Int.J.Curr.Microbiol.App.Sci (2016) *5*(9): 826-836

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 9 (2016) pp. 826-836**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.509.093

Study of Antimicrobial Resistance in *Enterococci* at Government Medical College, Bhavnagar, Gujarat, India

Ankita Nisarta*

Department of Microbiology, GMERS Medical College, Dharpur-Patan, Gujarat, India
**Corresponding author*

Keywords

Enterococci, Resistance, VRE and VSE Gujarat.

Article Info

Accepted:
25 August 2016
Available Online:
10 September 2016

A B S T R A C T

Enterococci were originally classified as enteric gram-positive cocci and later, included in the genus streptococcus. The intrinsic and acquired antimicrobial resistance properties of *Enterococci*, to several antibiotics, have enabled them to survive in clinical environment *Enterococci* acquire resistance to several available antimicrobial agents by either mutation or by receiving the foreign resistant determinations through plasmids & transposons. The Aim of this research work is to study antimicrobial resistance in *Enterococci* . The present prospective study was conducted on 125 pure isolates of *Enterococci* isolated consecutively from various clinical samples like Pus, Blood, wound Swab, Sputum, urine, etc. Received at Department of Microbiology of Govt Medical College, Bhavnagar for bacteriological culture and sensitivity. The samples obtained were processed for culture of the bacteria as per routine standards methods. Detection of VRE and VSE is done. Chi- square was used to compare differences in resistance to antibiotics among the entercoccal species. A þ value of <0.05 was used to indicate significant differences. 22 (2.3%) pure enterococcal isolates were recovered from 921 specimens. The most frequent source of enterococcal isolations in this study was urine (63.63%) and greater rate of isolation of *Enterococci* from patients admited in wards (88.80%) as compared to isolates from outdoor patients Overall, this study revealed *E.faecalis* as the most common species (71.42%) followed by *E.faecium* (28.57%).The isolates were resistant to Penicillin (43.75%), Ampicillin (37.5%), Gentamicin (50%), Erythromycin (96.87%), Tetracycline (28.1%), Ciprofloxacin (75%). None of the isolates were resistant to linezolid. Two (0.64%) strains were resistant to vancomycin and Teicoplanin. All the strains (100%) in this study were resistant to Erythromycin. It was reassuring that 98.73% and 77.21% of the *E.faecalis* and 97.67% and 13.95% of the *E.faecium* in this study were vancomycin and Ampicillin susceptible, respectively. None of the isolate was resistant to linezolid and Tetracycline resistance was found only in 20.00% of isolates, suggesting their possible role in VRE and multi-drug resistant infection. The most frequent source of enterococcal isolations in this study was urine (63.63%) and greater rate of isolation of *Enterococci* from patients admitted in wards (88.80%) highlights the organisms as one of the important cause of nosocomial urinary tract infections. Overall, in the present study, the isolates were resistant to Penicillin (43.75%), Ampicillin (37.5%), Gentamicin (50%), Erythromycin (96.87%), Tetracycline (28.1%), Ciprofloxacin (75%). None of the isolates were resistant to linezolid. Two (0.64%) strains were resistant to vancomycin and Teicoplanin.

Introduction

Enterococci are catalase negative gram positive cocci that occur singly or arranged in pairs or as short chains. They are ubiquitous in nature. *Enterococci* are traditionally regarded as low grade pathogens but have emerged as an increasingly important cause of nosocomial infections in the 1990s The ability of enterococi to have intrinsic resistance as well as to acquire resistance to several classes of antibiotics enhances their importance as human pathogen, especially in the nosocomial setting (Arthur *et al*., 1993).

The term 'enterococcus' probably originated with the discovery of the first organism of this group Thiercelin (1899) used this term to describe bacteria seen in pairs and short chains in human faeces. The name Streptococcus faecalis was used by Anderws and Horder (1906) (Agarwal *et al*., 1999) to identify an organism of faecal origin that clotted milk and fermented mannitol and lactose but not raffinose. Orla Jensen (1919) described a second organism, S.faecium which differed from the fermentation patterns of S-faecalis (Orla Jensen *et al*., 2005).

Murray BE (1990) reported that *Enterococci* were originally classified as enteric gram positive cocci and later included in the genus streptococcus (Murray, 1990).

Lancefield R.C (J Exp Med 1933) traces that in the early 1930's *Enterococci* were classified as group D steptococci and were differentiated from the non-enterococcal Group D streptococci by distinitive biochemical characteristics (Lancefield, 1933).

Sherman JM (1938) recommended that the term enterococcal group be specifically used for the streptococci that grow both at 10^0C and 45^0C at pH 9.6,in the presence of 6.5% NaCl, survive at 60^0C for 30 minutes and hydrolyseesculin (Sherman, 1937).

Materials and Methods

The present prospective study was conducted on 125 pure isolates (1 per patient) of *Enterococci* isolated consecutively from various clinical samples like Pus, Blood, wound Swab, Sputum, urine, etc. received at Department of Microbiology at Govt Medical college, Bhavnagar for bacteriological culture and sensitivity. The samples obtained were processed for culture of the bacteria as per routine standards methods.

These samples were obtained from patients attending the outpatient departments and admitted to the indoor wards of various facilities of Govt Medical college, Bhavnagaren compassing, specimen from all age groups & both sexes with various disease, over a period of 1 year from 2013-2014.

The samples obtained were processed for culture of the bacteria as per routine standards methods. Only one enterococcal isolate was analyzed from each patient.

A total of 125 isolates were obtained from different clinical samples 2013-2014.

The study was conducted under the following steps:-

1. Culture of the specimens and identification of Genus enterococcus.
2. Identification of enterococcal species.
3. Antimicrobial sensitivity testing by modified Kirby Bauer disc diffusion method.
4. Detection of VRE.

a) Vancomycin disc diffusion method using vancomycin (30ug) disc susceptibility testing by modified Kirby – Bauer disc diffusion method.

b) Vancomycin agar screen method – (vancomycin 6 ug/ml) was used for true detetion of vancomycin resistance.

5. Statistical Analysis: Chi- square was used to compare differences in resistance to antibiotics among the entercoccal species. A þ value of <0.05 was used to indicate significant differences.

Detection of VRE and VSE is done. Chi-square was used to compare differences in resistance to antibiotics among the entercoccal species. A þ value of <0.05 was used to indicate significant differences.

Culture of the specimen & identification of genus entercoccus

Isolates received from clinical samples were presumptively identified as *Enterococci* by colony morphology. Morphology on Gram's staining, the absence of catalase production, the presence of pyrrolidonyl arylamidase by hydrolysis of L- pyrrolidonyl – B-naphthylamide (Himedia Labs),tolerance to 65% sodium chloride.

(A) Clinical samples like urine, Pus, Body fluids, etc were inoculated on the following media:

a) Blood Agar (BA)
b) Mac Conckey Agar (MCA)
c) Blood was inoculated into blood culture bottle containing brain heart infusion broth.

(B) Culture media after inoculation were incubated at 35-37^{0}C for 18-24 hrs aerobically in an incubator.

(C) Media were examined for microbial growth.

(D) Identification of the colony as enterococcus was done on the basis of:

a) Colony characteristics.
b) Morphology on Gram's staining
c) Catalase test.
d) Tolerance to bile esculin.
e) Salt (6.5 % Sodium Chloride) Tolerance test.
f) PYR Test (Hydrolysis of L – Pyrrolidonyl – ß – napthylamide).

(E) Species identification of Enterococcal isolates was done on the basis of :

a) Sugar fermentation (lactose, mannitol, sucrose, maltose)
b) Pigmentation on Blood agar (BA).
c) Motility.
d) Arginine hydrolysis.
e) Pyruvate utilization.

Anti-microbial susceptibility testing of of Enterococcal Isolates

All the Enterococcal isolates were subjected to modified Kirby- Bauer disc diffusion susceptibility using standard techniques as per (CLSI 2009) recommendations.

Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method using antibiotic discs and Muller Hinton Agar (Himedia) as recommended by CLSI.

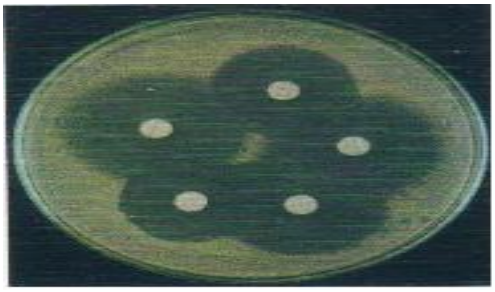
The antibiotic discs and their potency (antibiotic content) that was used for susceptibility testing of enterococcal isolates is listed below:

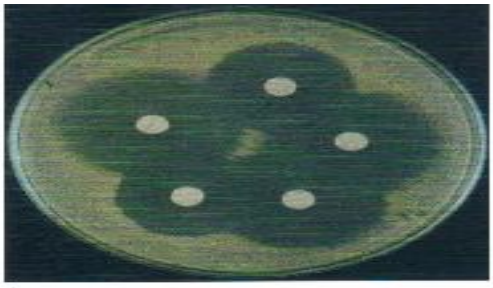
Anitbiotic discs	Potency
Ampicillin	10 ug.
Penicillin – G	10 units
Linezolid	30 ug.
Vancomycin	30 ug.
Gentamicin	30ug.
Ciprofloxacin	5 ug.
Norfloxacin	10 ug.
Nitrofurantoin	300 ug.
Tetracycline	30 ug.
Teicoplanin	30 ug.

After placing the antibiotic discs on the agar surface, the plates were incubated at 37^0C for 18-24 hrs.

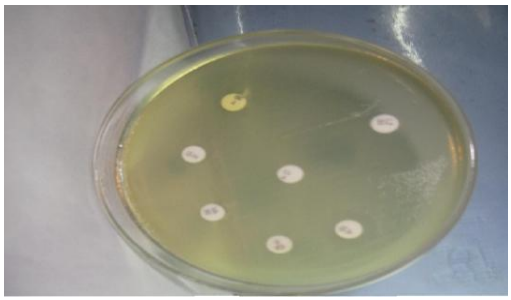
After 18 – 24 hrs of incubation, the plates were viewed with unaided eye using reflected light for the presence or absence of zones of inhibition around each of antibiotic discs.

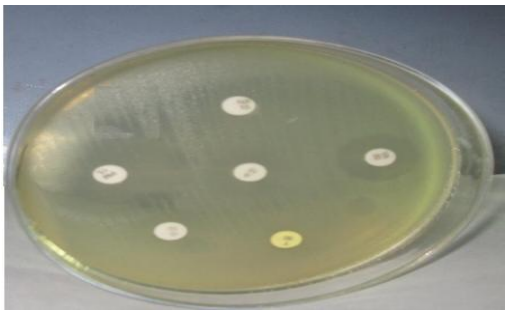
If present, the zone of inhibition around the respective antibiotics discs was measured using a ruler to within the nearest millimeters, including the diameter of the disc.





Modified Kirby: Bauer disc diffusion Susceptibilty testing with Standard strain of Staphylococcus aureus-ATCC 25923





Modified Kirby Bauer disc diffusion Susceptibility testing with Enterococcal strain

Result and Discussion

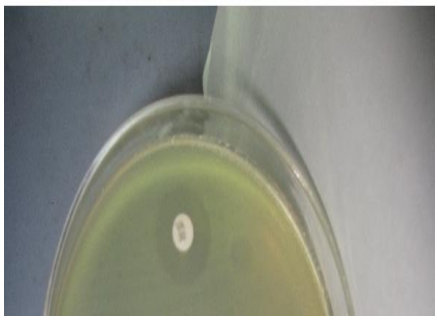
The zones of inhibition obtained by measurement were interpreted by comparison with reference zone sizes for the respective antibiotic disc when tested for *Enterococci*, as being susceptible, intermediate or Resistant. The antibiotic discs used for susceptibility testing and their reference zone sizes for interpretation as

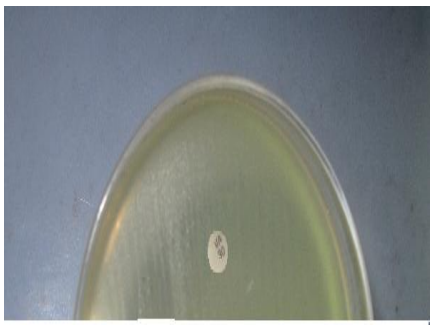
published by the manufacturer (Hi media laboratories Ltd.) are given below (these are as per CLSI recommended standards).

Zone Size Interpretative Chart

		Disc	**Zone diameter (mm)**			
S.No.	Antibiotic	Potency	Quality control staph aureus ATCC 25923	Susceptible	Intermediate	Resistance
1.	Penicillin - G	10 units	26 – 37	≥15	-------	≤14
2.	Ampicillin	10 ug.	27 – 35	≥17	------	≤16
3.	Erythromycin	15 ug.	22 – 30	≥23	14 – 22	≤13
4.	Tetracycline	30 ug.	24 – 30	≥19	15 – 18	≤14
5.	Linezolid	30 ug.	25 – 32	≥23	21 – 22	≤20
6.	Vancomycin	30 ug.	17 – 21	≥17	15 – 16	≤14
7.	Teicoplanin	30 ug.	15 – 21	≥14	11 – 13	≤10
8.	Ciprofloxacin	5 ug.	22 – 30	≥21	16 – 20	≤15
9.	Gentamicin	30 ug.	16 – 23 mm (*E.faecalis* ATCC29212)	≥10 mm	7 – 9 mm (Inconclusive)	≤6 mm.
10.	Nitrofurantoin	300 ug.	18 – 22	≥17	15 -16	≤14

S.No.	**Result**	**Inhibition zones (in mm)**		
		Susceptible	**Intermediate**	**Resistant**
1.	Vancomycin	≥17	15 – 16	≤14 and 1 or any discernable growth within the inhibition zone
2.	Teicoplanin	≥14	11 – 13	≤10





MHA Plate Showing Susceptibility Enterococcal strain showing resistant vancomycin (30mcg) vancomycin (30mcg) disc. Screening for vancomycin resistance was performed by the vancomycin disc diffusion method and vancomycin agar screen method

Detection of Vancomycin Resistance in *Enterococci*

Vancomycin was used for detection of vancomycin resistance.

Using 30 ug vancomycin disc in antimicrobial susceptibility testing by modified Kirby–Bauer disk diffusion method

After 24 hr of incubation, the Muller Hinton agar plate containing the vancomycin disc was examined with unaided eye using transmitted light for presence or absence of inhibition zone around the disc. Inhibition zones, if present, were measured with ruler. Any visible growth within the inhibition zones was also noted.

- Susceptible VSE
- Resistant VRE.
- Intermediate – *Enterococci* with intermediate susceptibility to vancomycin.

All enterococcal strains, including those strains that were vancomycin resistant by the vancomycin disc diffusion method were further tested for vancomycin resistance by the vancomycin agar screen method. Organism with intermediate zones should be tested by an MIC method as per CLSI recommendedation.

Vancomycin Agar screen method

Disc diffusion method had problems detecting low- level vancomycin resistance in *Enterococci*. The sensitivity & specificity of the vancomycin agar screen test to detect vancomycin resistance (low level) is very high 96-99% & 100% respectively.

ATCC1=E. faecalis ATCC 29212 (Vancomycin Susceptible)

ATCC2=*E.faecalis* ATCC 51299 (Vancomycin Resistant)

1, 3& 4 =VRE Strains

2 & 5 = VSE Strains

S.No.	Observation	Result	Interpretation
1	>1 colony or a film of growth	Positive (vancomycin resistant)	Presumptive Vancomycin resistance in entercocci (VRE)
2	Absence of growth	Negative (vancomycin sensitive)	Vancomycin susceptible Enterocci (VSE)
3	Unexpected or Inappropriate results with Quality control strains	Invalid	Test to be repeated

Inference – Thus evidence of small colonies (> 1 colony) or a film of growth indicate presumptive vancomycin resistance in *Enterococci* (VRE strains) .

Table.1 Isolation rate of *Enterococci* or Incidence of Enterococcal Isolates

S.No	**Total No of specimen**	**Total No of enterococcal isolates**	**Percentage**
1	921	22	2.3%

Table.2 Distribution of Enterococcal Isolates in various clinical samples

Out of 921 various clinical samples (1 per patient), 22 (2.3%) were identified as *Enterococci*.

S.No.	Specimen Type	No. of Speicmen type	No. (%) of Enterococcal isolates (Out of total enterococcal isolates)	Percentage (out of total specimens)
1.	Urine	569	14(63.63)	2.4 %
2.	Pus and Wound Swabs	166	05(22)	3%
3.	Blood	96	02(9)	2.0 %
4.	Specimens from lower respiratory tract	75	1(4.5)	1.3 %
5.	Body Fluids	10	0	0
6.	Cerebrospinal fluid (CSF)	05	0	0
	Total	921	22	2.3 %

Table.3 Department wise distribution of Enterococcal Isolates

Urine yielded the maximum number 14(2.4%) of enterococcal isolates.

S.No.	Department	No. of Isolates	Percentage
1.	Medical Wards	07	31.81 %
2.	Surgical Wards (ENT, GEN.)	05	22.72 %
3.	Nursery	02	9.09%
4.	Pediatrics	04	18 %
5.	Intensive Care Unit	01	4.5 %
6.	Burn	02	9.09 %
7.	Obstetrics & Gynecology	01	4.5%
	Total	22	100 %

Table.4 Distribution and Species identities of *Enterococci* from Various clinical samples

S.No.	Specimen Type	No. (%) of Isolates		Total
		E.faecalis	***E.faecium***	
1.	Urine	10 (71.42)	04 (28.57)	14(63.63%)
2.	Pus and Wound Swabs	04 (80)	1 (20)	05(22%)
3.	Blood	01 (50)	01 (50)	02(9%)
4.	Specimens from Lower respiratory Tract	1 (100)	0	1(4.5%)
5.	Body Fluids	0	0	0
6.	CSF	0	0	0
	Total	16(68.75)	06 (21.87)	22 (100%)

Table.5 Anti microbial Resistance pattern of *Enterococcus* species tested by Kirby Bauer disc diffusion method

S.No.	Anti Microbial agents	No. (%) of resistant strains			Total (n=22)
		***E.faecalis* (n= 16)**	***E.faecium* (n = 06)**		
1.	Penicillin – G	05 (31.64)	06 (95.34)		10 (43.75)
2.	Ampicillin	04 (22.78)	05 (86.04)		8(37.5)
3.	Gentamicin	7(44.30)	04 (72.89)		11(50)
4.	Erythromycin	15 (97.46)	06(100)		21(96.87)
5.	Vancomycin	1 (5.2)	1 (15.7)		1(0.64)
6.	Teicoplanin	1 (5.2)	1 (15.7)		1 (0.64)
7.	Linezolid	0	0		0
8.	Ciprofloxacin	11(72.15)	05 (81.39)		16(75)
9.	Tetracycline	5(29.11)	1 (15.7)		06(28.1%)

The distribution of isolates among all the clinical specimens is given in Table No.6. Of all the 22 enterococcal isolates 14 (63.63%) strains were isolated from Urine, 05(22%) from Pus and Wound swabs and 02(9%) from Blood and 1(4.5%) from lower respiratory tract (Table – 6)

E.faecalis 16(68.75%) was the most common species isolated from the clinical samples followed by *E.faecium* 06(21.87%).

No enterococcal isolate was recovered from body fluids and CSF.

Table.5a Results of Vancomycin (30 ug) disc diffusion test

Total tested	**% R**	**% I**	**% S**
No. of isolates	2	------	20
Percentage	0.64	------	99.36

Table.6 Results of Vancomycin agar screen test

Out of 22 enterococcal strains tested, 2 (0.64%) were resistant to vancomycin (VRE) in the disc diffusion method.

Vancomycin agar screen result	**Total**	**Percentage**
Resistant (VRE)	2	0.64
Susceptible (VSE)	20	99.36

Table.6a Species specific antibiotic resistance pattern of VRE isolates

VRE – Vancomycin resistant *Enterococci*
VSE – Vancomycin susceptible *Enterococci*

Distribution and incidence of VRE is more in males (3.03%). Out of 22 enterococcal strains tested from females, none was identified as VRE.

S No	**Antimicrobial Agents**	**No. (%) of VRE strains**			
		***E.faecalis* (n=1)**	***E.faecium* (N=1)**		**Total (n=2)**
1.	Penicillin-G	1(100)	1(100)		2(100)
2.	Ampicillin	1(100)	1(100)		2(100)
3.	Teracycline	1(100)	0		1(50)
4.	Teicoplanin	1(100)	1(100)		2(100)
5.	Linezolid	0	0		0
6.	Erthromycin	1(100)	1(100)		2(100)
7.	Gentamiun	1(100)	1(100)		2(100)
8.	Ciprofloxacin	1(100)	1(100)		2(100)

Table.6b Characteristics of vancomycin resistant *Enterococci* isolated in the present study

The VRE strains showed high degree of resistance to most of the antibiotics tested. All VRE strains were resistant to Penicillin-G, Ampicillin, Teicoplanin, Linezolid, Erythromycin, Gentamicin and Ciprofloxacin. Least resistance was seen for Tetracycline (50%) none of the strains shoved resistance to Linezolid.

Isolate No.	**Source**	**Zone diameter (mm) (Interpretation)**		**Vancomycin Screen agar**
		Vancomycin	**Teicoplanin**	
(1)	Blood	N (R)	N (R)	R
(2)	Urine	N (R)	N (R)	R
(3) *E. faecalis* ATCC 29212	----	22 (S)	18 (S)	S
(4) *E. faecalis* ATCC 51299	----	N (R)	10 (R)	R

Where, N= No zone; R = Resistant; S = Sensitive; MIC = Minimum inhibitory Concentration.

The wider spread use of glycopeptides in hospitals has lead to the emergence of vancomycin resistant enterocooci (VRE), which is a major concern for health care professionals. Treatment of infections caused by VRE is a challenging task especially because the resistance appears in strains, which are multi-resistant. The optimal therapy for such infections is not known. Thus, acquired resistance to vancomycin by *Enterococci* greatly reduces the number of treatment options for disease management and the problem is further compounded by the fact that resistance genes can potentially be transferred to toher pathogenic organisms, such as staphylococcus aureus and streptococcus species (Carias *et al*., 1998).

Thus, measures should be taken to prevent further development and transmission of these infections by strictly implementing infection control guidelines and antibiotic policies in hospitals. Prudent use of antibiotics and a proper surveillance for VRE may permit early recognition and containment of spread of this emergency pathogen in our country (Center for Disease control and Prevention, 1989).

Moreover it has become more difficult for treating physicians to treat such multi – resistant enterococcal strains due to the lack of adequate information regarding the species specific anti-microbial resistance pattern worldwide. There is also a paucity of information on species specific anti-microbial resistance pattern in *Enterococci* from our country.

Thus, looking to the impending need for constant monitoring of the species prevalence and antimicrobial resistance pattern (including VRE & VSE) of local enterococcal strains and its epidemiology, the present study was conducted in our setting. The present study results are consistent with other studies conducted elsewhere in India and abroad (Boyce *et al*., 2004).

Mathur *et al*., (2003) reported 66% *Enterococci* to be ampicillin resistant which is in accordance to the present study result. From India, Karmarkar *et al*., (2004) reported 100% *E.faecalis* and 85.7% E. faecium to be resistant to this drug. This incidence is much lower than that obtained in the present study but much higher than that obtained in another study from Delhi (Mathur *et al*., 2003) in which they reported only 26% E. Faecalis strains to be gentamicin resistant.

The incidence of VRE in the present study is 0.64%, which reflects the emergence of VRE in Govt medical college, Bhavnagar, Gujarat. Because of the limited therapeutic options for treating serious infections caused by VRE; it has emerged as one of the leading clinical challenge for physicians.

In conclusion, the most frequent source of enterococcal isolations in this study was urine (63.20%) and greater rate of isolation of *Enterococci* from patients admitted in wards (88.80%) highlights the organisms as one of the important cause of nosocomial urinary tract infections.Overall, in the present study, the isolates were resistant to Penicillin (43.75%), Ampicillin (37.5%), Gentamicin (50%), Erythromycin (96.87%), Tetracycline (28.1%), Ciprofloxacin (75%). None of the isolates were resistant to linezolid. Two (1.60%) strains were resistant to vancomycin and Teicoplanin.

References

Agarwal, V.A., Jain, Y.I., Pathak, A.A. 1999. Concomitant high level resistance to pencillin and aminoglycosides in *Enterococci* at Nagpur, Central India, *Indian J. Med. Microbiol.,* 17: 85 – 7.

Arthur, M., Courvalin, P. 1993. Genetics and mechanisms of glycopeptide resistance in *Enterococci. Antimicrob. Agents Chemother.,* 37: 1563-71.

Boyce, J.M., Opal, S.M., Chow, J.W., Zervos, M.J., Potter Bynoe, G., Sherman, C.B., *et al.* 1994. Outbreak of Multi – drug reistance E – faecium with transferable Van B Class Vancomycin resistance, *J. Clin. Microbiol.,* 32: 1148 – 53.

Carias, L.L., Rudin, S.D., Donskey, C., Rice, L.B. 1998. Genetic linkage and co-transfer of a novel, Van B encoding transposon (In 5382) and low affinity penicillin binding protein 5 gene in a clinical vancomycin –resistant E-faecium isolate. *J. Bacteriol.,* 180: 4426-34.

Center for Disease control and Prevention (COC). 1993. Nosocomial *Enterococci* resistant to vancomycin in United States, 1989 – 1993. *MMWR Morb. Mortal. WKLY Rep.,* 42: 597 – 9.

Karmarkar, M.G., Gersham, E.S., Mehta, P.R. 2004. Enterococcal Infections with special reference to Phenotypic characterization and drug resistance *Indian J. Med. Res.,* 119: 22-5.

Lancefield, R.C. 1933. A serological differentiation of human & other groups of hemolytic streptococci. *J. Exp. Med.,* 57: 571 – 95.

Mathur, P., Zervos, M. 1990. High Level gentamicin resistance in Enterococcus, Microbiology, genetic basis and epidemiology, *Rev. Infect. Dis.,*12: 644 – 51.

Murray, B.E. The life and times of the enterococcus, *Clin. Microbiol. Rev.,* 3: 46 – 65.

Orla Jensen, Randhawa, V.S., Deb, M. 2005. Antimicrobial resistance of enterococcal blood isolates at a paediatric care hospital in India, *Jpn J. Infect. Dis.,* 58: 101.3.

Sherman, J.M. 1937. The streptococci *Bacteriol. Rev.,* 1: 3-97.

How to cite this article:

Ankita Nisarta. 2016. Study of Antimicrobial Resistance in *Enterococci* at Government Medical College, Bhavnagar, Gujarat, India. *Int.J.Curr.Microbiol.App.Sci.* 5(9): 826-836.
doi: http://dx.doi.org/10.20546/ijcmas.2016.509.093