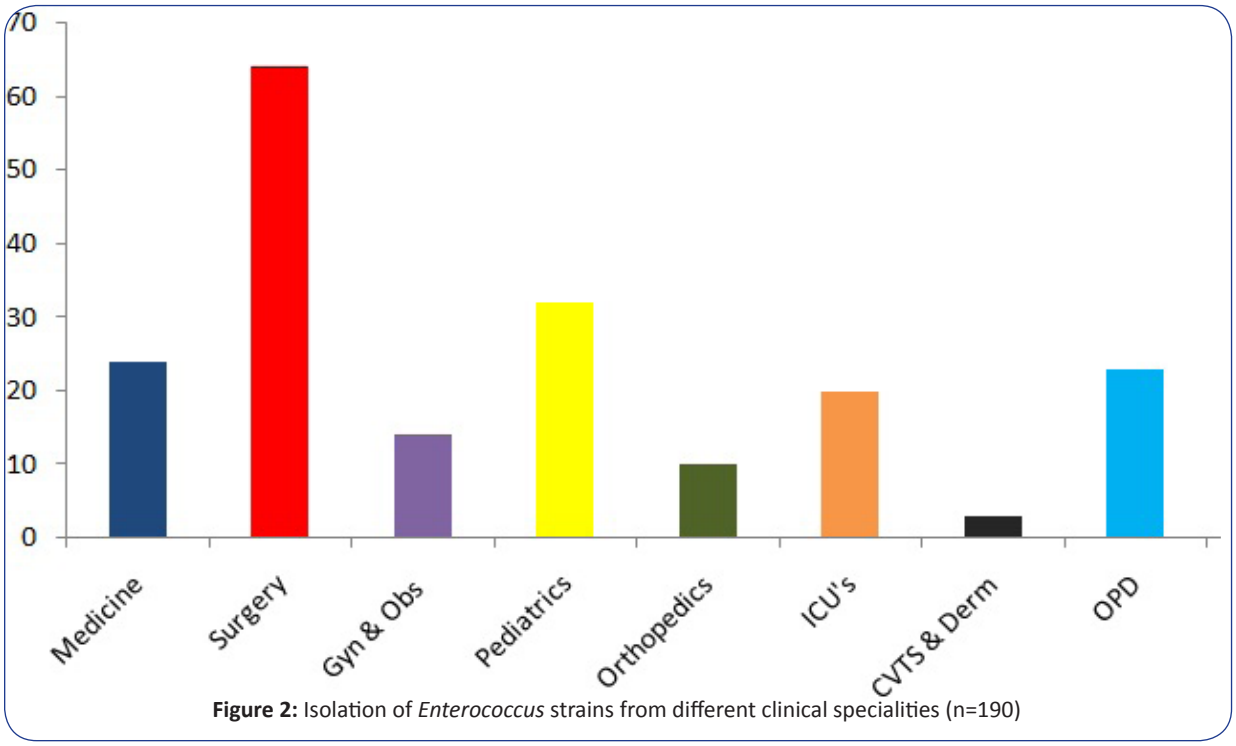


Figure 2: Isolation of *Enterococcus* strains from different clinical specialities (n=190)

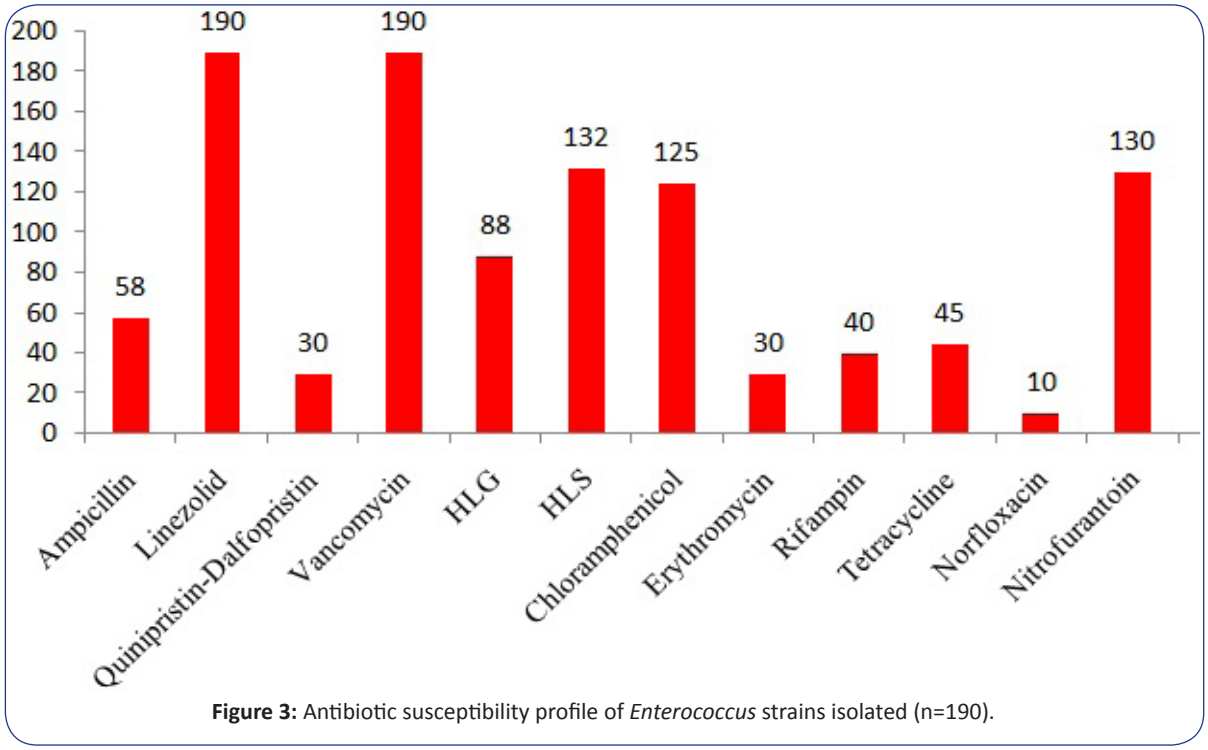


Figure 3: Antibiotic susceptibility profile of *Enterococcus* strains isolated (n=190).

species isolated was E. *faecalis* (55.8%) followed by *E. faecium* (43.8%). One relatively uncommon species has also been isolated; *E. hirae* (0.5%). Jain et al from Delhi reported 53% of their isolates as *E. faecium* and 33% as *E. faecalis*. They have also reported other Enterococcus species as *E, casseliflavus* (8%), *E. raffinosus* (4%) and *E. dispar* (2%) [12]. Maximum (33.7%) enterococcal strains were isolated from clinical specimens received from surgery ward followed by pediatrics and others. Our study correlated well with study conducted by J. Papaparaskevas et al who reported isolation of Enterococcus species from medicine and surgical wards as 19 % and 33% respectively [13].

All 190 (100%) *Enterococcus* strains were sensitive to vancomycin and linezolid. There were 14 enterococcal strains in the intermediate range for vancomycin by disc diffusion method (repeated thrice). But all 14 strains had vancomycin MIC < 4µg/ml by vancomycin

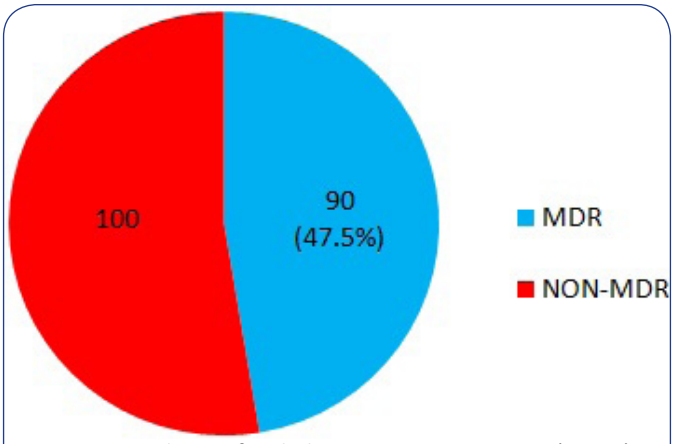


Figure 4: Incidence of multidrug resistant *Enterococci* (n=190).

Table 1: Antibiotic susceptibility profile of *Enterococcus* strains isolated from different clinical samples (n= 190)

	Enterococcus sp. sensitive (n=190)		E. faecalis sensitive (n=106)		E. faecium sensitive (n=83)		E. hirae** sensitive (n=1)
Antibiotic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**
Ampicillin	58	30.5%	45	42%	12	14%	1
Linezolid	190	100%	106	100%	83	100%	1
Q-D #	30	15.8%	14	13%	15	18%	1
Vancomycin	190	100%	106	100%	83	100%	1
Gentamicin (HLG)	88	46.3%	54	50.9%	33	39.7%	1
Streptomycin(HLS)	132	69.4%	70	66%	61	73.4%	1
Chloramphenicol	125	65.7%	54	50.9%	70	84.3%	1
Erythromycin	30	15.8%	18	16.9%	11	13.2%	1
Rifampin	40	21%	26	24.5%	14	16.8%	0
Tetracycline	45	23.6%	18	16.9%	26	31.3%	1
Norfloxacin*	10	7.1%	6	7.1%	4	7%	-
Nitrofurantoin*	130	92%	80	95%	50	87%	-

Quinupristin- dalfopristin

*Norfloxacin and Nitrofurantoin have been put for Urine samples only. Total number of Urine samples were 141(*E. faecalis* :84, *E. faecium* : 57). Calculations have been done accordingly.

E Test (BioMeireux) which is in the sensitive range and therefore considered vancomycin sensitive. The CLSI guidelines must be followed by all clinical microbiology laboratory for reporting vancomycin resistant or vancomycin intermediate *Enterococci* otherwise false positive VRE may be reported. The problem of VRE (vancomycin resistant *Enterococci*) may not be very high in India, especially in Central India. Mendiratta et al have reported 100% vancomycin sensitivity [14] which is also seen in our study. Rahangdale et al have reported 100 % sensitivity to linezolid [15], which was also reported in the present study. 95% of *E. faecalis* and 87% of *E. faecium* strains isolated from urine samples were sensitive to nitrofurantoin (Table 1). The very high percentage of sensitivity to nitrofuratoin was observed probably because Nitrofurantoin is seldom used now a days for treating urinary tract infections.

HLAR has been detected in 115 (60.5%) enterococcal strains [10]. Mendiratta et al reported 46% HLAR producing *Enterococcus* strains [14], whereas Vinod kumar C S et al reported 65.6% HLAR producing *Enterococcus* strains [16]. A common regime for treatment of serious enterococcal infections is the combination of cell-wall inhibitors, such as penicillin, ampicillin or vancomycin; with aminoglycosides, such as streptomycin or gentamicin. The addition of cell-wall inhibitor agent helps in the penetration of the aminoglycoside into the bacterial cytoplasm, making the

intrinsically resistant organism aminoglycoside sensitive. The presence of HLAR producing *Enterococcus* strains makes the synergism of cell-wall inhibitor and aminoglycoside ineffective [17]. In our study 47.5% of *Enterococcus* strains, are MDR. Out of 90 MDR strains, *E. faecium* and *E. faecalis* accounts for 50 (55.5%) and 40 (44.5%) of MDR strains respectively. Our study correlated well with the study conducted by Deshpande et al who reported 57% MDR *Enterococcus* [18].

Conclusion

In the present study, MDR and HLAR producing *Enterococcus* strains were detected by phenotypic methods. With the spread of *Enterococcus* strains showing HLAR, there is now rampant use of vancomycin since it is the last resort for treatment. The problem of VRE may not be very high in India especially in our hospital in a rural set-up. But monitoring of VRE according to CLSI Guidelines is need of the hour as it appears to be an emerging pathogen in India and has been already reported from Mumbai, Delhi and South India. Resistance to multiple antibiotics and inactivity to the synergistic killing of combination therapy of penicillin and aminoglycosides have given an excellent opportunity to *Enterococcus* species to survive and cause infetions.

Hence, to conclude *Enterococcus* strains which are commonly isolated from different clinical specimens must be screened routinely for HLAR, MDR and VRE by all clinical microbiology laboratories to prevent the emergence and spread of this multiple antibiotic resistant organism.

References

1. Giridhara UPM, Umapathy BL, Ravikumar KL (2012) Comparative Study for the presence of Enterococcal Virulence Factors Gelatinase, Hemolysin and Biofilm among Clinical and Commensal Isolates *Enterococcus faecalis*. Journal of Laboratory Physicians 2(2): 100-104.
2. Murray BE (1990) The Life and Times of the *Enterococcus*. Clinical Microbiology Reviews 3(1): 46-65.
3. Winn W Jr, Allen S, Janda W et al. (2006) Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins; Chapter 13, Gram-positive Cocci Part II: Streptococci, Enterococci and the 'Streptococcus-Like' Bacteria 672-764.
4. Marothi YA, Agnihotri H, Dubey D (2005) Enterococcal resistance: an overview. Indian Journal of Medical Microbiology 23(4): 214-219.
5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3): 268-281.
6. Uttley AHC, Collins CH, Naidoo J, George RC (1988) Vancomycin-resistant *Enterococci*. Lancet 1(8575-6): 57-58.
7. Facklam RR, Collins MD (1989) Identification of *Enterococcus* species isolated from human infections by conventional test scheme. J Clin Microbiol 27(4): 731-734.
8. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a single disc method. Am J Clin Pathol 36(3): 49-52.
9. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing: 22th informational supplement. CLSI document M100-S22 32(3).
10. Lall N, Basak S (2014) High level Aminoglycoside resistant *Enterococcus* species: A study. Int J Cur Res Rev 6 (3): 16-21.
11. Suresh K, Saipriya B, Viswanath G (2013) Isolation, Speciation and Determination of High Level Aminoglycoside Resistance of *Enterococci* among hospitalised patients in Davangere. National Journal of Laboratory Medicine 2(1): 12-15.
12. Jain S, Kumar A, Kashyap B, Kaur IR (2011) Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. International Journal of Applied and Basic Medical Research 1(2): 80-83.
13. Papaparaskevas J, Vatapoulos A, Tassios PT, Avlami A, Legakis NJ, et al. (2000) Diversity among high-level aminoglycoside- resistant enterococci. Journal of Antimicrobial Chemotherapy 45(3): 277-283.
14. Mendiratta DK, Kaur H, Deotale V, Thamke DC, Narang R, et al. (2008) Status of High level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a rural hospital of Central India. Indian Journal of Medical Microbiology 26(4): 369-371
15. Rahangdale VA, Agrawal G, Jalgaonkar SV (2008) Study of antimicrobial resistance in *enterococci*. Indian Journal of Medical Microbiology 26(3): 285-287.
16. Vinodkumar C S, Srinivasa H, Basavarajappa K G, Geethalakshmi S, Bandekar N (2011) Isolation of bacteriophages to multi-drug resistant Enterococci obtained from diabetic foot: A novel antimicrobial agent waiting in the shelf? Indian Journal of Pathology and Microbiology 54(1): 90-95.
17. Adhikari L (2010) High-level Aminoglycoside Resistance and Reduced Susceptibility to Vancomycin in Nosocomial *Enterococci*. J Glob Infect Dis 2(3): 231–235.
18. Deshpande VR, Karmarkar MG, Mehta PR (2013) Prevalence of multidrug-resistant enterococci in a tertiary care hospital in Mumbai, India. J Infect Dev Ctries 7(2): 155-158.