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Study of antimicrobial resistance in enterococci at J.L.N medical college and associated Group of Hospitals, Ajmer, Rajasthan

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Abstract
Background: 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.
Objective: The Aim of this research work is to study antimicrobial resistance in enterococci.
Methodology: 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 of JLN Medical & AG of Hospitals, Ajmer and Rajasthan for bacteriological culture and sensitivity. The samples obtained were processed for culture of the bacteria as per routine standards methods. Detection of VRE and HLAR is done. Chi-square was used to compare differences in resistance to anitibiotics among the entercoccal species. A þ value of <0.05 was used to indicate significant differences.
Result: 125 (3.53%) pure enterococcal isolates were recovered from 3534 specimens. 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%) as compared to isolates from outdoor patients (11.20%; representative of general population) Overall, this study revealed E.faecalis as the most common species (63.20%) followed by E.faecium (34.40%) and the rest species (2.46%) including E.hirae (1.60%) and E.durans (0.80%) the isolates were resistant to Penicillin (53.6%), Ampicillin (44.80%), high – level gentamicin (53.60%), Erythromycin (98.40%), Tetracycline (20.00%), Ciprofloxacin (76.00%), Quinupristin / Dalfopristin (55.20%). None of the isolates were resistant to linezolid. Two (1.60%) 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, HLGR and multi-drug resistant infection.
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 (53.6%), Ampicillin (44.80%), high – level gentamicin (53.60%), Erythromycin (98.40%), Tetracycline (20.00%), Ciprofloxacin (76.00%), Quinupristin / Dalfopristin (55.20%). None of the isolates were resistant to linezolid. Two (1.60%) strains were resistant to vancomycin and Teicoplanin.

Keywords: antimicrobial resistance, enterococci

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 amtibiotics enhances their importance as human pathogen, especially in the nosocomial setting [1].

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) [2] 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 thefermentation patterns of S-faecalis. [3]

Murray BE (1990) reported that enterococci were originally classified as enteric gram positive cocci and later included in the genus steptococcus. [4]

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. [5]

Sherman JM (1938) recommended that the term enterococcal group be specifically used for the streptococci that grow both at 10 ^{0}C and 45 ^{0}C at pH 9.6, in the presence of 6.5% NaCl, survive at 60 ^{0}C for 30 minutes and hydrolyse esculin. [6]

Material & Method

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 of JLN Medical & AG of Hospitals, Ajmer 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 out patient departments and admitted to the indoor wards of various facilities of JLN MC & AG of Hospitals, Ajmer encompassing, specimen from all age groups & both sexes with various disease, over a period of 7 months from 1st march 2009 to 30th Sept, 2009.

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 116 isolates was obtained from different from different clinical samples between March 1994 and 18 Sept 2009 in JLN Hospitals & AG, Ajmer.

The study was conducted under the following steps:-

1. Culture of the specimens and identification of Genius enterococcus.
2. Identification of enterococcal species.
3. Antimicrobial sensitivity testing by modified Kirby Bauer disc diffusion method.
4. Detecion 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.
(c) Microbroth dilution Method: Minimal inhibitory concentrations (MIC) of vancomycin were determined for VRE by microbroth dilution method using Mueller Hinton broth.
(d) Hi Comb MIC Test

5. Detection of HLAR: Gentamicin disc diffusion method using Gentamicin (120ug) disc discussion susceptibility testing by modified Kirby _Bauer disc diffusion method.
6. Statistical Analysis: Chi- square was used to compare differences in resistance to anitibiotics among the entercoccal species. A þ value of <0.05 was used to indicate significant differences.

Detection of VRE and HLAR 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 colonial morphology. Morphology on Gram's straining, the absence of catalase production, the presence of pyrrolidonyl arylamidase by hydrolysis of L- pyrrolidonyl – B- naphtylamide (Himedia Labs). Tolerance to 65% sodium chloride.

A. Clinical samples like urine, Pus, Body flids, etc were inoculated on the following media:

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

B. Culture medias after inoculation were incubated at 35-37^{0}C for 18-24 hrs aerobically in an incubator.
C. Medias were inspected for microbial growth Turbid Trypticase Soya broth was subcultured onto solid medias after 18-24 hrs. of incubation. Solid medias were inspected for colony growth.
D. Identifcation of the colony as enterococcus was done on the basis of :

(a) Colony character.
(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 (arabinose, raffinose, lactose, mamitol, sorbose, sucrose, sorbitol)
(b) Pigmentation on sheep blood agar (SBA).
(c) Motility.
(d) Arginine hydrolysis.
(e) Pyruvate utilization.

The colonies morphologically resembling enterococci on gram stained smear were subjected to –
Catalase Test.

- **Growth on BILE ESULINAGAR**
- **Salt (6.5% Nacl) TOLERANCE TEST.**
- **PYR TEST (L-pyrrolidonyl – β – napthylamide)**

Catalase Test

This test preliminarly helps in differentiating enterococci which are catalase negative from staphylococci (family Micrococcaceae) which are catalase positive.

Bile Esculin Test

This test is used to differentiate group D streptococci from other streptococci. The bile esculin test is based on the ability of certain bacteria, notably the group D streptococci to hydrolyze esculin in the presence of bile (4% bile salts or 40% bile)

Results Interpretations

(A) Positive BE test (esculin hydrolysis) Blackening of the agar slant

- Half or more of the medium is blackened
- In any time interval
- On plates black haloes will be observed around isolated colonies and any blackening is considered positive

(B) Negative BE test

- No blackening of the medium
- Blackening of less than half of the tube after 72 hours incubation (+/- rexn)
- Growth may occur but this does not indicate esculin splitting only indicates that the bile concentration did not inhibit usual growth of organisms other than the group D streptococci: Therefore growth alone on bile esculin agar does not constitute a positive test.

(3) Salt Tolerance Test (6.5% NaCl)
This test along with the bile esculin test, is used to distinguish enterococcus from the group D streptococci

Results & Interpretation
Positive test: Visible turbidity in broth, with or without acidification of the medium which is indicated by a change in colours of the medium from purple to yellow.
Negative test:-No turbidity and no colour change in NaCl broth. If the organism is bile esculin positive and fails to grow in the 6.5% NaCl broth, the organism is a group D streptococcus.

(4) PYR Test
Principle
The PYR test was first decribed by Facklam and coauthors in 1982 and, since that time has gained acceptance as a rapid test for the presumptive identification of both grp A ß.hemolytic streptococci and enterococci

Results & interpretation
Positive: The development of a deep cheery red color within 2 minutes of reagent addition
Negative: Yellow color or orange color.

Identification of Enterococcal species is done by following method:
(a) Sugar fermentation (arabinose, raffinose, lactose, mannitol, sorbose, sucrose, sorbitol)
(b) Pigmentation on SBA (yellow)
(c) Motility (on semi-solid media)
(d) Arginine dehydrolase test
(e) Pyruvate utilization

(a) Sugar fermentation tests
Principle
To determine the ability of an enterococcus to ferment (degrade) a specific carbohydrate incorporated in a basal medium producing acid carbohydrate utilization may aid in species differentiation.
1. **Positive:** Acid production is detected in the medium by the appearance of a yellow color (pH 6.8)
2. (Indicator change from purple to yellow)
3. **Delayed:** orange color if unsure compare with an uninoculated tube & reincubate
4. **Negative:** Alkaline & reddish pink color (growth but no change in color. Medium remains purple

(b) Pigment Production on Sheep Blood Agar Medium (SBA)
Enterococcus casseliflavus, E mundtii & E sulfureus produce a yellow pigment on blood agar medium.

Results & Interpretation
The pigment is detected by using a white sterile cotton swab to pick up the growth from blood agar medium & examination of the swab for a yellow colour.

(c) Motility Testing: These tests are used to determine if an enterococcus is motile.

(d) Arginine Dehydrolase test:
Principle: Decarboxylases are a group of Substrate – specific enzymes that are capable of reacting with the carboxyl (COOH) portion of amino acids, forming alkaline – reacting amines. This reaction, known as decarboxylation, forms carbon di oxide as a second product. Each decarboxylase enzyme is specific for an amino acid. The specific amine products are as follows:
Arginine –>Citrulline
Ornithine –> Putrescine

Results and Interpretation
Conversion of the control tube to a yellow color indicates that the organism is viable and that the pH of the medium has been lowered sufficiently to activate the decarboxylase enzymes. Reversion of the tube containing the amino acid to a blue-purple color indicates a positive test owing to the formation of amines from the decarboxylation reaction.
Thus, the medium first becomes yellow due to acid production during glucose fermentation and later if decarboxylation occurs, the medium becomes violet (deep purple). The control should remain yellow. Yellow color is not a positive reaction and this only indicates acid reaction, not deamination.

(5) Pyruvate Utilization
Result & Interpretation
Positive = When color changes from green to definite yellow.
Negative = Yellow – green indicates weak reaction & should be regarded as negative utilization of pyruvate.

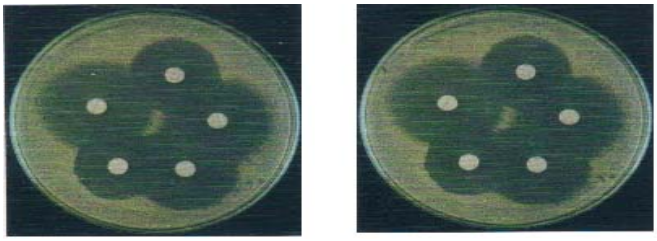
3. Anti-microbial susceptibility testing of of Enterococcal Isolates
All the Enterococcal isolates were subjected to modified Kirby- Bauer disc diffusion susceptibility using standard techniques as per (CLSI 2009) recommendations.
Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method using antibiotic disks and Mueller Hinton Agar (Himedia) as recommended by CLSI.
The panel of antibiotic discs and their potency (antibiotic content) that was used for susceptibility testing of enterococcal isolates is listed below:

S.No.	Anitbiotic discs	Potency
1.	Ampicillin	10 ug.
2.	Penicillin – G	10 units
3.	Linezolid	30 ug.
4.	Quninupristin – dalfopristin	15 ug.
5.	Vancomycin	30 ug.
6.	Gentamicin (High Level resistance Screen only)	120 ug.
7.	Ciprofloxacin	5 ug.
8.	Norfloxacin	10 ug.
9.	Nitrofurantoin	300 ug.
10.	Tetracycline	30 ug.
11.	Teicoplanin	30 ug.

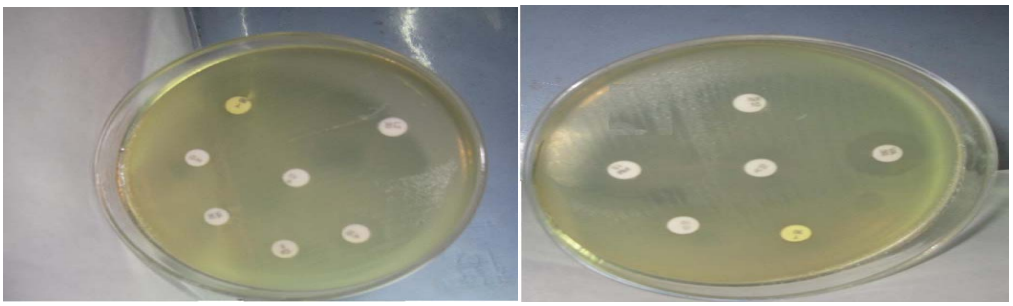
After placing the antibiotic discs on the agar surface, the plates were incubated at 37^0C for 16-18 hrs.

Observation

- After 16 – 18 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 disk.



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



Modified Kirby: Bauer disc diffusion Susceptibilty testing with Enterococcal strain

Result and Interpretation

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 diam (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.	Quinupristin/ dalfopristin	15 ug.	21 – 28	≥19	16 – 18	≤15
6.	Linezolid	30 ug.	25 – 32	≥23	21 – 22	≤20
7.	Vancomycin	30 ug.	17 – 21	≥17	15 – 16	≤14
8.	Teicoplanin	30 ug.	15 – 21	≥14	11 – 13	≤10
9.	Ciprofloxacin	5 ug.	22 – 30	≥21	16 – 20	≤15
10.	Gentamicin (high – Level screening only)	120 ug.	16 – 23 mm (E.faecalis ATCC29212)	≥10 mm	7 – 9 mm (Inconclusive)	≤6 mm.
11.	Nitrofurantoin	300 ug.	18 - 22	≥17	15 -16	≤14

Screening for vancomycin resistance was performed by the agar plate method and minimal inhibitory concentration of vancomycin was determined for vancomycin resistant strain by microbroth dilution method and Hi Comb MIC test.

Detection of Vancomycin Resistance in Enterococci

Vancomycin was used for detection of vancomycin resistance.

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

Observation

After full 24 hr of incubation the Mueller Hinton agar plate containing the vancomycin disk was viewed with unaided eye using transmitted light for presence or absence of inhibition zone around the disk. Inhibition zones, if present, were

measured with ruler. Any discernable/visible growth within the inhibition zones was also noted.

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 of enterococcal strain to vancomycin (30mcg) disk

Enterococcal strain showing resistance to vancomycin (30mcg) disk

Interpretation:

- 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.

(b) Vancomycin Agar screen method

Disk diffusion method have had problems detecting low-level vancomycin resistance in enterococci (both vanB & vanC types). The sensitivity & specificity of the vancomycin agar screen test to detect vancomycin resistance (esp 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

Result and Interpretation

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).

(c). Micro broth Dilution Method

This is a well standardized & reliable reference method for antimicrobial susceptibilty testing. Animal inhibitory concentration (MICs) of Vancomycin was determind using Mueller Hinton broth.

Result and interpretation

- Examine growth control wells for organism viability. For a test to be considered valid, acceptable growth (≥ 2 mm button or definite turbidity) must occur in the growth control well.
- Check inoculum purity by subculture.
- Verify inoculum size periodically by quantitative sub culture.
- Confirm appropriateness of MICs for the quality control strains

The CISI 2009 MIC interpretative criteria for dilution susceptibility testing:-

Anitimicrobial agent	MIC (Ug/ml)		
	Susceptible	Intermediate	Resistant
Vancomycin	≤4	8-16	≥32

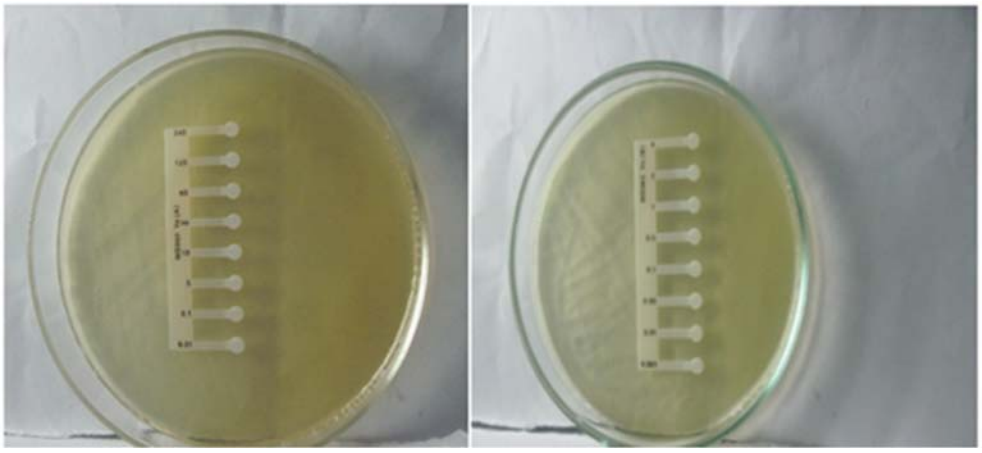
(d)Hi Comb MIC Test

Hi Comb is an innovative gradient-based technique. This system provides a set of 16 different concentrations in gradient that can be easily used to deduce the Minimum Inhibitory Concentration (MIC) in microgram levels, of a desired antibiotic against micro-organisms on agar medium following incubation at specified time interval.

Results and Interpretation

The zone of inhibition will be in the form of an ellipse. MIC value would be the value at which zone convenes the comb – Like projections of the strips and not at the handle. If there is no zone of inhibition observed, report the MIC as greater than the highest concentration on the strip. If the zone inhibition is below the lowest concentration then report the MIC as less than the lowest concentration.

The MIC value interpreted from Part A might not necessarily be the same for Part B. In that case white reporting the MIC value – report the value that is the lower of the two.



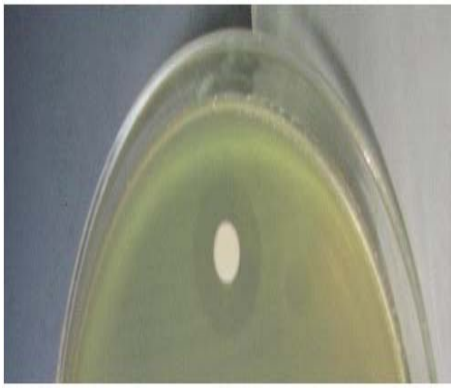
5. Detection of HLAR = High content gentamicin discs were prepared locally obtained fom Ranbaxy antibiotics.

Using 120ug Gentamicin disc in anti-micobial susceptibilitytesting by modified Kirby-Bauer disk diffusion method.

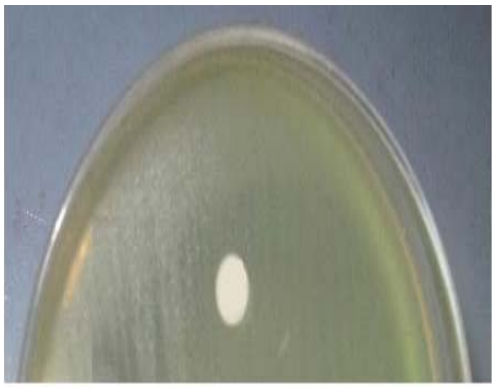
Observation

After 16-18 hours of incubation the Mueller Hinton Agar plate containg the Gentamicn disk was viewed with the unaided eye using transmitted light for presence or absence of inhibition zones, if present, were measured with ruler. Any discernable / visible growth within inhibition zones was also noted.

Results	Inhibition Zones (in mm)
Susceptible	≥10mm
Resistant	6mm &/ or any discernable growth within the inhibition zone.
Inconclusive	7-9 mm



MHA Plate Showing susceptibility resistants to Gentamicin (120mcg) disk



Enterococcal strain showing of enterococcal strain to Genamicin (120mcg) disk

Interpretation

Susceptible – Gentamicin sensitive Enterococci

Resistant – Gentamicin Resistance enterococci i.e. presence of HLGR.

The absence of zone of inhibition corresponds to the presence of HLR, MIC correlates: Resistant = ≥500ug/ml

Susceptible = ≤500ug/ml

Results

Table 1*:* Isolation rate of enterococci or Incidence of Enterococcal Isolates

S.No	Total No of specimen	Total No of enterococcal isolates	Percentage
1	3534	125	3.53%

Out of 3534 various clinical samples (1 per patient), 125 (3.53%) were identified as enterococci.

Table 2: Distribution of Enterococcal Isolates in various clinical samples.

S.No.	Specimen Type	No. of Speicmen type	No. (%) of Enterococcal isolates (Out of total enterococcal isolates)	Percentage (out of total specimens)
1.	Urine	1306	79 (63.20)	6.04 %
2.	Pus and Wound Swabs	307	17 (13.60)	5.53 %
3.	Blood	1339	26 (20.80)	1.94 %
4.	Specimens from lower respiratory tract	364	3 (2.40)	0.82 %
5.	Body Fluids	156	0	0
6.	Cerebrospinal fluid (CSF)	62	0	0
	Total	3534	125	3.53 %

Urine yielded the maximum number 79 (6.04%) of enterococcal isolates

Table 3: Department wise distribution of Enterococcal Isolates

S.No.	Department	No. of Isolates	Percentage
1.	Medical Wards	74	59.20 %
2.	Surgical Wards (ENT, GEN.)	15	12 %
3.	Nursery	5	4 %
4.	Pediatrics	19	15.20 %
5.	Intensive Care Unit	6	4.8 %
6.	Burn	2	1.6 %
7.	Obstetrics & Gynecology	4	3.2 %
	Total	125	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	E. hirae	E. durans	
1.	Urine	43 (54.43)	33 (41.77)	2 (2.53)	1 (1.26)	79 (63.20)
2.	Pus and Wound Swabs	16 (94.11)	1 (5.88)	0	0	17 (13.60)
3.	Blood	18 (69.23)	8 (30.76)	0	0	26 (20.80)
4.	Specimens from Lower respiratory Tract	2 (66.66)	1 (5.88)	0	0	3 (2.40)
5.	Body Fluids	0	0	0	0	0
6.	CSF	0	0	0	0	0
	Total	79 (63.2)	43 (34.4)	2 (1.60)	1 (0.80)	125 (100)

The distribution of isolates among all the clinical specimens is given in Table No.6. Of all the 125 enterococcal isolates 79 (63.20%) strains were isolated from Urine, 17 (13.60%) from Pus and Wound swabs and 26 (20.80%) from Blood and 3 (2.4%) from lower respiratory tract (Table – 6) E.faecalis (63.20) was the most common species isolated from the clinical samples followed by E.faecium (34.4). The other enterococcal species (2.46%) comprise of E.hirae (1.60%) and E.durans (0.80%). The 3 isolates of the unusual species were from Urine (2.46%).

The isolates were found to consist of E.faecalis (63.20%) E.faecium (34.4%) and other Enterococcus species (2.46%). No enterococcal isolate was recovered from body fluids and CSF.

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=125)
		E.faecalis (n= 79)	E.faecium (n = 43)	Other enterococci (n = 3)	
1.	Penicillin - G	25 (31.64)	4 (95.34)	1 (33.33)	67 (53.6)
2.	Ampicillin	18 (22.78)	37 (86.04)	1 (33.33)	56 (44.8)
3.	Gentamicin (HLGR)	35 (44.30)	31 (72.89)	1 (33.33)	67 (53.6)
4.	Erythromycin	77 (97.46)	43 (100)	3 (100)	123 (98.40)
5.	Vancomycin	1 (1.26)	1 (2.32)	0	2 (1.60)
6.	Teicoplanin	1 (1.26)	1 (2.32)	0	2 (1.60)
7.	Quinupristin / Dalfopristin	65 (82.27)	1 (2.32)	3 (100)	69 (55.2)
8.	Linezolid	0	0	0	0
9.	Ciprofloxacin	57 (72.15)	35 (81.39)	3 (100)	95 (76.00)
10.	Tetracycline	23 (29.11)	1 (2.32)	1 (33.33)	25 (20%)

HLGR = High Level Gentamicin resistance Include E – hirae (2) and E – Durans (1)

Table 5A: Results of Vancomycin (30 ug) disc diffusion test

Total tested	% R	% I	% S
No. of isolates	2	------	123
Percentage	1.6	------	98.4

Out of 125 enterococcal strains tested, 2 (1.60%) were resistant to vancomycin (VRE) in the disc diffusion method.

Table 6: Results of Vancomycin agar screen test

Vancomycin agar screen result	Total	Percentage
Resistant (VRE)	2	1.60
Susceptible (VSE)	123	98.4

VRE – Vancomycin resistant enterococci
VSE – Vancomycin susceptible enterococci
Distribution and incidence of VRE is more in males (3.03%). Out of 125 enterococcal strains tested from females none was identified as VRE.

Table 6A: Species specific antibiotic resistance pattern of VRE isolates

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

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, Quinupristin/Dalfopristin, Erythromycin, Gentamiun (HLGR) and Ciprofloxacin. Least resistance was seen for Tetracycline (50%) none of the strains shoved resistance to Linezolid.

Table 6B: Characteristics of vancomycin resistant enterococci isolated in the present study

Isolate No.	Source	Zone diameter (mm) (Interpretation)		Vancomycin Screen agar	MIC (ug/ml)		PYR Phenotype
		Vancomycin	Teicoplanin		Hi comb MIC Test	Broth dilution	
(1)	Blood	N (R)	N (R)	R	> 256	256	Van – A
(2)	Urine	N (R)	N (R)	R	> 256	512	Van – A
(3) E. faecalis ATCC 29212	----	22 (S)	18 (S)	S	4	4	--------
(4) E. faecalis ATCC 51299	----	N (R)	10 (R)	R	> 256	---	Van - A

Where,
N= No zone
R = Resistant
S = Sensitive
MIC = Minimum inhibitory Concentration.
Observations of the Table 16 shows that out of 2 (1.72%) strains one was E-faecium from blood and one was E-faecalis from urine. The MIC values of Vancomycin and Teicoplanin (as Calculated by micro broth dilution method and E-test). Were and respectively. All of the VRE strains carried the Van-A phenotype.

Discussion

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 to her pathogenic organisms, such as staphylococcus aureus and streptococcus species. [7]

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 and HLGR may permit early recognition and containment of spread of this emergency pathogen in our country. [8]

Moreover it has become more difficult for treating physicans to treat such multi – resistant enterococcal strains due to the lack of adequate information regarding the species specific anti-microbial resistance pattern (including VRE & HLGR) 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 prevelance and antimicrobial resistance pattern (including VRE & HLGR) of local enterococcal strains and its epidemiology, the present study was conducted in our setting.

Our results are consistent with other studies conducted else where in India and abroad. (Boyce etal, 2004). [9]

P. Mathur et al (2003) reported 66% enterococci to be ampicillin resistant which is in accordance to the present study result. [10]

In our study, 44.30% E.faecalis, 72.09% E.faecium and overall 53.60% enterococcal strains revealed resistance to high level gentamicin. From India, Karmarkar etal (2004) [11] 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 (P. Mathur etal, 2003) in which they reported only 26% E. Faeclais strains to be high-level gentamicin resistant.

The incidence of VRE in the present study is 1.60%, which reflects the emergence of VRE in J.L.N. hospital, Ajmer .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.

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 (53.6%), Ampicillin (44.80%), high – level gentamicin (53.60%), Erythromycin (98.40%), Tetracycline (20.00%), Ciprofloxacin (76.00%), Quinupristin/Dalfopristin (55.20%). None of the isolates were resistant to linezolid. Two (1.60%) strains were resistant to vancomycin and Teicoplanin.

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